Metabolic Alterations in Adipose Tissue During the Early Phase of Experimental Endotoxemia in Humans

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- metabolism
- endotoxemia
- sepsis
- microdialysis

Abstract

Adipose tissue plays an important role in energy homeostasis; however, there is only little knowledge about its metabolic activity during critical illness or sepsis. We assessed adipose tissue metabolic activity and local blood flow during experimental endotoxemia in otherwise healthy humans. In a prospective, placebo controlled and randomized experiment we measured changes in lactate, glycerol, and pyruvate concentrations in microdialysate samples of femoral adipose tissue after an intravenous bolus of lipopolysaccharide (LPS, 4 ng/kg). Intravenous endotoxin caused an early and constant increase in interstitial pyruvate, while formation of lactate in adipose tissue was not affected. In contrast, lactate levels in serum were elevated significantly after 90 min ($p < 0.05$) and likewise, serum glycerol concentrations rose 90 min after LPS treatment ($p < 0.05$) and 60 min earlier than in adipose tissue. Subcutaneous adipose tissue blood perfusion increased 2-fold while there was a strong decline in skin blood flow. Pyruvate accumulation in subcutaneous adipose tissue is an early marker of endotoxemia. While adipose tissue is a major source of serum glycerol and lactate in humans during physiological conditions, it contributes only little to increased serum lactate and glycerol levels during endotoxemia.

Introduction

Sepsis is the result of a profound systemic inflammation in response to an infection and associated with marked alterations of protein-, carbohydrate-, and lipid-metabolism. Endotoxemia caused by infection with gram-negative bacteria is one of the many causes for the sepsis syndrome and the early effects of gram-negative sepsis like fever, rigors, cytokine, and cortisol release can be mimicked by intravenous injection of purified bacterial endotoxin [1,2]

During endotoxemia, the anabolic effects of insulin are suppressed while protein turnover is stimulated leading to a decrease of lean body mass despite high caloric intake [3,4]. Multiple factors are responsible for the catabolic state in the septic patient. Cytokines are important mediators of septic shock, having a pivotal role in regulating host response to sepsis. Important among these are tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6), all of which influence adipose tissue metabolism directly or via release of metabolic active hormones like catecholamines, adrenocorticotropin, cortisol, and glucagon. Although, in patients with sepsis, fat is the preferred fuel for oxidation, little is known about the metabolic changes in adipose tissue that accompany early sepsis.

The injection of a single dose of endotoxin to human volunteers has been used as an experimental model to study the septic host response in vivo [1,2,5,6], and there is a well-described response including flu-like symptoms, hyperthermia and a typical cascade of cytokine release accompanied by a parallel increase in plasma catecholamines and cortisol. However, there are no detailed studies regarding the alterations of adipose tissue metabolism in response to intravenous endotoxin and likewise little is known about the effect of endotoxemia on adipose tissue perfusion which plays an important role in its metabolic activity [7]. For the study of local metabolic or paracrine changes in various compartments of the body like brain, skeletal muscle or adipose tissue microdialysis has been proven to be a useful tool [8–12]. Additionally, we have recently shown that adipose tissue perfusion can be conveniently monitored using laser-Doppler flowmetry (LDF) [13].

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The aim of this study was to investigate the effects of intravenous endotoxin on adipose tissue metabolism and adipose tissue perfusion in vivo. In order to exclude confounding factors that are usually present in critically ill patients, experiments were performed in healthy subjects who were challenged with a low-dose of lipopolysaccharide (LPS) bolus injection in a randomized, placebo controlled study. We used the microdialysis technique to monitor metabolic changes in femoral subcutaneous adipose tissue and LDF to assess dynamic changes in femoral adipose tissue blood flow.

Subjects and Methods

In order to investigate the effect of early endotoxemia on adipose tissue metabolism and blood flow we performed a randomized, double-blind experiment in 16 male, healthy and nonsmoking volunteers [n = 8, mean age 28.7 ± 2.6 years (range: 22–36)] and a control group [n = 8, mean age 26.3 ± 5.3 years (range: 23–38)] receiving placebo (saline).

