Intracellular Survival of \textit{Staphylococcus aureus} in Adipocyte-Like Differentiated 3T3-L1 Cells Is Glucose Dependent and Alters Cytokine, Chemokine, and Adipokine Secretion

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Although obesity and type 2 diabetes mellitus are associated with Gram-positive infections and a worse clinical outcome, it is unknown whether adipocytes can be infected by Gram-positive bacteria. Adipocyte-like differentiated 3T3-L1 cells and \textit{Staphylococcus aureus} were used for infection experiments under normoglycemic (100 mg/dl) and hyperglycemic (450 mg/dl) conditions in the presence/absence of insulin (1 \textmu M). Intracellular presence and survival of \textit{S. aureus} was investigated quantitatively. Supernatant cytokines, chemokines, and adipokines were measured by ELISA. Lipid metabolism and cellular morphology of infected adipocytes were investigated by different techniques. The present study provides the proof of principle that adipocyte-like cells can be infected by \textit{S. aureus} dose dependently for up to 5 d. Importantly, low bacterial inocula did not affect cell viability. Intracellular survival of \textit{S. aureus} was glucose dependent but not insulin dependent, and insulin receptor expression and insulin receptor signaling were not altered. Infection increased macrophage chemoattractant protein-1, visfatin, and IL-6 secretion, whereas resistin and adiponectin were decreased. Infected adipocytes had higher intracellular triacylglycerol concentrations and larger lipid droplets because of a decreased lipolysis. Taken together, infection of adipocytes by \textit{S. aureus} is glucose dependent, inhibits cellular lipolysis, and affects the secretion of immunomodulating adipokines differentially. Because cell viability is not affected during infection, adipose tissue might function as a host for chronic infection by bacteria-causing metabolic, proinflammatory, and prodiabetic disturbances. (Endocrinology 152: 4148–4157, 2011)

\textit{Staphylococcus aureus} is one of the most predominant pathogens implicated both in community-acquired and hospital-associated infections. \textit{S. aureus} frequently causes severe infections with a high mortality. Staphylococcal infections are associated with metabolic disorders such as diabetes mellitus (1, 2). On the other hand, severe bacterial infections with poor outcome have been associated with metabolic disturbances such as cachexia, insulin resistance, increased lipolysis, and elevated systemic free fatty acids (3, 4). In contrast, recent clinical data suggest that increased adipose tissue mass [reflected by a body mass index higher than 24–25 kg/m\(^2\)] might be associated with a better outcome in patients with septic shock (5) or in patients with \textit{S. aureus} bacteremia (6). Obesity might favor a positive outcome during infection because enlarged adipose tissue mass might provide defense tools such as antiinflammatory adipokines to fight bacterial infection. Adipocytes can be regarded not only as endocrine (7, 8) but also immunologically (9) active cells, and these cells exhibit physiological functions that go beyond lipid and energy storage. Adipocytes share common antigens and differentiation receptors with macrophages and are able to secrete inflammatory cytokines and chemokines such as macrophage chemoattractant protein-1 (MCP-1), TNF, RANTES, and IL-6 upon stimulation (reviewed in Ref. 9). Furthermore, several immunomodulating and

Abbreviations: CFU, Colony forming unit; IR, insulin receptor; IRS, insulin receptor substrate; JNK, c-Jun N-terminal kinase; LDH, lactate dehydrogenase; MCP-1, macrophage chemoattractant protein-1; NCS, newborn calf serum; P, phosphorylation; TLR, Toll-like receptor.
metabolically active adipokines (e.g., leptin, C1q/TNF-related protein-3, adiponectin, resistin, and visfatin) are synthesized by adipocytes and specifically regulate innate immune functions (10).

