Subacute endotoxemia induces adipose inflammation and changes in lipid and lipoprotein metabolism in cats

Osto, M; Zini, E; Franchini, M; Wolfrum, C; Guscetti, F; Hafner, M; Ackermann, M; Reusch, C E; Lutz, T A

Postprint available at:
http://www.zora.uzh.ch

Posted at the Zurich Open Repository and Archive, University of Zurich.
http://www.zora.uzh.ch

Originally published at:
Subacute endotoxemia induces adipose inflammation and changes in lipid and lipoprotein metabolism in cats

Abstract

Acute inflammation in humans is associated with transient insulin resistance (IR) and dyslipidemia. Chronic low-grade inflammation is a pathogenic component of IR and adipose tissue dysfunction in obesity-induced type 2 diabetes. Because feline diabetes closely resembles human type 2 diabetes, we studied whether lipopolysaccharide (LPS)-induced subacute inflammation, in the absence of obesity, is the potential primary cause of IR and metabolic disorders. Cats received increasing iv doses (10-1000 ng/kg(-1) \cdot h(-1)) of LPS (n = 5) or saline (n = 5) for 10 d. Body temperature, proinflammatory and metabolic markers, and insulin sensitivity were measured daily. Tissue mRNA and protein expression were quantified on d 10. LPS infusion increased circulating and tissue markers of inflammation. Based on the homeostasis model assessment, endotoxemia induced transient IR and β-cell dysfunction. At the whole-body level, IR reverted after the 10-d treatment; however, tissue-specific indications of IR were observed, such as down-regulation of adipose glucose transporter 4, hepatic peroxisome proliferative activated receptor-γ1 and -2, and muscle insulin receptor substrate-1. In adipose tissue, increased hormone-sensitive lipase activity led to reduced adipocyte size, concomitant with increased plasma and hepatic triglyceride content and decreased total and high-density lipoprotein cholesterol levels. Prolonged LPS-induced inflammation caused acute IR, followed by long-lasting tissue-specific dysfunctions of lipid-, glucose-, and insulin metabolism-related targets; this ultimately resulted in dyslipidemia but not whole-body IR. Endotoxemia in cats may provide a promising model to study the cross talk between metabolic and inflammatory responses in the development of adipose tissue dysfunction and IR.
Subacute Endotoxemia Induces Adipose
Inflammation and Changes in Lipid and Lipoprotein Metabolism in Cats

Institute of Veterinary Physiology (M.O., T.A.L.), Clinic of Small Animal Internal Medicine (E.Z., M.H., C.E.R.), Institute of Virology (M.F., M.A.), and Institute of Veterinary Pathology (F.G.), Vetsuisse Faculty, University of Zurich, 8057 Zurich, Switzerland; and Institute of Food, Nutrition and Health (C.W.), Swiss Federal Institute of Technology, 8603 Schwerzenbach, Switzerland

Address all correspondence and requests for reprints to: Professor Dr. med. vet. Thomas A. Lutz, Institute of Veterinary Physiology, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 260, 8057 Zurich, Switzerland. E-mail: tomlutz@vetphys.uzh.ch.

Abstract
Acute inflammation in humans is associated with transient insulin resistance (IR) and dyslipidemia. Chronic low-grade inflammation is a pathogenic component of IR and adipose tissue dysfunction in obesity-induced type 2 diabetes. Because feline diabetes closely resembles human type 2 diabetes, we studied whether lipopolysaccharide (LPS)-induced subacute inflammation, in the absence of obesity, is the potential primary cause of IR and metabolic disorders. Cats received increasing iv doses (10–1000 ng/kg·h) of LPS (n = 5) or saline (n = 5) for 10 d. Body temperature, proinflammatory and metabolic markers, and insulin sensitivity were measured daily. Tissue mRNA and protein expression were quantified on d 10. LPS infusion increased circulating and tissue markers of inflammation. Based on the homeostasis model assessment, endotoxemia induced transient IR and β-cell dysfunction. At the whole-body level, IR reverted after the 10-d treatment; however, tissue-specific indications of IR were observed, such as down-regulation of adipose glucose transporter 4, hepatic peroxisome proliferative activated receptor-γ1 and -2, and muscle insulin receptor substrate-1. In adipose tissue, increased hormone-sensitive lipase activity led to reduced adipocyte size, concomitant with increased plasma and hepatic triglyceride content and decreased total and high-density lipoprotein cholesterol levels. Prolonged LPS-induced inflammation caused acute IR, followed by long-lasting tissue-specific dysfunctions of lipid-, glucose-, and insulin metabolism-related targets; this ultimately resulted in dyslipidemia but not whole-body IR. Endotoxemia in cats may provide a promising model to study the cross talk between metabolic and inflammatory responses in the development of adipose tissue dysfunction and IR.

In recent years, chronic activation of the innate and adaptive immune system has been causally linked to the development of insulin resistance (IR) in metabolic diseases such as diet-induced obesity and type 2 diabetes (1, 2). In mice, infiltration of adipose tissue by neutrophils and macrophages and activation of toll-like receptors (TLRs), e.g., TLR4, in these cells induce a local release of inflammatory cytokines (3–5). The activation of inflammatory cascades is believed to increase the activity of serine/threonine kinases and to promote phosphorylation of insulin receptor substrate (IRS) sites, which results in impaired insulin receptor signaling and defective insulin-mediated glucose metabolism (6, 7). Interestingly, excess circulating nonesterified fatty acids (NEFAs) and triglycerides (TGs), which may be derived from increased lipolytic activity in adipose tissue, also block insulin action through direct or cytokine-mediated activation of serine kinases. Indeed, saturated NEFAs can elicit TLR4-dependent synthesis of cytokines in murine adipose cells and macrophages (8, 9). As a consequence, the low-grade chronic increase of cytokines and chemokines and the increased circulating levels of TGs and NEFAs gradually impair insulin sensitivity in obesity. Infection and inflammation lead to changes in lipid and lipoprotein profile comparable with those observed in obesity and type 2 diabetes. High-plasma TG levels or hypercholesterolemia combined with low levels of high-density lipoprotein (HDL) cholesterol are typical features of dyslipidemia and contribute to IR in the human metabolic syndrome.
The activation of a chronic inflammatory state may not only be the primary cause of obesity-induced IR, but any disease involving inflammation may also eventually be linked to IR and alterations in lipid and lipoprotein metabolism. This hypothesis is supported by clinical and experimental evidence showing that glucose intolerance, IR, and dyslipidemia naturally develop during acute infection and critical illness in humans (10). However, whether inflammation is sufficient as the key initiating event in the development of IR and abnormalities in lipid or glucose metabolism in the absence of obesity or whether it is a secondary event to obesity-related disorders remains to be determined. Especially, the role of tissue-specific inflammation, e.g. in adipose tissue, in the impairment of insulin signaling pathways during infection or critical illness needs to be addressed.