Exclusion criteria were chronic or acute illness, current medication of any kind, any vaccination during the last 4 weeks, smoking, and alcohol or drug abuse. On the first visit prior to the experiment, all volunteers underwent a careful physical examination and their written informed consent was obtained. To exclude any clinically not apparent illness routine blood tests were performed. In detail, we measured serum electrolytes, glucose, liver enzymes, creatinine, C-reactive protein (CRP), red and white blood count. The study was approved by the local ethics committee and subjects gave their written consent to participate in the study.

After a 10-h overnight fast participants arrived at our medical research unit at 08:00 AM of the experimental day to receive either placebo or LPS. The subjects were placed in a comfortable position with the upper body raised 30 °. An i. v. catheter (Vasofix, Braun Melsungen, Germany) was placed in the antecubital vein to allow regular blood sampling. A second catheter was inserted into a vein of the opposite arm and isotonic saline was infused at a low rate in order to guarantee good venous access in case of any unwanted side effects like septic or allergic shock during the experiment.

All experiments were carried out under the supervision of an experienced physician. Criteria to stop the experiment were defined as systolic hypotension below 100 mmHg, heart rate below 55 or above 120 for more than 5 min, and body temperature below 36 °C and above 39 °C.

After randomization by a physician blinded to the experimental condition, purified lipopolysaccharide (LPS) prepared form *Escherichia coli* O:113 (U.S. Standard Reference Endotoxin, Food and Drug Administration, Bethesda, MD, USA) was injected as a bolus intravenously (4 ng per kilogram of body weight), and flushed by normal saline to ensure complete delivery. Control subjects received saline only.

Blood pressure (BP) was measured plethysmographically every 15 min (Welch Allyn, Tycos Instruments, Arden, USA). On the opposite arm finger BP (Finapres 2 300, Ohmeda, Louisville, USA) was monitored continuously throughout the whole experiment. Heart rate and ventilation frequency were recorded online using a 3 lead ECG and a respiratory-belt (all ADinstruments GmbH, Germany). Body temperature was registered every 30 min using an oral digital thermometer.

Microdialysis

Microdialysis was performed in subcutaneous femoral adipose tissue with a commercially available catheter (CMA 60 microdialysis catheter, pore diameter 20 000 Dalton, CMA, Solna, Sweden) and perfused with isotonic dialysis fluid (CMA dialysis fluid) at a constant flow rate of 0.3 µl/min. In a pilot study we controlled correct insertion depth of the canula with ultrasound (Hitachi EUB 6000, 10 MHz probe). After insertion of the microdialysis catheter, a calibration time of 120 min was allowed. Dialysates were collected in 30-min intervals and immediately analyzed using an automated analyzer (CMA 600) .

Laser-Doppler flowmetry

In order to monitor blood flow at the site of microdialysis we used laser-Doppler flowmetry (LDF, Perimed 6005, Järsfalla, Sweden). An LDF probe (Perimed needle probe 402) was inserted via a cannula into the adipose tissue, 1 cm in depth and 2 cm next to the microdialysis catheter. A second LDF probe (Perimed small straight probe 407-1) was taped on the skin next to the needle probe in order to simultaneously assess skin blood flow. LDF probes were calibrated using a motility standard and blood flow was measured as arbitrary perfusion units. For inter-individual comparisons baseline values before bolus injection were set to 100%.

Data for heart rate, finger blood pressure, ventilation rate, tissue- and skin perfusion were registered with a multi-channel recorder (PowerLab 16Sp, Adinstruments, Castleville, Australia) and a standard personal computer (CHART software 4.2, Adinstruments).

Blood was collected in 30-min intervals for the determination of differential blood count, CRP, TNF-α, IL-1, Il-6 catecholamines, lactate, pyruvate, glycerol, and glucose. Serum was kept at –20 °C until final analysis.

