Effect of short-term intralipid infusion on the immune response during low-dose endotoxemia in humans

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Krogh-Madsen R, Plomgaard P, Akerstrom T, Møller K, Schmitz O, Pedersen BK. Effect of short-term intralipid infusion on the immune response during low-dose endotoxemia in humans. Am J Physiol Endocrinol Metab 294: E371–E379, 2008. First published December 4, 2007; doi:10.1152/ajpendo.00507.2007.—Novel anti-inflammatory effects of insulin have recently been described, and insulin therapy to maintain euglycemia suppresses the plasma levels of free fatty acids (FFA) and increases the survival of critically ill patients. We aimed to explore the effect of short-term high levels of plasma FFA on the inflammatory response to a low dose of endotoxin. Fourteen healthy male volunteers underwent the following two trials in a randomized crossover design: 1) continuous infusion of 20% Intralipid [0.7 ml·kg−1·h−1 (1.54 g/kg)] for 11 h, and 2) infusion of isotonic saline for 11 h (control). In each trial, heparin was given to activate lipoprotein lipase, and an intravenous bolus of endotoxin (0.1 ng/kg) was given after 6 h of Intralipid/saline infusion. Blood samples and muscle and fat biopsies were obtained before the Intralipid/saline infusion and before as well as after infusion of an endotoxin bolus. Plasma levels of FFA, triglycerides, and glycerol were markedly increased during the Intralipid infusion. Endotoxin exposure induced an increase in plasma levels of TNF-α, IL-6, and neutrophils and further stimulated gene expression of TNF-α and IL-6 in both skeletal muscle and adipose tissue. The systemic inflammatory response to endotoxin was significantly pronounced during Intralipid infusion. Short-term hyperlipidemia enhances the inflammatory response to endotoxin, and skeletal muscle and adipose tissue are capable of producing essential inflammatory mediators after endotoxin stimulation.

HIGH LEVELS OF FREE FATTY ACIDS (FFA) are often observed in critically ill patients with (45, 60, 64) and without sepsis (78) and are associated with insulin resistance (6, 7, 61). The effect of FFA on the systemic inflammatory response to an exogenous challenge, such as endotoxin, has not been established; however, FFA (rich in n-6 fatty acids) induce oxidative stress and have a proinflammatory effect (72), whereas FFA (rich in n-3 fatty acids) blunts the inflammatory response to endotoxin (56). Moreover, type 2 diabetics, who have elevated levels of FFA, also exhibit a low-grade inflammation, including an elevation of proinflammatory cytokines such as TNF-α (34, 50, 55). The recent finding that intensive insulin treatment reduces mortality in critically ill surgical patients may be related to a reduction in the plasma level of FFA, with a subsequent downregulation of inflammation. Insulin has been shown to have an anti-inflammatory effect by decreasing NF-κB in mononuclear cells, plasma concentrations of soluble intracellular adhesion molecule-1 (sICAM-1), and monocyte chemoattractant protein-1 (MCP-1) in obese humans (13), as well as by reducing C-reactive protein (CRP) levels in patients with myocardial infarction (10).

TNF-α, IL-6, and neutrophils are present in elevated concentrations in plasma during acute inflammatory conditions such as sepsis (9, 25), as are plasma levels of sICAM-1, soluble E-selectin (sE-selectin) (1, 24, 77), and soluble CD-14 (sCD-14) (42). E-selectin and ICAM-1 are located in endothelial cells. E-selectin is responsible for the transient stage of leukocyte adhesion to endothelial cells (the rolling phenomenon), while ICAM-1 is important for the firm adhesion of leukocytes (15, 30). CD14 binds lipopolysaccharide (LPS) at the cell surface and facilitates an interaction between the Toll-like receptor-4 (TLR4) and the MD-2 molecule (a nonmembrane spanning molecule) (4, 32, 63). This initiates a complex signal-transduction cascade, which ultimately leads to NF-κB activation and hence transcription of proinflammatory cytokines (33, 41, 73). sCD14 is released primarily from monocytes, macrophages, and neutrophils and exerts both proinflammatory (27) and anti-inflammatory actions (38). It is suggested that this diversity in action is dependent on the plasma concentration of sCD14. Thus, low concentrations of sCD14 promote proinflammatory actions, whereas high concentrations suppress the response to LPS (37, 38). TLR4 is located mainly on neutrophils, monocytes, macrophages, dendritic cells, endothelial, and epithelial cells (73), but it is also expressed in skeletal muscle (43, 54) and adipose tissue (5, 12).