Experimental endotoxemia has been widely used as model of bacterial infection, inflammation, and sepsis and was shown to change glucose and lipid metabolism in a way that resembles that observed in humans with the metabolic syndrome (10-12). In most studies, however, bacterial lipopolysaccharide (LPS) has been administered at high doses for no longer than a few hours (13), mimicking more an acute-phase response than a subacute or chronic inflammatory condition. Only a single study has been published indicating that chronic endotoxemia may be sufficient to induce IR and obesity in mice (14). However, deeper insights into the regulatory mechanisms of insulin, glucose, and lipid metabolism during prolonged LPS exposure in humans or in animal models which spontaneously develop type 2 diabetes are needed.

In contrast to current rodent models, cats naturally develop a form of diabetes that shares many similarities to human type 2 diabetes, including obesity-induced IR and dyslipidemia, impaired β-cell function and pancreatic amyloid deposition (15, 16). Recently we have shown that sustained hyperglycemia and hyperlipidemia promotes inflammatory responses in experimental cats and led to increased circulating and tissue levels of inflammatory markers (17, 18). In addition, some studies reviewed by Rand et al. (19) suggested that IR may be ameliorated in diabetic cats if concurrent inflammatory diseases are successfully treated.

In the present study, we investigated whether, in the absence of obesity, a 10-d infusion of LPS in cats induces a low-grade inflammatory response similar to that in humans with the metabolic syndrome. We hypothesized that continuous exposure to small doses of LPS in cats, besides activating inflammation and impairing insulin sensitivity, has a detrimental effect on tissue-specific genes linked to glucose and lipid metabolism.

**Materials and Methods**

Ten neutered male, 12- to 15-month-old healthy domestic shorthair cats (Liberty Research, Waverly, NY) were used. All cats were healthy based on clinical examination and hematological and serum biochemical analyses. During the study cats were housed individually in single cages. The experiment was approved by the Veterinary Office, Zurich, Switzerland (no. 116/2007).

The experimental paradigm is shown in Fig. 1. Animals were adapted to housing and feeding conditions for 5 wk before the baseline iv glucose tolerance test (ivGTT) was performed as described (17). After a 2-wk recovery period, animals were randomly allocated to two groups (n = 5 each) to receive either increasing doses of iv LPS or saline infusion. Groups were matched based on their glucose half-life during the baseline ivGTT and based on body weight at the end of the recovery period (d 0). On d 0, a jugular catheter was implanted and a telemetric transmitter (Data Sciences International, St. Paul, MN; see Supplemental Materials and Methods, published on The Endocrine Society's Journals Online web site at http://endo.endojournals.org) to record body temperature was placed sc on the back of the cats' neck under general anesthesia, as described (17). Infusion of increasing doses of LPS derived from Escherichia coli 0127:B8 [10 (0–6 h), 200 (7–24 h), 500 (25–140 h), and 1000 (141–240 h) ng/kg h⁻¹ diluted in saline and infused at 2–4 ml/kg h⁻¹; Sigma-Aldrich, St. Louis, MO] or saline (2 ml/kg h⁻¹) was started after complete recovery from anesthesia and was maintained for 10 d. LPS doses had been previously determined in a pilot study (Supplemental Fig. 1). During the pilot study, the LPS concentration of the solution and the infusion rate were gradually increased to raise and maintain rectal temperature just above...
normal range for cats, i.e. to targeted levels between 39.2 and 40.5 C. During the 10-d infusion, daily blood samples (0.5 ml) were collected after overnight fasting for biochemical analysis. A second ivGTT was performed 1 h after the end of the infusion. Thereafter cats were euthanized and tissue biopsies were excised, as described (17).

Fig. 1. Overview of the study design. Animals were adapted to the housing and feeding conditions for 5 wk before a baseline ivGTT was performed. After a recovery period of at least 2 wk, a catheter was implanted in the jugular vein and a telemetric transmitter was placed sc under general anesthesia (d 0). Animals were randomly divided into two groups of five cats each to receive either increasing doses of LPS (10, 200, 500, and 1000 ng/kg$^{-1} \cdot h^{-1}$) or saline. During the 10-d infusion, daily blood samples (0.5 ml) were collected after overnight fasting for biochemical analysis. On d 10, a second ivGTT was performed 1 h after the end of the infusion. At the end of the second ivGTT, tissue biopsies were excised.

Biochemical analysis and assessment of insulin sensitivity and β-cell function
Daily fasting values of glucose, insulin, TGs, NEFAs, and α1-acid glycoprotein (AGP) were measured as described elsewhere (17). Plasma lipoproteins and characterization of lipoproteic fractions were performed on d 10 as outlined in Supplemental Materials and Methods (Diagnostic Service, University of Bristol, Langford, Bristol, UK). Serum cortisol was measured on d 0, 1, and 10 by a competitive chemiluminescent immunoassay (Bayer, Tarrytown, NY). On d 10, plasma monocyte-chemotactic protein-1 (MCP-1) and serum amyloid A (SAA) and liver TGs were assessed as outlined in Supplemental Materials and Methods. Fasting glucose and insulin values were used to calculate daily baseline insulin sensitivity and β-cell function with the homeostasis model assessment (HOMA-IR and HOMA-B, respectively) (20-22). The ivGTTs were performed to estimate insulin sensitivity, which was calculated with the whole-body insulin sensitivity index as described by Matsuda and DeFronzo (23) and to estimate β-cell function by using the insulin secretion index (24).

Tissue samples
Tissue biopsies from pancreas, liver, skeletal muscle, and visceral and subcutaneous fat were collected immediately after the second ivGTT as described (17). One aliquot of each tissue was fixed in 10% buffered formalin for 24 h and embedded. One aliquot was embedded in Tissue-Tek O.C.T. (optimal cutting temperature) compound (Leica Microsystems, Nussloch, Germany), and one additional aliquot of each tissue was immediately frozen in liquid nitrogen and stored at −80 C (see Supplemental Materials and Methods).