Laboratory measurements

Glycerol, glucose, lactate, and pyruvate in dialysate and plasma were analyzed using a colorimetric enzymatic method in a CMA/600 analyzer (CMA Microdialysis, Sweden) (precision > 94% accuracy > 90%). Plasma norepinephrine and epinephrine levels were determined by high-performance liquid chromatography with subsequent electrochemical detection. The sensitivity was 15.64 pmol/l for norepinephrine and 15.46 pmol/l for epinephrine. Plasma TNF-alpha and IL-6 levels were measured with the human TNF-alpha UltraSensitive Kit and the Il-6 UltraSensitive Kit (both BioSource, Camarillo, CA, USA) The sensitivity of both kids was < 0.1 pg/ml.

Statistical evaluation

We calculated a sample size of 10 volunteers in each group to achieve a power of 80% to detect a difference of 20% in interstitial metabolite concentrations between verum and placebo with a significance level of $p < 0.05$. Statistical analysis of the effects of LPS vs. placebo relied on ANOVA with a repeated measure factor 'time' and the condition 'treatment' (LPS vs. placebo). When ANOVA showed significant treatment effects, subsequent post-hoc pairwise comparisons were performed. Degrees of freedom were corrected according to the method of Greenhouse-Geisser. Results are presented as mean ± SEM.

Results

LPS induced flu-like symptoms starting 60 min after injection with malaise, headache, and rigors. There was a significant elevation of body temperature 180 min after endotoxin administration from 36.2 ± 0.1 °C to 37.0 ± 0.3 °C ($p < 0.05$). Compared to treatment with placebo, endotoxin caused a significant rise in mean blood pressure after 60 min from 95 ± 3 mm Hg to 103 ± 5 mm Hg ($p < 0.05$) but no change in heart rate. There was also a relative increase in subcutaneous adipose tissue blood flow of 243 ± 46 % but a 36 ± 7 % decrease in skin perfusion. Changes in blood pressure, heart rate, adipose tissue- and skin perfusion were not significant after placebo (data not shown).

Cytokines and hormones

Similar to earlier experiments cytokine release peaked 90 min after endotoxin i. v. with a maximum value for TNF-α and IL-6 of 611.58 pg/l ± 113.55 and 96.00 pg/l ± 2.03, respectively. Likewise there was a rapid increase of cortisol and adrenocorticotropin (ACTH) after endotoxin injection with a maximum of 103.7 ± 4.7 nmol/l for cortisol and of 228.5 ± 17.6 pmol/l for ACTH after 160 min. Baseline plasma concentrations of norepinephrine and epinephrine were 1313.79 ± 148.34 pmol/l and 345.62 ± 63.88 pmol/l, respectively. Maximum plasma concentrations for norepinephrine were found 90 min after LPS infusion (4349.76 ± 770.07 pmol/l) and for epinephrine after 160 min (655.75 ± 93.91 pmol/l). Catecholamine levels remained elevated until the end of the experiments.

Glucose, lactate, glycerol, and pyruvate

Baseline glucose concentrations in WAT and serum were 4.03 ± 0.86 mmol/l and 4.67 ± 0.22 mmol/l, respectively. There were no significant changes in glucose concentrations neither in femoral adipose tissue nor in serum after i. v. endotoxin or placebo.

Initial lactate concentrations were higher in WAT (2.06 ± 0.26 mmol/l) than in serum (1.01 ± 0.11 mmol/l). Interstitial lactate rose slightly after endotoxin injection, but this effect failed to reach statistical significance when compared to placebo. In contrast to interstitial lactate, serum lactate increased significantly 90 min after i. v. endotoxin from 1.4 ± 0.2 mmol/l to a maximum of 1.9 ± 0.1 mmol/l towards the end of the experiment ($p < 0.05$). However, there was no difference in the lactate gradient between tissue and serum (**Fig. 3**, vide infra).