Adiponectin, leptin, and visfatin have been shown to regulate monocyte/macrophage functions such as cytokine secretion, adhesion, phagocytosis, and expression of surface markers (reviewed in Ref. 11). Adiponectin and leptin have also been implicated in modulating B and T cell responses. Resistin was able to limit the activation of dendritic cells by S. aureus in vitro (12), whereas leptin improved bacterial clearance in an animal model of pneumonia (13). In an animal model of Listeria monocytogenes infection, adiponectin regulated macrophage recruitment (14), and endotoxin levels were negatively correlated with adiponectin levels in a murine sepsis model (15). Adipokines might thus be involved in the modulation of infectious diseases.

Adipocytes express Toll-like receptors (TLR) as important pathogen pattern recognition receptors. In vitro, stimulation of adipocytes with several TLR agonists including lipopolysaccharide, flagellin, Poly(U), polyinosinic-polycytidylic acid, macrophage-activating lipopeptide from Mycoplasma fermentans, Pam3Cys, and CpG modified cytokine, chemokine and adipokine release (16). However, the direct interaction of pathogenic Gram-positive bacteria with adipocytes has not been studied yet. It is currently unknown whether S. aureus is able to infect adipocytes directly and to survive intracellularly. Neither the conditions facilitating intracellular survival of S. aureus in adipocytes nor the potential consequences on adipocyte cell function have been examined. Therefore, it was the aim of the present study: 1) to clarify in an in vitro infection model whether adipogenic-differentiated 3T3-L1 fibroblasts can be directly infected by S. aureus in a dose-dependent manner, 2) to investigate whether S. aureus is able to survive intracellularly in these adipocyte-like cells, 3) to determine whether intracellular survival is modified by carbohydrate metabolism, and 4) to investigate whether S. aureus infection affects important inflammatory and metabolic functions of adipogenic cells.

Materials and Methods

Cell culture media, bacteria, and reagents

S. aureus strain PS80 (a type 8 encapsulated clinical isolate) was kindly provided by Professor J. C. Lee (Channing Laboratory, Brigham and Women’s Hospital, Boston, MA). DMEM with low glucose (1.0 g/liter concentration) was purchased from Biochrom AG (Berlin, Germany), DMEM with high glucose (4.5 g/liter concentration) was from PAA Laboratories (Pasching, Austria), and DMEM/F12/glutamate was from Lonza (Basel, Switzerland).

3-Isobutyl-methyl-xanthine was from Serva (Heidelberg, Germany), and Pedersen-fetuin was from MP Biomedicals (Illkirch, France). Rabbit antimouse insulin receptor antibodies, antiphospho-ERK, antiphospho-c-Jun N-terminal kinase (JNK), antiphospho-p38 MAPK, antiphospho-Akt, antinsulin receptor substrate (IRS), and anti-β-actin antibodies were all from Cell Signaling Technologies (Danvers, MA), and horseradish peroxidase-coupled goat antirabbit antibodies were from Santa Cruz Biotechnology (Heidelberg, Germany). All other reagents were from Sigma-Aldrich (Munich, Germany).

Adipogenic differentiation and infection assays

3T3-L1 fibroblasts, a murine cell line derived from disaggregated mouse embryos, were cultured at 37°C and 5% CO2 in DMEM supplemented with 10% newborn calf serum (NCS) and 1% penicillin/streptomycin. At confluence, cells were seeded into 12-well tissue culture plates and differentiated into adipocyte-like cells by DMEM/F12/glutamate medium supplemented with 0.5 mM 3-isobutyl-methyl-xanthine, 10⁻⁷ M corticosterone, 10⁻⁷ M insulin, 200 mM ascorbate, 2 μg/ml transferrin, 5% NCS, 1 mM biotin, 17 mM panthothenate, 1% penicillin/streptomycin, and 300 mg/liter Pedersen-fetuin for 8 d as described previously (17). Phenotype was controlled by light microscopy (typical appearance of extensive lipid droplet accumulation) and by measuring the secretion of adipogenic differentiation markers such as adiponectin and resistin. Only terminally differentiated cells at d 8 of differentiation were used for subsequent experiments.