Laser capture microdissection (LCM) of pancreatic islets, pancreatic islet clusters, RNA isolation, and real-time PCR
Pancreatic islets were isolated with two methods. First, pancreatic islet-like clusters (ILCs) were isolated as described (25, 26), RNA was isolated and cDNA was subjected to quantitative real-time PCR for the macrophage marker F4/80, for MCP-1 and for IL-8. The second method of pancreatic islet isolation consists of microdissecting islets with the LCM technique; RNA was isolated and cDNA was subjected to quantitative real-time PCR for insulin, IL-6, and IL-8 (see Supplemental Materials and Methods).
From insulin-sensitive tissues (liver, skeletal muscle, and visceral and sc fat), RNA was extracted and cDNA was obtained according to standard protocols (27). Quantitative real-time PCR was performed for adiponectin, resistin, glucose transporter (GLUT)-1, GLUT4, peroxisome proliferative activated receptor (PPAR)-γ1, PPARγ2, MCP-1, neutrophil elastase (NEla), the macrophage-specific antigen F4/80, TLR-4, IL-8, IL-1β, IL-6, TNF-α, hormone-sensitive lipase (HSL), suppressor of cytokine signaling (SOCS)-3, and perilipin (see Supplemental Materials and Methods). 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.

**Western blotting**

Whole liver and sc and visceral fat were homogenized in lysis buffer to obtain nuclear protein, enriched plasma membrane protein, or whole-cell protein lysates (see Supplemental Materials and Methods). Nuclear protein lysates were used for Western blot analysis of PPARγ; and enriched plasma membrane protein lysates for analysis of GLUT4; whole-cell protein lysate for analysis of total and phosphorylated HSL. Nuclear protein, enriched plasma membrane protein and whole-cell protein lysates (35, 20, and 50 µg, respectively) were run on a 10% sodium dodecyl sulfate-polyacrylamide gel, membranes were incubated overnight at 4°C with the following antibodies: PPARγ (E-8; Santa Cruz Biotechnology, Santa Cruz, CA), primary antisera to GLUT4 (kindly supplied by Dr. L. Reagan) (28), and GLUT4 (GT41-S; α Diagnostic, San Antonio, TX), HSL, and phospho-HSL (Ser660) (4107 and 4126; Cell Signaling Technology, Danvers, MA). Western blotting was quantified by densitometry using β-actin as a loading control. Densitometric analysis was performed using Quantity One software (Bio-Rad Laboratories, Hercules, CA).

**Histopathology and immunohistochemistry**

Paraffin sections (3 µm) from all tissues tested were stained with hematoxylin and eosin for histomorphometry and with myeloperoxidase to count neutrophils as described (17). Adipocyte cross-sectional size was measured with ImageJ software (http://rsb.info.nih.gov/ij/). Average cell size was measured on five pictures collected from each cat’s visceral and sc fat tissue at ×40 magnification; all adipocytes in one microscopic field were included in the analysis. Neutrophils in islets or exocrine pancreas sections were counted in 50 microscopic fields at ×40 magnification, excluding neutrophils in large blood vessels and interlobular tissue. The number of neutrophils was similarly counted as in samples collected from the other insulin-responsive tissues tested. Histological sections were evaluated in a blinded manner.

**HSL activity assay**

Visceral and sc fat (0.5 g) was homogenized in a homogenization buffer, and the fat-free infranatant (cytosolic-fraction) was collected and used for HSL activity assays as described elsewhere (29). Briefly, the cytosolic fraction (0.2 ml) was incubated with assay buffer containing a final concentration of 330 µM triolein and [3H] triolein. The reaction was terminated by adding 3.25 ml of extraction buffer and 1.05 ml of a 0.1 m potassium carbonate, and 0.1 m boric acid solution. After vortexing and centrifugation (800 × g, 15 min), radioactivity was determined in 1 ml of the upper phase by liquid scintillation counting.

**Statistical analysis**

Data are expressed as mean ± se. Group differences were compared using the Mann-Whitney test. Data were analyzed using GraphPad Prism 4.0 (GraphPad, San Diego, CA). Significance was set at $P < 0.05$. 


Results
Is a LPS-induced subacute endotoxemia sufficient to induce systemic inflammation in cats?
Based on daily physical examinations and routine blood work, LPS infusion was well tolerated in cats. The mean body weight did not differ between baseline and after 10 d of the LPS or saline infusion (data not shown). Subcutaneous temperature progressively increased during LPS and significantly differed from controls from 15 to 121 h after the onset of infusion; afterward temperature gradually decreased to preinfusion values (Fig. 2A). LPS induced a rapid and marked increase in circulating AGP, which lasted throughout the infusion (Fig. 2B). On d 10, LPS-infused cats had elevated circulating levels of MCP-1 (99.5 ± 15.2 saline vs. 260.7 ± 55.7 LPS; P < 0.01) and SAA (24.4 ± 21.2 saline vs. 158.7 ± 86.2 LPS; P < 0.01) compared with controls (Fig. 2, C and D). These findings confirmed our hypothesis that the infusion of LPS in normal-weight cats is associated with a systemic inflammation that resembles, to some extent, the inflammatory states of obesity and type 2 diabetes in human patients.

Fig. 2.
Subacute endotoxemia induces systemic and tissue inflammation in cats. A, Mean change in sc body temperature after LPS (n = 5; filled circle) and saline (n = 5; open circle) infusion. Subcutaneous temperature was assessed every 2 h throughout the infusion period by telemetry. B, Circulating concentration of α1-acid glycoprotein during the 10-d infusion of LPS or saline. Values are expressed as mean ± se. C and D, Circulating concentration of MCP-1 (C) and SAA (D) of LPS- and saline-infused cats on d 10. E, Number of neutrophils relative to high-power field in visceral and sc adipose tissue, skeletal muscle, and liver of LPS- and saline-infused cats. Individual and mean values are shown; representative myeloperoxidase liver stainings are shown. Arrows show positively stained neutrophils. F, Relative quantities of F4/80 mRNA in pancreatic ILCs. The values are expressed as mean ± se and are relative to one cat infused with saline. *, P < 0.05 and †, P < 0.01 vs. saline-infused cats.