Also glycerol concentrations were found to be higher in WAT (84.84 ± 15.15 mmol/l) than in serum (43.27 ± 7.33 mmol/l) under baseline conditions. 90 min after LPS treatment glycerol release from adipose tissue began to increase rapidly and peaked after 120 min (192.74 ± 40.79 µmol/l) indicating accelerated lipolysis in femoral adipose tissue. However, the rise of interstitial glycerol was preceded by an increase of serum glycerol which started 30 min after LPS injection and reached its peak after 90 min.

Baseline concentrations of pyruvate were similar in WAT and serum (66.81 ± 10.08 mmol/l vs. 56.36 ± 3.89 mmol/l). Interstitial pyruvate rose continuously starting 30 min after endotoxin and had not reached its peak at the end of the experiment period. Similarly, there was a continuous increase in serum pyruvate. However, this increase in serum was delayed when compared to adipose tissue (**Fig. 1**, **2**).

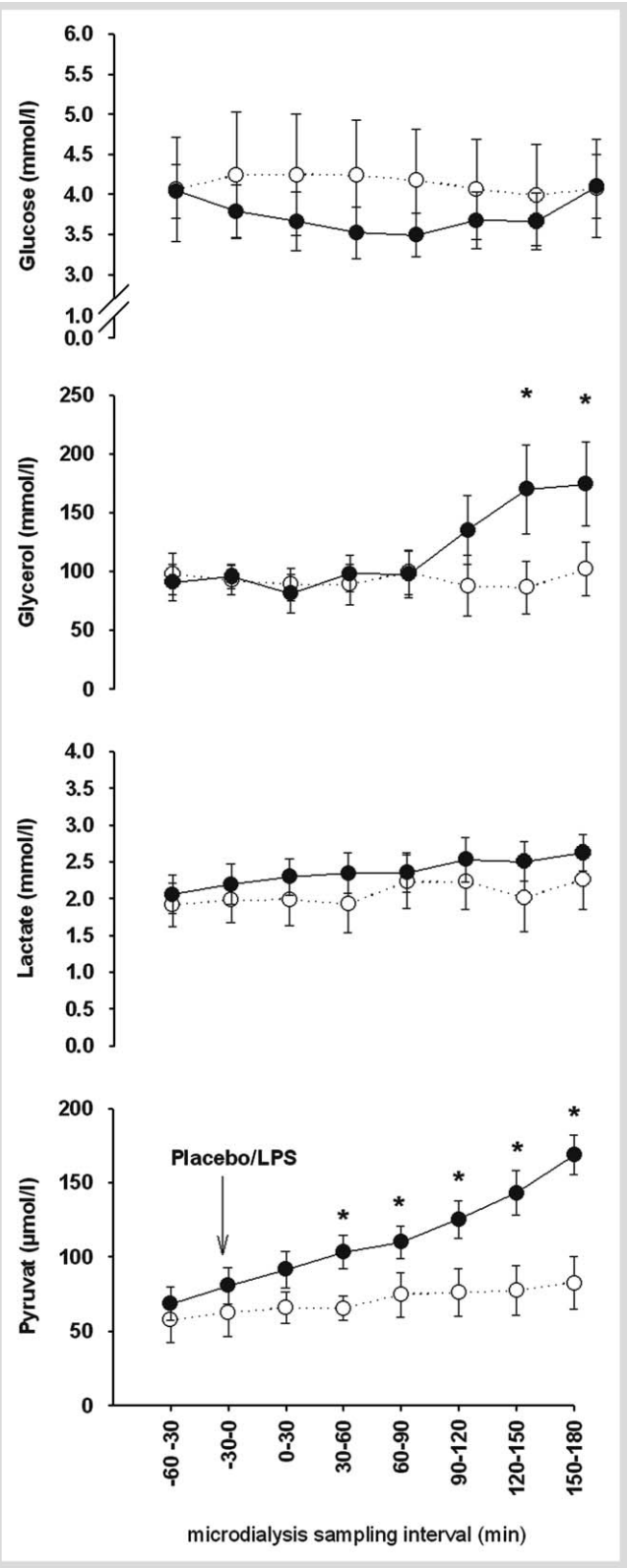

Fig. 1 Interstitial concentrations in adipose tissue of glucose, glycerol, lactate, and pyruvate after bolus injection of either placebo (open circles) or purified lipopolysaccharide (4 ng/kg body weight, closed circles). Microdialysis flow rate 3 µl/min, sampling interval of probes 30 min *$p > 0.05$.

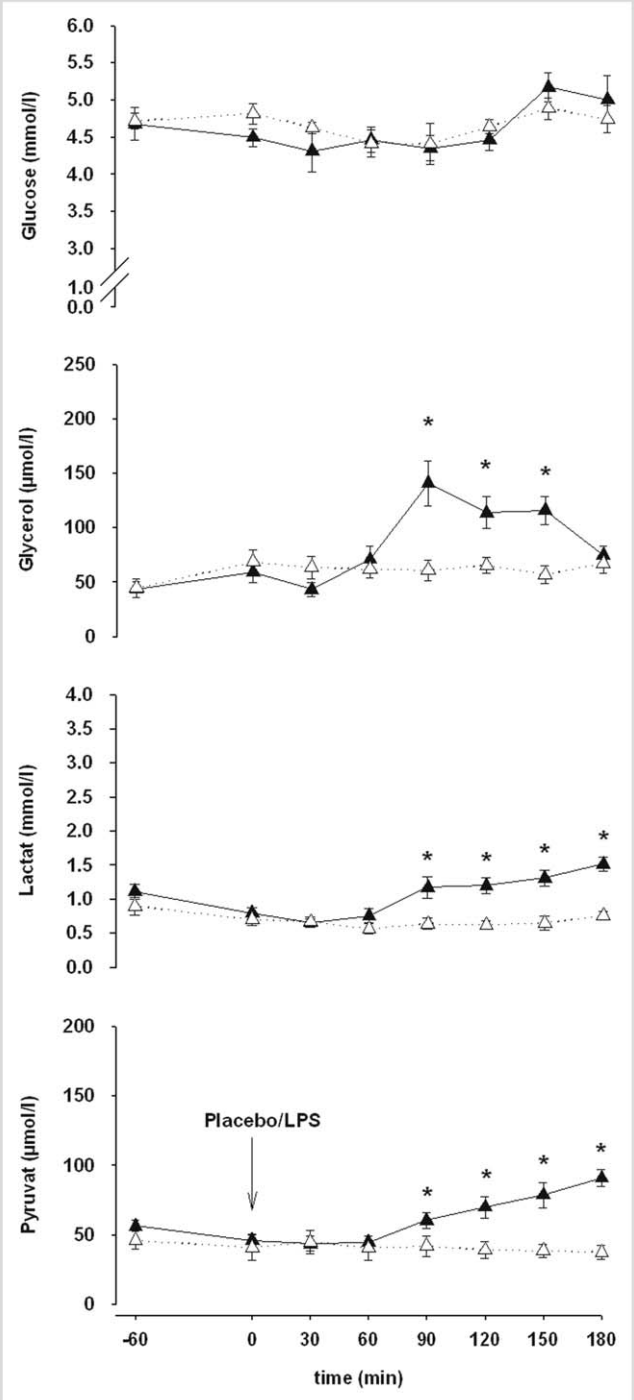

Fig. 2 Serum glucose, glycerol, lactate, and pyruvate after bolus injection of either placebo (open triangles) or purified lipopolysaccharide (4 ng/kg body weight, closed triangles) * $p < 0.05$.