An intravenous endotoxin injection in humans triggers a well-defined inflammatory response (16) in which TNF-α and IL-6 appear early in the cytokine cascade (21, 75). We have established a human low-dose endotoxemia model resulting in a low-grade systemic inflammation with a significant cytokine response in plasma in the absence of clinical signs or symptoms of inflammation (39, 65). It is now recognized that TNF-α and IL-6 are produced and released not only by a variety of immune cells such as macrophages, monocytes, and lymphocytes (26, 47, 52) but also by cells that were previously considered not to take part in the inflammatory response, such as adipose tissue (22, 31, 40, 51) and skeletal muscle (19, 59, 79).
It has recently been shown that endotoxemia in rats stimulates the gene expression of TNF-α and IL-6 in skeletal muscle (43). Furthermore, Brix-Christensen et al. (8) demonstrated an increase in the protein content of TNF-α and IL-6 in both muscle and adipose tissue at 9.5 h after induction of endotoxemia in pigs. In the latter model, the highest protein content was found in adipose tissue (8).

The present study was performed to test the hypothesis that a condition with elevated plasma levels of FFA would result in a more marked systemic inflammatory response to endotoxemia. Therefore, we monitored the cytokine response and neutrophil count, as well as the soluble marker of activity of the monocyte/macrophage system (sCD14) and the vascular endothelium (sE-selectin and sICAM-1), to a low dose bolus of intravenous endotoxin in plasma during acute highly elevated plasma concentrations of FFA compared with fasting levels of FFA. We furthermore investigated whether adipose and skeletal muscle tissue were involved in mediating the inflammatory response to endotoxin.

MATERIALS AND METHODS

Subjects. Fourteen healthy human males [mean age = 25.1 yr (±0.9 SE); mean body mass index (BMI) = 23.2 kg/m² (±0.6 SE)] were included after oral and written informed consent was obtained. None of the subjects had a history of medical problems. Before the study, all 14 subjects underwent a thorough clinical examination. Blood samples for renal, hepatic, and thyroid function, hemoglobin, white blood cells counts, electrolytes, and plasma glucose were analyzed as well. All tests were normal. The study was approved by the Scientific-Ethics Committee of Copenhagen and Frederiksborg Municipalities (file number: KF 11–085/04) in accordance with the Helsinki Declaration.

Study design. The 14 volunteers underwent the following two trials: I) continuous infusion of Intralipid 200 mg/ml (0.7 ml·kg⁻¹·h⁻¹; Fresenius Kabí, Uppsala, Sweden) for 11 h. Intralipid is composed of 20% soybean oil (12% palmitic acid, 4% stearic acid, 21% oleic acid, 53% linoleic acid, 7% linolenic acid, and 3% other acids), 12 g of egg phospholipids (P1), and 21.3 g of glycerol; and 2) continuous infusion of normal saline 9 mg/ml (0.7 ml·kg⁻¹·h⁻¹, Sygehus Apóteke, Copenhagen, Denmark) for 11 h (control trial).

To activate lipoprotein lipase (LPL), which catalyzes the conversion of triglycerides (TG) into FFA, heparin 5,000 U/ml, (bolus of 3.5 U/kg) was infused intravenously in both trials (53). In each trial an intravenous bolus of endotoxin (0.1 mg/kg; endotoxin Escherichia coli, Lot G2 B274, United States Pharmacopoeia Convention, Rockville, MD) was given after 6 h of Intralipid/saline infusion. Every trial lasted 5 h after injection of the endotoxin bolus. The total amount of Intralipid during the 11 h of infusion ranged from 67 to 152 g.

The trials took place in the following randomized order: trial 1, trial 2 (n = 7) and trial 2, trial 1 (n = 7). The interval between the two trials was 7 days. On the study day, the subjects reported to the laboratory (at 5.00 a.m.) after an overnight fast (from 9 p.m.). A peripheral catheter (I.V. Cannula 18 GA, Beckton-Dickinson, Helsingborg, Sweden) was placed in an antecubital vein for blood sampling, and isotonic saline 9 mg/ml (500 ml) was infused at a constant rate. In the contralateral antecubital vein, a peripheral catheter (I.V. Cannula 18 GA, Beckton-Dickinson) was placed for infusion of Intralipid/saline and heparin. ECG was continually monitored; heart rate, noninvasive blood pressure, and tympanic temperature were recorded at baseline and every second hour during the first 6 h and every hour for the last 5 h.