Is a LPS-induced subacute endotoxemia sufficient to enhance expression of inflammation- and immune response-related genes particularly in sc fat?
After 10 d of infusion, the number of neutrophils was increased in paraffin-embedded tissues of LPS-infused cats (visceral fat: 0.04 ± 0.01 vs. 0.08 ± 0.01; P = 0.09; sc fat: 0.06 ± 0.01 vs. 0.11 ± 0.01; P < 0.05; skeletal muscle: 0.04 ± 0.01 vs. 0.07 ± 0.01; P < 0.05; liver: 20.2 ± 3.4 vs. 33.0 ± 2.5 neutrophils per high power field; P < 0.05; saline- vs. LPS-infused cats; Fig. 2E) except in islets and exocrine pancreas (data not shown). Transcripts of MCP-1, NEla, and IL-1β were increased in sc fat in the LPS group. Transcripts of the macrophage marker F4/80
were augmented in pancreatic ILCs (0.5 ± 0.2 saline vs. 1.9 ± 0.5 LPS; *P* < 0.05; Fig. 2F) and in all insulin-sensitive tissues tested, except visceral fat. TLR-4 mRNA was reduced in skeletal muscle. In parallel, liver mRNA of IL-8 was increased (Table 1). These data clearly confirmed our hypothesis that the LPS-induced increase in circulating levels of inflammatory markers is paralleled by the tissue-specific activation of tissue markers of inflammation in the insulin-sensitive tissues tested and particularly in sc fat.

Table 1.
Inflammation- and insulin sensitivity-related genes in visceral and sc fat, skeletal muscle, and liver

<table>
<thead>
<tr>
<th>Gene</th>
<th>Visceral fat (mean ± st)</th>
<th>Subcutaneous fat (mean ± st)</th>
<th>Skeletal muscle (mean ± st)</th>
<th>Liver (mean ± st)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N.ELa</td>
<td>1.4 ± 0.7</td>
<td>0.7 ± 0.2</td>
<td>n.d.</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>IL-8</td>
<td>1.7 ± 0.4</td>
<td>0.4 ± 0.2</td>
<td>n.d.</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>MCP-1</td>
<td>3.7 ± 0.9</td>
<td>1.2 ± 0.3</td>
<td>n.d.</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td>F480</td>
<td>1.4 ± 0.6</td>
<td>0.5 ± 0.2</td>
<td>0.7 ± 0.2</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>IL-6</td>
<td>1.9 ± 0.7</td>
<td>0.9 ± 0.4</td>
<td>5.5 ± 3.3</td>
<td>n.d.</td>
</tr>
<tr>
<td>IL-1β</td>
<td>1.7 ± 0.5</td>
<td>1.1 ± 0.5</td>
<td>2.8 ± 0.6</td>
<td>n.d.</td>
</tr>
<tr>
<td>TNF-α</td>
<td>4.2 ± 2.8</td>
<td>1.2 ± 0.3</td>
<td>7.1 ± 0.8</td>
<td>n.d.</td>
</tr>
<tr>
<td>TLR-4</td>
<td>4.9 ± 1.5</td>
<td>4.4 ± 1.4</td>
<td>2.8 ± 0.6</td>
<td>n.d.</td>
</tr>
<tr>
<td>SGC53</td>
<td>0.9 ± 0.3</td>
<td>0.9 ± 0.3</td>
<td>1.1 ± 0.2</td>
<td>n.d.</td>
</tr>
<tr>
<td>GLUT1</td>
<td>2.0 ± 1.2</td>
<td>0.9 ± 0.3</td>
<td>1.0 ± 0.2</td>
<td>n.d.</td>
</tr>
<tr>
<td>GLUT4</td>
<td>0.9 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>1.0 ± 0.2</td>
<td>n.d.</td>
</tr>
<tr>
<td>PPARγ1</td>
<td>0.9 ± 0.2</td>
<td>0.9 ± 0.2</td>
<td>1.0 ± 0.2</td>
<td>n.d.</td>
</tr>
<tr>
<td>PPARγ2</td>
<td>0.8 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>1.0 ± 0.3</td>
<td>n.d.</td>
</tr>
<tr>
<td>IRS-1</td>
<td>1.7 ± 0.3</td>
<td>0.9 ± 0.2</td>
<td>1.0 ± 0.3</td>
<td>n.d.</td>
</tr>
<tr>
<td>HSL</td>
<td>1.0 ± 0.3</td>
<td>0.9 ± 0.2</td>
<td>1.0 ± 0.3</td>
<td>n.d.</td>
</tr>
<tr>
<td>Resistin</td>
<td>4.8 ± 1.9</td>
<td>2.2 ± 0.5</td>
<td>1.0 ± 0.2</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

Table 1.
Inflammation- and insulin sensitivity-related genes in visceral and sc fat, skeletal muscle, and liver

Is a LPS-induced subacute endotoxemia sufficient to induce transient IR and β-cell dysfunction?
Fasting plasma glucose significantly increased, during the first 5 d of LPS infusion; glucose then progressively decreased back to baseline by d 7 (Fig. 3A). Plasma insulin concentration did not differ between groups (Fig. 3B). IR, as assessed by HOMA-IR, was significantly increased in the LPS group on d 2 and 3 and showed a trend toward an increase on d 4 (*P* = 0.09 vs. saline) and 5 (*P* = 0.055 vs. saline); β-cell function based on HOMA-B was significantly decreased in the LPS cats on d 1 and 4 (Table 2). After 10 d of infusion, iv glucose tolerance as well as insulin secretion did not differ between LPS- and saline-infused cats (Fig. 3, C and D). Insulin mRNA expression was up-regulated by LPS compared with saline in islets excised by LCM (*P* = 0.057; Supplemental Fig. 2).
Subacute endotoxemia induces transient IR and β-cell dysfunction. A and B, Fasting plasma glucose (A) and insulin (B) levels during the 10-d LPS or saline infusion period in cats. C and D, Glucose (C) and insulin (D) levels during the final ivGTT in LPS- and saline-infused cats. Graphs E and F show the area under the curve (AUC) of glucose and insulin, respectively. Values are expressed as mean ± se. *, P < 0.05 vs. saline-infused cats.