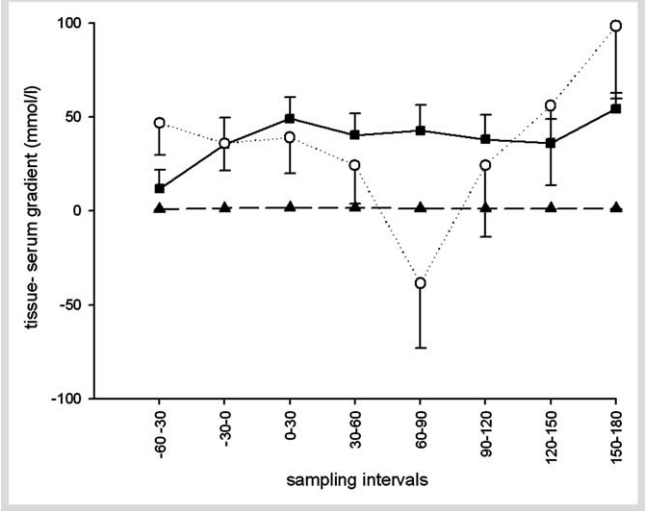

Fig. 3 Tissue-serum gradients of glycerol (open circles), lactate (triangles) and pyruvate (squares).

Discussion

In our study, we evaluated local adipose tissue metabolism in otherwise healthy humans after a low dose challenge with endotoxin. Endotoxin is a cell wall component of gram-negative bacteria and plays an essential role in the pathogenesis of septic shock, and its experimental administration to humans is a commonly used model to study early inflammatory host responses to sepsis [2, 14–17]. As expected, all volunteers experienced flu-like symptoms after intravenous (i.v.) endotoxin with rigors, nausea, and alteration in body temperature. This was accompanied by a typical rise of cytokines, cortisol, and catecholamines. Onset of symptoms was approximately 60 min after injection of endotoxin and lasted for about 3 h.

Fat is the preferred fuel for oxidation in patients with sepsis [18–20] and interstitial glycerol release is a more accurate index of the lipolytic rate than FFA release because glycerol, in contrast to FFA, is not re-utilized by fat cells in an important way. Microdialysis of adipose tissue has been proven to be an excellent tool to monitor glycerol release [21–23]. In our experiments, bolus injection of endotoxin caused an increase in interstitial and serum glycerol concentration reflecting accelerated lipolysis. Several factors might have contributed to the observed increase in glycerol generation. Plasma cytokines, such as TNF-α, Il-1, and Il-6 increased rapidly after i.v. endotoxin and previous studies have shown that cytokines have a direct effect on lipid metabolism in adipose tissue, muscle and liver [5, 24, 25]. Additionally, cytokines stimulate lipolysis indirectly through liberation of lipolytic hormones like cortisol and plasma catecholamines [26]. Although microdialysis perfusion flow was slow, concentration of glycerol in the dialysates did probably not accurately reflect glycerol release because of dynamic changes in adipose tissue blood flow. For example, a rise in tissue blood perfusion would prevent accumulation of metabolites in the interstitial tissue and hence yield a lower metabolite recovery [7]. In fact, laser-Doppler-flowmetry at the site of microdialysis sampling showed a significant rise in local blood perfusion starting 40 min after i.v. endotoxin and hence the true release of glycerol might have been actually higher.

Lipolysis is under neuronal and endocrine control and regulated differently in different tissues. Experiments in rats show that lipolysis in subcutaneous adipose tissue is greater than in visceral adipose tissue [27] and in humans, for example, glycerol release in skeletal muscle is differently regulated than in fat [28]. Lipolysis during endotoxemia can be either caused by an increased activity of the sympathetic nervous system, or the release of lipolytic hormones like cortisol and cytokines in the blood stream or both.