For measurement of cytokines, neutrophils, sCD14, sICAM, sE-selectin, FFA, TG, glycerol, glucose, insulin, and cortisol, blood samples were obtained at baseline and every 2 h before endotoxin bolus and hourly after endotoxin bolus (0-, 2-, 4-, 6-, 7-, 8-, 9-, 10-, and 11-h time points). To assess blood coagulation [activated partial thromboplastin time (APPT) in seconds], blood was drawn at baseline and after the study (0- and 11-h time points). Muscle biopsies obtained from musculus quadriceps, vastus lateralis, and fat biopsies obtained from the abdominal subcutaneous adipose tissue were taken at baseline (before Intralipid/saline) infusion, before endotoxin bolus, and 3 and 5 h, respectively, after endotoxin bolus (0-, 6-, 9-, and 11-h time points). The biopsies were analyzed for IL-6, TNF-α, and TLR4 mRNA using real-time PCR (Fig. 1). Blood samples for neutrophil count were drawn into EDTA containing tubes and sent for immediate analysis at the hospital laboratory. The remaining blood samples were also drawn into tubes containing EDTA but were immediately centrifuged, and plasma was stored at −80°C until analyzed.

Measurements

All ELISA determinations were measured in duplicates, and mean concentrations were calculated.

Plasma inflammation markers. Plasma concentrations of TNF-α, IL-6, sCD14, sICAM-1, and sE-selectin were measured by the ELISA technique (R&D Systems, Minneapolis, MN).

Plasma cortisol. Plasma concentrations of cortisol were measured using the ELISA technique (DSL, Webster, TX).

Lipid metabolism. Plasma concentrations of FFA, glycerol, and TG were determined using an automatic analyzer (Cobas Fara, Roche, Basel, Switzerland).

Glucose metabolism. Plasma concentrations of glucose were determined using an automatic analyzer (Cobas Fara, Roche). Plasma concentrations of insulin were analyzed using the ELISA technique (Dako, Glostrup, Denmark).

APPT and neutrophil count. Standard laboratory procedures were used.

Tissue biopsies. Abdominal subcutaneous adipose tissue and muscle tissue (m. vastus lateralis) were obtained by use of the percutaneous biopsy technique (4) with suction and were immediately frozen in liquid nitrogen and stored at −80°C until analyzed for IL-6, TNF-α, and TLR4 mRNA as described below.

RNA extraction. Adipose and muscle tissue RNA extraction was performed with TRIzol (Invitrogen, Carlsbad, CA) according to the manufacturer’s directions. In brief, 50–100 mg of the tissue were dissolved in 1 ml of TRIzol and homogenized with a Brinkman Polytron (version PT 2100, Kinematica, Luzern, Switzerland) rotating at 26,000 rpm. The muscle tissue samples were directly transferred to a fresh tube, 100 μl of chloroform/isoamyl alcohol (24:1) were added, and the tubes were vigorously shaken. The adipose tissue samples were allowed to sit for a few minutes for a TG phase to form. The lower aqueous phase was then transferred to a tube, 100 μl of...
chloroform/isoamyl alcohol (24:1) was added, and the tubes were shaken. Samples were allowed to sit for 5 min and were subsequently spun at 16,060 G (Heraeus Biofuge Pico, DJB Labcare, Buckinghamshire, UK) for 15 min at 4°C after which the upper aqueous phase was transferred to a new tube. The aqueous phase was mixed with 0.5 ml of ice-cold isopropanol, and samples were placed at −20°C for 1 h. They were then centrifuged at 16,060g for 15 min at 4°C, and the resulting pellet was washed with 0.5 ml of cold 75% ethanol in diethylpyrocarbonate (DEPC)-treated water (0.05%). After centrifugation at 9,883 g for 10 min, pellets were redissolved in 15 μl of DEPC-treated water and allowed to dissolve on ice. RNA was dissolved in DEPC water after which the concentration of RNA was measured spectrophotometrically at an optical density of 260 nm.

Reverse transcription. Two micrograms of total RNA were reversely transcribed using the Applied Biosystems Taqman RT-Kit (Foster City, CA).