### Table 2. Insulin sensitivity and β-cell function calculated with the homeostasis model assessment indices (HOMA-IR and HOMA-B, respectively)

<table>
<thead>
<tr>
<th>Days</th>
<th>Saline (mean ± se)</th>
<th>LPS (mean ± se)</th>
<th>Saline (mean ± se)</th>
<th>LPS (mean ± se)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.9 ± 0.2</td>
<td>1.3 ± 0.4</td>
<td>99.6 ± 16.6</td>
<td>253.6 ± 71.4</td>
</tr>
<tr>
<td>1</td>
<td>1.9 ± 0.2</td>
<td>2.7 ± 0.1</td>
<td>185.7 ± 37.6</td>
<td>54.2 ± 9.8a</td>
</tr>
<tr>
<td>2</td>
<td>1.0 ± 0.1</td>
<td>2.1 ± 0.3b</td>
<td>160.9 ± 49.3</td>
<td>50.6 ± 6.9</td>
</tr>
<tr>
<td>3</td>
<td>0.9 ± 0.2</td>
<td>3.8 ± 1.5a</td>
<td>184.9 ± 69.7</td>
<td>82.5 ± 23.4</td>
</tr>
<tr>
<td>4</td>
<td>1.2 ± 0.2</td>
<td>2.0 ± 0.4</td>
<td>338.1 ± 29.9</td>
<td>69.6 ± 22.8a</td>
</tr>
<tr>
<td>5</td>
<td>0.8 ± 0.2</td>
<td>1.4 ± 0.2</td>
<td>32.0 ± 114.7</td>
<td>83.0 ± 17.2</td>
</tr>
<tr>
<td>6</td>
<td>1.4 ± 0.4</td>
<td>1.4 ± 0.3</td>
<td>466.1 ± 345.2</td>
<td>77.8 ± 13.8</td>
</tr>
<tr>
<td>7</td>
<td>1.3 ± 0.5</td>
<td>1.3 ± 0.2</td>
<td>285.1 ± 107.0</td>
<td>136.1 ± 32.9</td>
</tr>
<tr>
<td>8</td>
<td>0.8 ± 0.2</td>
<td>1.1 ± 0.3</td>
<td>166.9 ± 67.1</td>
<td>134.8 ± 42.0</td>
</tr>
<tr>
<td>9</td>
<td>0.7 ± 0.1</td>
<td>1.5 ± 0.4</td>
<td>63.6 ± 118.0</td>
<td>314.9 ± 195.1</td>
</tr>
<tr>
<td>10</td>
<td>1.2 ± 0.2</td>
<td>1.3 ± 0.2</td>
<td>-21.3 ± 264.6</td>
<td>162.2 ± 55.7</td>
</tr>
</tbody>
</table>

Different letters indicate significant difference vs saline-infused cats. \( I_0 \), basal fasting insulin levels; \( G_0 \), basal fasting glucose levels.

\( a P < 0.05 \)

\( b P < 0.01 \) vs. saline-infused cats.
Is a LPS-induced subacute endotoxemia sufficient to modulate expression of insulin sensitivity-related genes in a tissue-specific manner in the insulin-sensitive tissues tested?

LPS-infused animals had decreased mRNA levels of GLUT4 in visceral fat and increased transcript levels of SOCS3 in sc fat. IRS-1 expression was reduced and SOCS3 was augmented in skeletal muscle. LPS significantly decreased PPARγ1 and -2 levels in liver (Table 1). Protein levels of GLUT4 were lower in visceral fat after LPS ($P = 0.056$; data not shown); total PPARγ was significantly decreased in liver (Fig. 4A). These data confirmed our hypothesis that 10 d of LPS down-regulate the expression of important insulin signaling-related targets in a tissue-specific manner and point toward the development of tissue-specific reduction in insulin sensitivity.

Fig. 4.
Subacute endotoxemia modulates expression of PPARγ in liver and alters lipid metabolism in cats. A, Protein content of total PPARγ in liver extracts of LPS- and saline-infused cats. B, A representative Western blot is shown. Results are normalized to β-actin. Values are expressed as mean ± se. C and D, Fasting plasma cortisol (C) and TG (D) levels during the 10-d LPS or saline infusion in cats. Cortisol levels were measured daily in pooled plasma samples from the two treatment groups and only on d 0, 1, and 10 in individual cat plasma samples. Values are expressed as mean ± se. E and F, Average cross-sectional area of adipocytes in visceral (E) and sc fat (F) of LPS- and saline-infused cats. Individual and mean values are shown. Values are expressed relative to one cat infused with saline. *, $P < 0.05$ vs. saline-infused cats. G and H, Representative hematoxylin and eosin visceral (G) and sc (H) adipose tissue staining in cats after LPS or saline infusion.

Is a LPS-induced subacute endotoxemia sufficient to alter lipid and lipoprotein metabolism in cats?

In response to LPS, plasma TGs and cortisol sharply increased during the first 24 h and then rapidly decreased to baseline levels already by d 2 (Fig. 4, C and D). Fasting NEFA concentrations did not differ from controls at any time (data not shown). After 10 d LPS infusion, the average cross-sectional size of both visceral and sc adipocytes was significantly reduced by 33 and 37%, respectively, compared with saline (visceral fat: 0.9 ± 0.1 vs. 0.6 ± 0.1; $P < 0.05$; sc fat: 0.8 ± 0.1 vs. 0.5 ± 0.04 adipocyte size normalized to one cat; $P < 0.05$, saline- vs. LPS-infused cats; Fig. 4, E–H). Decreased mRNA expression of perilipin in both adipose fat tissues (Fig. 5A) was accompanied by a 50% increase in HSL activity in sc adipose tissue extracts from the LPS cats (Fig. 5B). Western blot analysis confirmed an enhanced expression of total HSL in visceral adipose tissue (Fig. 5C) and of total and phosphorylated HSL protein in sc adipose tissue (Fig. 5D). The content of TGs in liver was increased by 24% compared with the saline-infused cats (25.6 ± 0.9 saline vs. 31.8 ± 0.4 mg/g
Furthermore, plasma concentration of phospholipids and total and esterified cholesterol were significantly lower in the LPS-infused than in the saline-infused cats on d 10.