We calculated gradients of tissue und serum metabolites to quantify the contribution of WAT to systemic metabolite levels (**Fig. 3**). Lactate concentrations in WAT were higher than in

serum under basal conditions reflecting the role of WAT as a major source for lactate generation [29]. However lactate gradients remained unchanged after LPS injection which suggests that WAT has only a minor role in lactate production during endotoxemia. Likewise, glycerol concentrations in adipose tissue were also higher than serum levels at baseline; however, after injection of LPS the glycerol gradient fell rapidly owing to its sharp increase in serum, which was not accompanied, but followed by an increase in WAT. As serum levels declined and WAT concentrations of glycerol increased after 120 min the gradient turned positive, which suggests that only during this later period most glycerol originated from adipose tissue. However, one of our main findings was that LPS caused a significant increase of pyruvate in adipose tissue. This is also reflected by rising pyruvate WAT/serum gradient early during the experiment. Because serum concentrations rose 60 min later than in adipose tissue the gradient remained unchanged from there on and we believe that adipose tissue is a major source of pyruvate generation during LPS treatment.

In adipose tissue, pyruvate is the end product of the glycolytic pathway. However, pyruvate can also be generated through lipolysis since glycerol can enter the glycolytic pathway via metabolism to dihydroxyacetone-phosphate.

Pyruvate has a variety of metabolic fates itself. Under physiological conditions it is oxidized by the pyruvate dehydrogenase complex (PDH) to give acetyl-CoA, which is either used for de novo fatty acid synthesis or enters the citrate cycle to generate energy-rich phosphates. Under conditions of low oxygen supply, however, pyruvate is metabolized to lactate rather than to acetyl-CoA. Apart from tissue hypoxia lactate generation during sepsis can also be the result of an accelerated glycolysis, which yields high amounts of pyruvate and NADH leading to a shunting towards lactate formation despite normal tissue oxygenation [30]. The accumulation of pyruvate in adipose tissue observed in our experiments corresponds with data from severely burned patients where pyruvate production increased threefold when compared to controls [31].

Endogenous pyruvate inhibits NF kappa B signaling pathways and could possess an anti-inflammatory role during sepsis [32]. Similarly, externally administered ethyl pyruvate inhibits the release of inflammatory cytokines and attenuates lethal systemic inflammation in animal experiments [33]. With respect to those and other studies increased interstitial pyruvate formation observed in our experiments might have a protective role during sepsis.

In our experiments, basal concentrations of lactate in interstitial adipose tissue were about 3-fold higher than serum lactate, and this finding is in accordance with data from other research [9, 34]. Human adipose tissue is a major source of lactate production under resting conditions and the basal serum lactate is proportional to the overall amount of adipose tissue mass [35, 36]. Recently published observations show that adipose tissue lactate, glycerol, and pyruvate are elevated in patients with severe sepsis and that these metabolites relate to a poor clinical outcome [37]. In earlier studies using an endotoxin challenge in otherwise healthy humans, however, adipose tissue did not seem to contribute significantly to the hyperlactatemia typically seen during sepsis [38], which is confirmed by our observations using a similar model. Additionally, experimental endotoxin injection can only be a model for pathophsiolical processes during gram negative sepsis. Hence, experimental endotoxemia might not accurately reflect metabolic changes in adipose tissue when compared to patients with severe septic shock. In experimental endotoxemia other compartments than adipose tissue must have contributed to the increase in serum lactate. Data from rodents suggest that during endotoxemia this source might be skeletal muscle [39–41]. Of course, monitoring lactate and pyruvate release during experimental endotoxemia in skeletal muscle would have been interesting and might have added valuable data to our experiment. However, we decided against placing microdialysis probes in skeletal muscle because we were concerned about coagulation abnormalities after LPS injection with possible bleeding complications and local hematoma in our study volunteers.

In summary early endotoxemia accelerates glycolysis and pyruvate accumulation in white adipose tissue in humans. After bolus injection of LPS there is also a local increase in lipolysis reflected by rising interstitial glycerol levels. Although adipose tissue is one of the major sources of lactate release during physiological conditions it contributes only little to elevated serum lactate levels during early endotoxemia.

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