For infection assays, S. aureus strain PS80 was grown on tryptic soy agar overnight, resuspended in PBS, and adjusted to an OD at 650 nm of 0.34. CFU (colony forming units) were verified by serial plate counts. Bacteria were pelleted at 10,000 rpm for 5 min, resuspended in 1 ml DMEM with 5% NCS at concentrations of 1 × 10⁸ CFU/ml, 5 × 10⁸ CFU/ml, or 2 × 10⁹ CFU/ml, overlaid on adherent differentiated adipocytes in 12-well tissue culture plates and incubated at 37°C. After 90 min, the supernatant was removed and replaced with serum-free DMEM for 4 h. Differentiated 3T3-L1 cells were then stimulated with 10 nM, 50 nM, or 100 nM insulin for 5–60 min before preparing cell lysates for Western blots.

Insulin stimulation assays

For insulin stimulation assays, the cell supernatant was removed from differentiated 3T3-L1 cells infected 24 h before and replaced with serum-free DMEM for 4 h. Differentiated 3T3-L1 cells were then stimulated with 10 nM, 50 nM, or 100 nM insulin for 5–60 min before preparing cell lysates for Western blots.

Enzyme-linked immunosorbent assays

Cytokines, chemokines, and adipokines were measured in cell culture supernatants using commercially available ELISA sets (R & D Systems, Wiesbaden, Germany). For the detection of cytotoxic effects and to assess cell viability during the infection experiments, lactate dehydrogenase (LDH) concentration was measured in supernatants (cytotoxicity detection kit; Roche Diagnostics, Mannheim, Germany).
Western blot analysis

*S. aureus*-infected and -noninfected adipocyte-like cells were harvested into ice-cold PBS, washed once, and lysed in radioimmunoprecipitation assay buffer (150 mM NaCl; 1% Nonidet P-40; 0.5% sodium deoxycholate; 0.1% sodium dodecyl sulfate; 50 mM Tris, pH 7.5). Equal amounts of total protein were submitted to gel electrophoresis after dilution in Laemmli buffer. SDS-PAGE was performed following standard procedures. Proteins were transferred to a nitrocellulose membrane (Invitrogen, Darmstadt, Germany). Primary antibodies were diluted 1:1000 (β-actin 1:2000) in 5% nonfat dry milk/PBS, and the secondary horseradish peroxidase-coupled antirabbit antibodies of goat origin was used with a 1:3000 dilution in a 5% nonfat dry milk/PBS suspension. Detection of the immune complexes was carried out with the enhanced chemiluminescence Western blot detection system (Amersham Corp., Aylesbury, UK). For densitometric quantification of bands, ImageJ software (National Institutes of Health, Bethesda, MD) was used.

Oil-red-O staining, glycerol, and intracellular triacylglycerol measurement

Cell culture supernatants were removed; cells were washed with PBS, fixed for 5 min with 10% formaldehyde in PBS, washed with 60% isopropanol, and dried. Oil red O (Sigma-Aldrich) was added for 20 min, and cells were washed with sterile water and analyzed using light microscopy.

Intracellular triacylglycerol concentrations were measured using the triacylglycerol GPO-PAP microtest (Roche, Mannheim, Germany). Triacylglycerol concentrations were normalized to total protein content. Glycerol concentrations in cell culture supernatants were measured using the free glycerol assay (Biocat, Heidelberg, Germany).

Statistical analysis

Means were compared using a Student’s *t* test, and paired *t* tests were used to compare mean densitometric measurements between different Western blots. Multiway ANOVA was used to perform simultaneous tests of the independent effects of time and inoculum dose in the *in vitro* infection model. A statistical software package (SPSS version 17; SPSS Inc., Chicago, IL) was used to calculate *P* values, and a *P* < 0.05 was considered to be statistically significant. Results are expressed as means ± SEM.