Fig. 5. Subacute endotoxemia increases lipolysis in fat tissues. A, Relative quantities of perilipin mRNA in adipose tissues of LPS- and saline-infused cats. B, HSL activity in sc fat after LPS or saline infusion. Values are expressed as mean ± se and are relative to one cat infused with saline. C and D, Protein content of total and phosphorylated HSL in visceral (C) and sc (D) adipose tissue extracts of cats infused with LPS or saline; the graphs show the average values across the groups and representative Western blots. Results are normalized to β-actin. Values are expressed as mean ± se. E, Liver TG content (milligrams per gram liver). Individual and mean values are shown. *, $P < 0.05$ and †, $P < 0.01$ vs. saline-infused cats. Plasma HDL concentration and free and esterified cholesterol in the HDL$_3$ subfraction were decreased after LPS (Table 3). Levels of HDL$_2$ free and esterified cholesterol were slightly decreased in the LPS-treated cats. These data confirmed our hypothesis that LPS-induced lipolysis in adipose tissues increases lipid mobilization and causes not only adipose tissue dysfunction and dyslipidemia but also adipose and hepatic IR.

Table 3. Concentration and composition (mean percentage weight) of plasma lipid and lipoprotein fractions

<table>
<thead>
<tr>
<th></th>
<th>Plasma (mean ± se)</th>
<th>LDL (mean ± se)</th>
<th>HDL$_2$ (mean ± se)</th>
<th>HDL$_3$ (mean ± se)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>LPS</td>
<td>Saline</td>
<td>LPS</td>
</tr>
<tr>
<td>Concentration (mmol/liter)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>6.4 ± 0.6</td>
<td>4.0 ± 0.4*</td>
<td>0.3 ± 0.0</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>Cholesterol ester</td>
<td>4.9 ± 0.5</td>
<td>2.9 ± 0.3*</td>
<td>0.6 ± 0.1</td>
<td>0.4 ± 0.0</td>
</tr>
<tr>
<td>TGs (mmol/liter)</td>
<td>0.5 ± 0.2</td>
<td>0.4 ± 0.1</td>
<td>0.3 ± 0.2</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>Phospholipids (mmol/liter)</td>
<td>4.2 ± 0.3</td>
<td>2.8 ± 0.2*</td>
<td>0.3 ± 0.1</td>
<td>0.3 ± 0.0</td>
</tr>
<tr>
<td>Protein (g/liter)</td>
<td>n.d.</td>
<td>(1.5 ± 0.2</td>
<td>1.3 ± 0.3</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>Total LP mass (mmol lipid/liter)</td>
<td>n.d.</td>
<td>1.2 ± 0.2</td>
<td>1.4 ± 0.2</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>Composition (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>n.d.</td>
<td>5.3 ± 0.8</td>
<td>4.4 ± 0.5</td>
<td>3.3 ± 0.5</td>
</tr>
<tr>
<td>Cholesterol ester</td>
<td>n.d.</td>
<td>29.1 ± 1.4</td>
<td>25.7 ± 1.0</td>
<td>25.1 ± 1.2</td>
</tr>
<tr>
<td>TGs</td>
<td>n.d.</td>
<td>1.4 ± 0.4</td>
<td>0.8 ± 0.3</td>
<td>0.3 ± 0.0</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>n.d.</td>
<td>23.3 ± 1.9</td>
<td>25.7 ± 0.7</td>
<td>23.5 ± 0.9</td>
</tr>
<tr>
<td>Protein</td>
<td>n.d.</td>
<td>40.7 ± 2.8</td>
<td>43.3 ± 1.5</td>
<td>47.8 ± 2.0</td>
</tr>
</tbody>
</table>

* $P < 0.05$ vs. saline-infused cats. n.d., Not determined; n.det., not detected; HDL tot, HDL total (HDL$_2$ + HDL$_3$); LP, Lipoprotein.
Discussion
The present study is the first to investigate the influence of a chronic 10-d systemic infusion of increasing doses of LPS on IR and on a variety of parameters for carbohydrate and lipid metabolism in cats.

Subacute endotoxemia induces systemic inflammation in cats
LPS infusion induced a mild increase in sc temperature, which was paralleled by a significant increase in circulating and tissue markers of inflammation. A 4-h infusion of LPS (2 µg/kg · h, iv) in cats, hence double of the highest LPS dose used in our study, has been previously demonstrated to induce systemic inflammation including increased temperature and plasma cytokines concentrations (13). In our study, the continuous infusion of increasing doses of LPS was sufficient to trigger a low-grade inflammatory response without seriously affecting the clinical conditions of the cats. Although the pyrogenic response normalized after about 5 d of LPS infusion, levels of AGP and SAA, the two most important acute-phase proteins in cats (30), and MCP-1 remained markedly elevated throughout the infusion. These findings confirm the presence of a low-grade systemic inflammatory response that fully resembles the state of inflammation typical of obesity and type 2 diabetes (31).

Of note, our study shows these effects of LPS infusion in healthy, normal-weight cats, i.e. independent of obesity or other underlying metabolic disturbances. In a similar experimental setting, a 4-wk infusion of endotoxin had been shown to increase whole-body, liver, and adipose depot weight to a similar extent as in high fat-fed mice (14). Similar findings were not observed after 10 d of endotoxemia in cats.

Subacute endotoxemia enhances expression of inflammation- and immune response-related genes, particularly in sc fat
Chronic low-grade inflammation is recognized as a key factor in the pathogenesis of obesity-induced IR. This low-grade inflammation is thought to be mediated by the activation of infiltrated neutrophils and macrophages with a subsequent increase in the expression of cytokines in insulin-sensitive tissues tested (3, 4). Consistent with the systemic inflammatory state, we found that transcripts of the chemokine IL-8 and the most potent neutrophil-specific serine proteinase, NEla, were increased in liver and sc fat after LPS. The number of neutrophils was significantly higher in liver, sc fat, and skeletal muscle and tended to be higher in visceral fat, confirming neutrophil migration and activation in those tissues.