Real-time PCR. IL-6, TNF-α, and TLR4 gene expressions were analyzed using semiquantitative real-time PCR using an ABI PRISM 7900 sequence detector (Applied Biosystems). β-Actin mRNA served as the internal reference gene. We used the predeveloped, primer-limited assay reagents for β-actin, TNF-α, and TLR4 mRNA determination. The IL-6 primers and probe sequences used were obtained from Starkie et al. (66). All PCR reagents were obtained from Applied Biosystems.

An 81-bp fragment was amplified using IL-6 forward primer: 5’-GGTACATCCTGACGGCATCT-3’; IL-6 reverse primer: 5’-GTGCCCTTITTGGCTTTCAC-3’; and IL-6 probe: 5’-FAM-TGTTACTCTTGTACATGCTCTTCTTCTCAGGGCT-TAMRA-3’. A reagent mixture of 75 μl was made up for each sample with 1× MasterMix, 900 nM IL-6 forward primer, 100 nM IL-6 probe, 1× β-actin mix (primers and probe), and 50–100 ng of sample and made up to a final volume of 38.5 μl with water. Each sample was run in triplicates in a reaction volume of 10 μl for 5 cycles using standard real-time PCR cycling conditions. All samples were run in triplicates and normalized to a relative standard curve, and the transcript quantity of genes was then normalized to β-actin mRNA.

Statistical Analysis

Data were tested for normal distribution by the Kolmogorov-Smirnov analysis. All data (except data on the cortisol plasma concentrations, where absolute data were used) were normally distributed when log transformed before analysis. Data were analyzed using parametric methods and reported values are geometric mean (95% confidence interval).

Parametric methods. Within-subject variation over time and variation between trials were analyzed using the following repeated-measures (two-way ANOVA, time-by-trial) approach: Procedure: “Proc mixed”; Class: person trial time; and Model: parameter = time trial time × trial. If a significant interaction (time × trial) was found, Bonferroni-corrected P values were obtained as appropriate to identify significant differences between trials and to identify significant differences from baseline values. P < 0.05 was considered statistically significant. Analysis was performed using a statistical software package (SAS version 9.1. SAS Institute, Cary, NC).

RESULTS

Clinical Signs

No difference was observed between trials with regard to tympanic temperature, heart rate (HR), and mean arterial pressure (MAP), but a significant variation occurred over time in temperature (due to a difference at the 6- to 11-h time points compared with baseline, P < 0.05 for all comparisons) and HR (due to a difference at the 9- to 11-h time points compared with baseline, P < 0.001 for all comparisons; Table 1).

<table>
<thead>
<tr>
<th>Measurement</th>
<th>0 h (Baseline)</th>
<th>2 h</th>
<th>4 h</th>
<th>6 h</th>
<th>8 h</th>
<th>10 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>36.3 (36.0–36.6)</td>
<td>36.3 (36.0–36.6)</td>
<td>36.3 (36.0–36.6)</td>
<td>36.4 (36.0–36.6)</td>
<td>36.3 (36.0–36.6)</td>
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<tr>
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<td></td>
<td>36.3 (36.0–36.6)</td>
<td>36.3 (36.0–36.6)</td>
<td>36.3 (36.0–36.6)</td>
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<tr>
<td>Mean</td>
<td>Control</td>
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<td>36.3 (36.0–36.6)</td>
<td>36.3 (36.0–36.6)</td>
<td>36.4 (36.0–36.6)</td>
<td>36.3 (36.0–36.6)</td>
</tr>
<tr>
<td>Intralipid</td>
<td></td>
<td>36.3 (36.0–36.6)</td>
<td>36.3 (36.0–36.6)</td>
<td>36.3 (36.0–36.6)</td>
<td>36.4 (36.0–36.6)</td>
<td>36.3 (36.0–36.6)</td>
</tr>
<tr>
<td>Mean</td>
<td>HR, beats/min</td>
<td>Control</td>
<td>60 (54–67)</td>
<td>61 (56–66)</td>
<td>64 (58–70)</td>
<td>61 (55–69)</td>
</tr>
<tr>
<td></td>
<td>Intralipid</td>
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<td>61 (55–69)</td>
<td>65 (59–72)</td>
<td>67 (61–73)</td>
<td>70 (63–78)</td>
</tr>
<tr>
<td>Tympanic temperature</td>
<td>Control</td>
<td>36.3 (36.0–36.6)</td>
<td>36.3 (36.0–36.6)</td>
<td>36.3 (36.0–36.6)</td>
<td>36.4 (36.0–36.6)</td>
<td>36.3 (36.0–36.6)</td>
</tr>
<tr>
<td></td>
<td>Intralipid</td>
<td>36.3 (36.0–36.6)</td>
<td>36.3 (36.0–36.6)</td>
<td>36.3 (36.0–36.6)</td>
<td>36.4 (36.0–36.6)</td>
<td>36.3 (36.0–36.6)</td>
</tr>
</tbody>
</table>