Transient infiltration of neutrophils has been shown to occur in adipose tissues of obese mice and to precede macrophages infiltration (32). Although macrophages could not be counted because of the lack of cross-reacting antibodies (17), LPS-infused cats showed a marked up-regulation of MCP-1 in sc fat, a crucial chemokine involved in the recruitment of monocytes in tissues, and transcripts of the macrophage-specific antigen, F4/80, were up-regulated in tissues in which neutrophils were also increased; this suggests that both cell types were recruited in those tissues. Surprisingly, myeloperoxidase-immunostained neutrophils were not increased in LPS-infused cats in pancreatic islets; however, F4/80 gene expression was up-regulated in pancreatic ILCs. Interestingly, cytokine transcripts were increased only in sc fat of LPS-infused cats. Desensitization of cytokine synthesis, which is well documented during prolonged LPS administration (33), may have occurred, despite the increasing doses of LPS that were given to our cats. A recent study in humans demonstrated that a low-dose acute endotoxemia induces adipose tissue inflammation leading to local and systemic IR (34). In this study, the increase in TNF-α and IL-6 mRNA levels was rapid and transient in adipose tissue. TNF-α transcripts returned to baseline levels 12 h after the LPS bolus. Moreover, in the study by Metha et al. (34), as in most of the studies conducted in humans, LPS was administered as a single bolus, and its effects were observed for not more than a few hours (60 h in the cited study).

To our knowledge, there are no studies in humans or cats that demonstrate whether TNF-α mRNA expression remains increased in adipose tissue or in any other tissue after prolonged (a few days) continuous infusion of LPS. The fact that transcripts of TLR4, the innate
receptor of LPS, were decreased in the skeletal muscle supports the idea that endotoxic desensitization had occurred, at least in some of the tested tissues (35). However, desensitization to the effects of LPS may not have been the same for all the inflammatory and metabolic parameters. It is noteworthy that in our animals, inguinal sc fat exhibited the strongest up-regulation of inflammatory genes, potentially indicating a greater sensitivity of this fat depot to LPS. In contrast to rodent or human models of obesity (36), an increase in fat mass in obese neutered cats results from a proportional increase in intraabdominal and abdominal subcutaneous fat (37) and increased inguinal sc fat. To our knowledge, no such studies have been performed in sexually intact cats. So far, it is unclear which fat depot is more critical for changes in insulin sensitivity in obese cats. Overall, these findings provide evidence that 10-d LPS infusion, in normal-weight cats, was sufficient to induce tissue-specific inflammatory responses similar to those associated with the metabolic endotoxemia of obesity and type 2 diabetes (1-5).

**Subacute endotoxemia induces transient IR and β-cell dysfunction**

In humans, the acute inflammatory response to infection and endotoxemia is accompanied by alterations of glucose metabolism, which are partly mediated by catecholamines and glucocorticoids (10, 11, 38-40). In line with the latter observation, cortisol levels transiently increased during LPS infusion and may possibly have contributed to the transient impairment of insulin sensitivity, increasing glycemia without decreasing insulin levels. In healthy humans, the acute administration of TNF-α is known to induce similar changes in glucose metabolism (41). Nevertheless, the responsiveness to insulin was normal after 10 d of LPS infusion, based on the whole-body insulin sensitivity index. The reason for the lack of whole-body IR after prolonged LPS infusion is not clear. However, this is in principle consistent with recent findings of Cani et al. (14), who showed that 4-wk endotoxemia induced hepatic but not whole-body IR in mice. Because tissue-specific glucose use or tissue-specific changes in fat metabolism were not assessed during or after LPS infusion, we cannot exclude the presence of tissue-specific IR. On the other hand, the fact that insulin gene transcription was augmented in pancreatic LCM islets of LPS-infused cats may suggest an adaptive response of β-cells to a condition of increased peripheral insulin demand as, for example, occurring in inflammation-induced IR.

**Subacute endotoxemia modulates expression of insulin sensitivity-related genes in a tissue-specific manner in the insulin-sensitive tissues tested**

Proinflammatory cytokines and endotoxins are known to activate various kinases (1, 42, 43) that phosphorylate the serine/threonine of IRS-1, ultimately inhibiting insulin signaling and the insulin-dependent cell membrane translocation of GLUT4. In addition, cytokines increase the expression of SOCS family proteins, known to reduce insulin signaling by suppressing the tyrosine phosphorylation of IRS-1 (44). Recent publications reported increased SOCS1 and SOCS3 expression in insulin-sensitive tissues during obesity and endotoxemia in humans and mice (34, 44); this suggests that SOCS family proteins may promote IR in both conditions. We observed an up-regulation of SOCS3 transcripts in skeletal muscle and sc fat of the LPS-treated cats; mRNA levels of IRS-1 were reduced in skeletal muscle; similarly to obese humans and cats (2, 45), gene and protein expression of GLUT4 was decreased in visceral fat. In addition, the gene and protein expression of the nuclear receptors PPARγ1 and -2, key transcriptional regulators preserving insulin sensitivity and reducing cytokine expression (46), were decreased in the liver of LPS-treated cats. In our study, we extend previous findings in mice (14) by demonstrating that although whole-body insulin sensitivity was not changed at d 10 of LPS infusion, the expression of important genes that preserve insulin sensitivity was reduced by LPS in major insulin-responsive tissues (liver, skeletal muscle, and visceral and sc adipose tissue) and that the degree of the response to LPS varied with respect to the tissue and to the target.
**Subacute endotoxemia alters lipid and lipoprotein metabolism in cats**

Among the target tissues of insulin, adipose tissue has a predominant role in the development of IR and disorders of glucose and lipid metabolism in human obesity. Adipose tissue IR in obesity is linked to the inflammation-mediated impairment of insulin action in respect to lipolysis and glucose uptake. Under physiological conditions, it has been shown that visceral adipose tissue has higher basal lipolysis activity than sc fat in rats (47). Similarly, HSL activity was significantly higher in visceral (1.3 ± 0.1) than in sc adipose tissue (0.7 ± 0.17; \( P < 0.01 \)) of our saline-infused cats (data not shown). During infections and obesity, increased cytokines and endotoxin levels activate lipolysis in adipose tissues through protein kinase A-mediated phosphorylation of the lipolytic enzyme HSL and perilipin, a protein associated with the cytoplasmatic lipid droplet (48). As a result, instead of being oxidized, mobilized fatty acids are reesterified into TGs in the liver (49) and are likely to cause IR through the activation of kinases involved in the serine/threonine phosphorylation of IRS-1 in this tissue (50). Furthermore, the lipolytic state and the biological characteristics of visceral and sc depots may change under inflammatory conditions.