Table 1. Vital signs
Lipid and Glucose Metabolism

Plasma levels of FFA, glycerol and TG rose during the Intralipid infusion (Fig. 2) (due to a difference at the 2-, 4-, and 6- to 11-h time points compared with baseline, $P < 0.001$ for all comparisons; difference between trials due to the 2-, 4-, and 6- to 11-h time points, $P < 0.001$ for all comparisons). Plasma levels of FFA, glycerol and TG rose during the Intralipid infusion (Fig. 2) (due to a difference at the 2-, 4-, and 6- to 11-h time points compared with baseline, $P < 0.001$ for all comparisons; difference between trials due to the 2-, 4-, and 6- to 11-h time points, $P < 0.001$ for all comparisons). Plasma levels of TG decreased during the saline infusion (due to a difference at the 2-, 4-, and 6- to 11-h time points compared with baseline, $P < 0.001$ for all comparisons). Confirming a previous study (39), plasma levels of FFA increased in the control trial after endotoxin bolus (due to a difference at the 9- to 11-h time points compared with baseline, $P < 0.05$ for all comparisons). Plasma levels of insulin decreased during the Intralipid infusion (due to a difference at the 9 to 11-h time points compared with baseline, $P < 0.01$ for all comparisons) and in the control trial (due to a difference at the 2-, 4-, and 6- to 11-h time points compared with baseline, $P < 0.05$ for all comparisons); there was a difference between trials at the 2-, 4-, and 6- to 11-h time points ($P < 0.05$ for all comparisons). Plasma levels of glucose increased during the Intralipid infusion (due to a difference at 2-h time point compared with baseline, $P < 0.001$) and decreased in the control trial (due to a difference at the 9- to 11-h time points compared with baseline, $P < 0.05$ for all comparisons). There was a difference between trials at the 9- to 11-h time points ($P < 0.05$ for all comparisons).

Fig. 2. Plasma concentrations of free fatty acids (FFA), glycerol, triglycerides (TG), glucose, and insulin. Plasma concentrations are presented as geometric mean [95% confidence interval (CI)]. *Significant difference over time in the control trial. #Significant difference over time in the Intralipid trial. €Denotes significant difference over time in the Intralipid trial. *Significant difference between trials.

Plasma Cytokine Levels and White Blood Cell Count

The cytokine, the leukocyte, and the neutrophil responses to endotoxemia were more pronounced during the Intralipid infusion (Fig. 3). Plasma levels of TNF-$\alpha$ increased after endotoxin bolus in both trials (due to a difference at the 7- to 11-h time points compared with baseline, $P < 0.001$ for all comparisons). There was a difference between trials at the 7- and 8-h time points ($P < 0.05$ for both). Plasma levels of IL-6 increased after endotoxin bolus in the control trial (7- to 11-h time points compared with baseline, $P < 0.001$ for all comparisons) and before and after endotoxin bolus in the Intralipid trial (4- and 6- to 11-h time points compared with baseline, $P < 0.01$ for all comparisons). There was a difference between
trials at the 7- and 8-h time points (P < 0.01 for both). The leukocyte count decreased before and increased after endotoxin bolus in the control trial (2-, 4-, and 6- to 11-h time points compared with baseline, P < 0.001 for all comparisons) and before and after endotoxin bolus in the Intralipid trial (2- and 6- to 11-h time points compared with baseline, P < 0.05 for all comparisons). There was a difference between trials at the 9-h time point regarding both leukocytes and neutrophils (P < 0.001). Monocyte, lymphocyte, and eosinophile counts decreased significantly before and after endotoxin bolus; basophile count decreased significantly after endotoxin bolus; there were no differences between trials (data not shown). The neutrophil count increased after endotoxin bolus in the control trial (2- to 11-h time points compared with baseline, P < 0.001 for all comparisons) and increased with endotoxin bolus in the Intralipid trial (7- to 11-h time points compared with baseline, P < 0.001 for all comparisons; data not shown).

Table 2. Plasma concentrations of sICAM-1, sE-selectin, and sCD14 and gene expression levels of TLR4 in muscle and adipose tissue

<table>
<thead>
<tr>
<th>Measurement</th>
<th>0 h (Baseline)</th>
<th>2 h</th>
<th>4 h</th>
<th>6 h</th>
<th>7 h</th>
<th>8 h</th>
<th>9 h</th>
<th>10 h</th>
<th>11 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>sCD-14, pg/ml</td>
<td>Control</td>
<td>953 (841–1,079)</td>
<td>943 (821–1,082)</td>
<td>918 (791–1,064)</td>
<td>945 (837–1,067)</td>
<td>949 (850–1,060)</td>
<td>901 (800–1,015)</td>
<td>940 (839–1,053)</td>
<td>911 (781–1,064)</td>
</tr>
<tr>
<td></td>
<td>Intralipid</td>
<td>1,008 (880–1,156)</td>
<td>870 (774–979)</td>
<td>856 (746–981)</td>
<td>903 (790–1,033)</td>
<td>900 (787–1,029)</td>
<td>883 (785–993)</td>
<td>903 (775–1,053)</td>
<td>1,019 (938–1,107)</td>
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<tr>
<td>sICAM-1, ng/ml</td>
<td>Control</td>
<td>9.4 (8.8–10.0)</td>
<td>9.0 (8.4–9.7)</td>
<td>8.7 (8.3–9.2)</td>
<td>8.9 (8.3–9.4)</td>
<td>8.9 (8.5–9.3)</td>
<td>9.1 (8.5–9.7)</td>
<td>9.2 (8.5–9.9)</td>
<td>9.1 (8.5–9.9)</td>
</tr>
<tr>
<td></td>
<td>Intralipid</td>
<td>9.4 (8.8–10.2)</td>
<td>8.6 (8.0–9.3)</td>
<td>8.7 (8.1–9.2)</td>
<td>8.9 (8.4–9.5)</td>
<td>8.9 (8.4–9.5)</td>
<td>9.2 (8.7–9.7)</td>
<td>9.5 (9.1–9.9)</td>
<td>9.3 (8.9–9.6)</td>
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<tr>
<td>sE-selectin, ng/ml</td>
<td>Control</td>
<td>2.2 (1.9–2.7)</td>
<td>2.2 (1.8–2.6)</td>
<td>2.1 (1.7–2.5)</td>
<td>2.1 (1.8–2.6)</td>
<td>2.0 (1.5–2.5)</td>
<td>2.0 (1.7–2.4)</td>
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<td>2.0 (1.7–2.5)</td>
<td>2.0 (1.7–2.5)</td>
<td>2.0 (1.6–2.4)</td>
<td>2.1 (1.7–2.5)</td>
<td>2.1 (1.7–2.6)</td>
<td>2.1 (1.8–2.7)</td>
<td>2.3 (1.9–2.9)</td>
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<tr>
<td>Muscle TLR4 mRNA-to-BA mRNA ratio</td>
<td>Control</td>
<td>2.18 (1.72–2.77)</td>
<td>3.00 (2.35–3.84)</td>
<td>2.76 (2.13–3.58)</td>
<td>2.81 (2.12–3.71)</td>
<td></td>
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<tr>
<td></td>
<td>Intralipid</td>
<td>2.15 (1.54–3.00)</td>
<td>2.88 (2.27–3.65)</td>
<td>3.44 (2.53–4.69)</td>
<td>2.97 (2.28–3.88)</td>
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<tr>
<td>Adipose TLR4 mRNA-to-BA mRNA ratio</td>
<td>Control</td>
<td>1.43 (1.20–1.72)</td>
<td>1.60 (1.40–1.83)</td>
<td>1.93 (1.54–2.42)</td>
<td>1.74 (1.25–2.43)</td>
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<td>Intralipid</td>
<td>1.46 (1.24–1.71)</td>
<td>1.62 (1.40–1.83)</td>
<td>1.95 (1.54–2.42)</td>
<td>2.02 (1.63–2.49)</td>
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Toll-like receptor 4 (TLR4) mRNA are expressed as a TLR4 mRNA-to-β-actin (BA) mRNA ratio. sICAM-1, soluble (s) intracellular adhesion molecule-1. Data are presented as geometric mean (95% confidence interval). *Denotes significant difference over time (from baseline values). †Denotes significant difference over time in both trials. **Denotes significant difference over time in both trials.
Plasma Levels of sCD-14, sICAM-1, and sE-Selectin

Plasma levels of sICAM and sCD14 changed significantly over time with no difference between trials (Table 2). Plasma levels of sICAM decreased before the endotoxin bolus (due to a difference at the 2-, 4-, 6-, and 7-h time points compared with baseline, $P < 0.05$ for all comparisons). Plasma levels of sCD14 increased after the endotoxin bolus (due to a borderline difference at the 11-h time point compared with baseline, $P = 0.07$). Plasma levels of sE-selectin increased after endotoxin bolus in the control trial (7-h time point compared with baseline, $P < 0.05$) and in the Intralipid trial (11-h time point compared with baseline, $P < 0.001$). There were no differences between trials.