In fact, after the LPS treatment, HSL activity and protein expression increased significantly in sc fat, whereas in visceral fat the increase in HSL activity did not reach statistical significance. In the present study, 10 d endotoxemia activated lipolysis as demonstrated by the increased activity and protein expression of HSL (in particular in sc fat) and decreased mRNA expression of perilipin; both may have led to the significantly reduced size of adipocytes in visceral and sc fat. In agreement, recent data showed that 4 wk LPS treatment increased the number of sc adipocytes of smaller size in LPS-infused mice (14). Moreover, an increased proportion of small adipose cells in sc adipose tissue has been associated with inflammation and IR independently of obesity (51).

In LPS-infused cats, serum TGs were increased initially, although the increase was followed by a rapid decline to preinfusion levels. Nonetheless, the TG content of the liver was significantly increased at d 10. These data support the notion that, in response to LPS, fatty acid oxidation decreases, whereas TG synthesis increases in the liver. Decreased fatty acid oxidation in response to LPS is consistent with the reduced expression of PPAR\( \gamma \) in the liver because PPAR\( \gamma \) has been shown to trigger the expression of proteins involved in fatty acid oxidation, whereas decreased hepatic PPAR\( \gamma \) transcription contributes to reduce fatty acid oxidation (52, 53). These findings suggest that the infusion of LPS in normal-weight cats was sufficient to impair the antilipolytic action of insulin in adipose tissue and to induce hepatic steatosis, both considered obesity-related manifestations of adipose and hepatic IR (54).

Acute infection and inflammation are associated with plasma lipid and lipoprotein profiles, which resemble those observed in the human metabolic syndrome (55). Cats share a lipoprotein profile that is qualitatively similar to that in humans; this is, for example, reflected by the presence of separable HDL fractions and similar dyslipidemic changes in obesity (56).

LPS-infused cats had decreased total plasma cholesterol, HDL, and HDL-cholesterol, which is similar to humans with endotoxemia (55, 57). In humans with sepsis or the metabolic syndrome, low levels of HDL cholesterol and elevated levels of acute-phase proteins have been identified as independent risk factors for the future development of IR and type 2 diabetes (58). The mechanism by which inflammation decreases total and HDL cholesterol levels is not completely understood. Similar findings have been reported in nonhuman primates and humans, whereas in rodents cholesterol levels increased during the acute-phase response.

Interestingly, it has been shown that lipoprotein composition changes with infection. Acute-phase HDL contains less esterified cholesterol and apolipoprotein A-I, with the latter being partly replaced by apolipoprotein containing the inflammatory protein SAA (apolipoprotein SAA). The presence of SAA in HDL was suggested to increase HDL clearance and decrease the ability of HDL to inhibit LDL oxidation (58, 59). The protective capacity of HDL, especially of the HDL\(_3\) fraction, is also diminished in metabolic syndrome patients (59). In LPS-infused cats, the HDL\(_3\) and HDL\(_2\) fractions contained less free and esterified cholesterol. Because HDL, besides its antioxidant effects, has antiinflammatory properties including the
down-regulation of cytokines and the synthesis of adhesion molecules (58), the decreased and modified composition of HDL may have contributed to amplifying the inflammatory reaction in LPS-infused cats.

In conclusion, we confirmed, to some extent, previous findings from Cani et al. (14) and showed that despite normal whole-body insulin sensitivity, the local and systemic inflammation induced by prolonged LPS infusion resulted in tissue-specific IR. Our study provided further important insight into the tissue-specific modulation of glucose and insulin metabolism-related targets. In addition, we provided new evidence that LPS-induced adipose tissue inflammation, in particular in the sc depot, induced severe alterations in lipid metabolism, which included the disruption of TG storage and the increase in lipolysis; this in turn led to the development of dyslipidemia and hepatic steatosis in normal-weight cats.

Whether a more prolonged endotoxemia may permanently impair adipose function and lipid and lipoprotein metabolism and may therefore contribute to the development of systemic IR to a similar extent as in obese cats requires further investigation. Additional studies of the biological processes and the molecular mechanisms that underlie tissue-specific development of inflammation and the related metabolic alterations in cats may allow the development of more targeted therapeutic approaches to managing IR in infection, obesity, and type 2 diabetes. LPS infusion in cats thus provides a promising model to study the cross talk between metabolic and inflammatory responses in the development of adipose tissue dysfunction and IR in the absence of obesity.

Acknowledgments
We thank K. Kaufmann (Clinic for Small Animal Internal Medicine, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland), M. Bednar (Institute of Veterinary Pathology, Vetsuisse Faculty), S. Behnke (Department of Pathology, University Hospital Zurich, Switzerland) Dr. U. Sauer (Carl Zeiss MicroImaging GmbH, Munich, Germany), and S. Dodkin (Diagnostic Service, University of Bristol, Langford, Bristol, UK) for excellent technical assistance. We also thank Dr. L. Reagan (Department of Pharmacology, Physiology and Neuroscience, University of South Carolina) for the generous gift of the primary antisera to GLUT4.

This work was supported by the University of Zurich. Disclosure Summary: The authors have nothing to disclose.

References

16. Lutz TA, Rand JS 1997 Detection of amyloid deposition in various regions of the feline pancreas by different staining


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


44. Ueki K, Kondo T, Kahn CR 2004 Suppressor of cytokine signaling 1 (SOCS-1) and SOCS-3 cause insulin resistance through inhibition of tyrosine phosphorylation of insulin receptor substrate proteins by discrete mechanisms. Mol Cell Biol 24:5434–5446


47. Anthony NM, Gaidhu MP, Ceddia RB 2009 Regulation of visceral and subcutaneous adipocyte lipolysis by acute AICAR- induced AMPK activation. Obesity 17:1317–1317


50. Itani SI, Ruderman NB, Schmieder F, Boden G 2002 Lipid-induced insulin resistance in human muscle is associated with
changes in diacylglycerol, protein kinase C, and I\textsubscript{B\textminus}. Diabetes 51:2005–2011
53. Kim MS, Sweeney TR, Shigenaga JK, Chui LG, Moser A, Grunfeld C, Feingold KR 2007 Tumor necrosis factor and interleukin 1 decrease RXR\textsubscript{\alpha}, PPAR\textsubscript{\alpha}, PPAR\textsubscript{\gamma}, LXR\textsubscript{\alpha}, and the coactivators SRC-1, PGC-1\textsubscript{\alpha}, and PGC-1\textsubscript{\beta} in liver cells. Metabolism 56:267–279