Cytokine and TLR4 Gene Expression in Skeletal Muscle and Adipose Tissue

TNF-$\alpha$ and IL-6 mRNA levels in skeletal muscle tissue as well as in adipose tissue increased in response to endotoxin (Fig. 4); however, there were no differences in the levels between trials. In skeletal muscle, TNF-$\alpha$ mRNA levels were increased at the 9-h time point compared with baseline ($P < 0.05$), and IL-6 mRNA levels were increased at the 9- and 11-h time points compared with baseline ($P < 0.01$ for both comparisons). In adipose tissue, TNF-$\alpha$ and IL-6 mRNA levels were increased at the 11-h time point compared with baseline ($P < 0.01$ and $P < 0.001$, respectively). TLR4 mRNA levels in muscle and adipose tissue did not change in response to endotoxin or between trials (Table 2).

APPT and Cortisol

There was no difference in APPT during and between trials despite the heparin infusion [Intralipid trial: 29 (27–31) and 28 (26–31) s at the 0- and 11-h time points, respectively (NS); saline: 30 (28–31) and 29 (28–30) s at the 0- and 11-h time points, respectively (NS)]. Plasma levels of cortisol revealed a well-described circadian rhythm (58) with no difference between trials (data not shown).

DISCUSSION

The main findings of this study were that 1) the systemic inflammatory response to low-dose endotoxemia is elevated during acute Intralipid infusion, and 2) muscle and adipose tissue responds to endotoxemia by increasing TNF-$\alpha$ and IL-6 gene expression. These results support the hypothesis that high plasma levels of FFA may alter the inflammatory response.

Our results agree with previous findings. Mesotten et al. (49) found that intensive insulin therapy improved lipid control in critically ill patients and suggested that this partially explained the improved morbidity and mortality in these patients. Conventional n-6 lipid emulsion enhances the release of proinflammatory cytokines from monocytes, while n-3 fatty acid-based lipid emulsion suppresses the release (48), and high levels of FFA induce proinflammatory effects by an increase in NF-$\kappa$B binding activity in mononuclear cells (72). Infusion of n-3 fatty acids 48 and 24 h before endotoxin bolus injection (2 ng/kg) has recently been shown to decrease the TNF-$\alpha$ response to endotoxin with no effect on IL-6 plasma concentrations (56), and high dose n-3 fatty acid infusion improves bacterial clearance from the blood compared with n-6 fatty acid infusion in rabbits (35). In accordance, infusion of Intralipid alters bacterial clearance in rabbits (36) and mice (20) and decreases neutrophil bacterial killing in human cells (76). Intralipid infusion for only 2 h increased the IL-6 and IL-8 response but not TNF-$\alpha$ response to high-dose endotoxemia in humans (74); however, this study addressed the effect of highly elevated TG levels and did not activate LPL with heparin, which was used in the present study to induce persistent and high levels of

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**Fig. 4.** Gene expression levels of TNF-$\alpha$ and IL-6 in muscle and adipose tissue. TNF is expressed as a TNF/mRNA/BA mRNA ratio and IL-6 as an IL-6 mRNA/BA mRNA ratio. Data were normalized to a relative standard curve and then normalized to $\beta$-actin mRNA. Data are presented as geometric mean (95% CI). *Significant difference over time (from baseline values) in both trials.
FFA. Thus, we applied a study design allowing us to evaluate the effect of short-term elevated levels of FFA on the inflammatory response to low-dose endotoxemia and discovered that FFA caused a mild but significant increase in the inflammatory response, including TNF-α, IL-6, and neutrophil count. It should be noted that the plasma levels of FFA in the present study were acutely elevated and that the infusion time was shorter than the infusion time used in patients at the Intensive Care Unit, which most often is 24 h. Rapid infusion (6 h) of soybean-based fat emulsions increases proinflammatory actions compared with slow infusion (24 h; Refs. 68, 69), and the present study does not exclude the possibility that 24 h of infusion of Intralipid would lead to different results. In addition, we did not determine the different types of FFA in plasma. As previously mentioned, different FFA may have different effects on the immune response (48, 56, 72). Thus the result of the present study may not be representative of all types of hyperlipidemia. Patients with sepsis have FFA plasma levels of −6,450 μM (±1,600 SE) and TG levels on −5,020 μM (±970 SE) (60). Although lower levels were achieved in the present study (FFA levels at −2,900 μmol/l and TG levels at −3,200 μmol/l), such levels may be associated with an enhanced response to an inflammatory stimulus in septic patients as well. On the contrary, patients with hyperlipidemia, such as persons with type 2 diabetes, exhibit somewhat lower levels of circulating FFA [563 μM (±74 SE)], and a direct extrapolation, therefore, would probably not be justified for such patients. Although we cannot rule out that the elevated plasma levels of TG in the present study have an effect on the inflammatory response as well, animal studies (28, 29, 57) indicate that high TG-rich lipoprotein levels work in the opposite direction by binding and inactivating endotoxin. Saturated FFA modulate NF-κB activation of monocytes and macrophages through TLR4 (44). According to our study (where a combination of saturated and unsaturated FFA was applied), this activation may not be related to the release of CD14 from the cells, since we found no effect of elevated plasma levels of FFA on the concentration of sCD14.

Increased levels of FFA induce endothelial cell apoptosis (3), trans fatty acid intake increases plasma concentrations of sICAM and sE-selectin (46), and unsaturated fatty acids induce a proinflammatory environment in endothelial cells in vitro (71). However, contrary to our expectations, we found no differences between trials regarding plasma concentrations of sE-selectin and sICAM-1. We hypothesize that activation of these cells may require a longer follow-up than the 5 h applied in the present study, as indicated by the plasma concentrations in Table 2, or that a higher dose of endotoxin would be needed to induce differences between trials.

Interestingly, we were able to demonstrate that human skeletal muscle and adipose tissues are activated by endotoxemia and are capable of expressing TNF-α and IL-6. Skeletal muscle tissue is capable of expressing and releasing IL-6 by a TNF-α-independent pathway during exercise (67). In endotoxemic rats, the expression of TNF-α precedes that of IL-6 in skeletal muscle (43). Thus, IL-6 expression in skeletal muscle after endotoxin administration is most likely induced by TNF-α, implying that the expression of IL-6 in skeletal muscle occurs via different pathways during inflammation and exercise. Adipokines, including TNF-α and IL-6, are primarily produced by the macrophages in the adipose tissue (17, 18). The present study does not exclude the possibility that the adipose tissue would respond more readily to endotoxin in individuals with preexisting inflammation of the adipose tissue (e.g., patients with type 2 diabetes).

Insulin has potent anti-inflammatory effects (10, 13). In the present study, plasma insulin and glucose were higher during Intralipid infusion, although the levels were still within the normal range (2, 11). This finding supports the increase in TNF-α and IL-6 during Intralipid infusion but does not exclude the possibility that insulin and glucose levels in septic patients have an effect on the inflammatory response as well.

TLR4 are expressed on the surface of the adipocytes (5) but are also expressed in macrophages (70). FFA and LPS activate TLR4 signaling in 3T3-L1 adipocytes (62, 70), while LPS decreases the TLR4 expression in porcine adipose tissue 36 h after administration of the endotoxin (23). We found no difference between groups in the gene expression of TLR4. In accordance, it has been shown that endotoxemia in rats stimulates the gene expression of TNF-α and IL-6 but not TLR4 in skeletal muscle (43). However, the TLR4 pathway, e.g., the activation of NF-κB, was not examined; thus we were unable to determine whether the inflammatory response in muscle and adipose tissues was mediated via TLR4 activation or not.

In conclusion, in healthy humans short-term infusion of Intralipid combined with heparin, resulting in an acute highly elevated plasma lipid profile, increases the plasma cytokine response and the neutrophil count to endotoxin. This observation is consistent with the notion that inflammatory pathways are closely linked to metabolic control in humans. Additionally, human skeletal muscle and adipose tissue are activated by endotoxemia and are capable of expressing essential inflammatory mediators in this setting. These mediators, in turn, might exert important metabolic regulatory roles during inflammation.

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