Results

Demonstration of dose-dependent infection of differentiated 3T3-L1 cells by *S. aureus*

To address whether differentiated 3T3-L1 cells can be infected by *S. aureus* directly, we incubated these cells with different concentrations of *S. aureus* for 90 min before substituting with fresh medium containing 100 µg/ml gentamicin to kill the remaining extracellular bacteria. Viable staphylococci were detected in cell lysates 2, 5, 22, 28, 50, 71, and 120 h after infection (Fig. 1A). All cell culture supernatants sampled in parallel before lysing the cells were sterile. The number of intracellular colony-forming units was dependent on the colony-forming units of the initial inoculum and decreased over time (Fig. 1A). Intracellular numbers of *S. aureus* were significantly higher when differentiated 3T3-L1 cells were infected with 5 × 10⁸ CFU compared with 1 × 10⁸ CFU at all time points tested (Fig. 1A). Although statistically not significant, infection with *S. aureus* at 2 × 10⁹ CFU/well was associated with a trend toward an increased LDH release 4 h after infection, whereas lower inocula did not result in increased LDH release. Therefore, inocula of 5 × 10⁸ CFU/well and lower were used for subsequent experiments.

FIG. 1. Intracellular survival of *S. aureus* in 3T3-L1 adipocytes. A, Adherent adipocytes were incubated with the indicated bacterial inocula for 90 min before replacing the cell culture supernatant with medium containing 100 µg/ml gentamicin to kill the remaining extracellular bacteria. Cells were lysed with 0.1% Triton X-100 at various time points after infection, and intracellular bacteria were counted. Intracellular *S. aureus* could be demonstrated for up to 5 d after infection. B, Cell viability is not affected by bacterial inocula with low colony-forming units. LDH concentrations in cell culture supernatants of infected and uninfected cells were measured. High bacterial inocula (2 × 10⁹ CFU/well) were associated with a trend (statistically not significant) toward increased cell damage 4 h after infection, whereas lower inocula did not result in increased LDH release. Therefore, inocula of 5 × 10⁸ CFU/well and lower were used for subsequent experiments.
control cells (Fig. 1B). Therefore, inocula of $5 \times 10^8$ CFU/well or lower were used in subsequent experiments.

**Intracellular survival of S. aureus in differentiated 3T3-L1 cells is affected by glucose but not by insulin concentrations in culture media**

Intracellular survival of *S. aureus* was strongly affected by supernatant glucose concentrations in the cell culture medium (Fig. 2A). Differentiated 3T3-L1 cells cultured in DMEM/NCS with high-glucose concentrations (450 mg/dl) contained significantly fewer viable bacteria than those cultured in DMEM with low-glucose concentrations (100 mg/dl) 48 and 72 h after infection with the same inoculum ($P < 0.001$ and $P < 0.01$, respectively; Fig. 2A). In contrast, cells cultured in the presence or absence of 1 μM insulin showed no differences in the number of intracellular viable staphylococci at all time points (Fig. 2B).

**Infection of differentiated 3T3-L1 cells with S. aureus stimulates the secretion of MCP-1, IL-6, and visfatin but inhibits the secretion of resistin and adiponectin**

Cellular supernatants of differentiated 3T3-L1 cells infected with $1 \times 10^8$ CFU, $5 \times 10^8$ CFU, or $2 \times 10^9$ CFU were collected after 24 and 48 h. The concentrations of MCP-1, IL-6, visfatin, resistin, and adiponectin were measured by ELISA. IL-6 concentrations were significantly increased in supernatants of cells infected with $5 \times 10^8$ CFU or $2 \times 10^9$ CFU after 48 h ($P < 0.001$ each, Fig. 3A). Similarly, the concentrations of MCP-1 ($P = 0.001$, $P < 0.001$, and $P < 0.001$) and visfatin ($P = 0.036$, $P < 0.01$ and $P = 0.037$) were significantly increased in supernatants of infected cells after 48 h; (Fig. 3, B and E). In contrast, these supernatants contained significantly less resistin and adiponectin (all $P < 0.001$ for resistin, $P = 0.019$, $P < 0.001$, and $P < 0.001$ for adiponectin; Fig. 3, D and C). Supernatants of cells infected with high doses of *S. aureus* had already reduced levels of resistin ($P < 0.01$ for $5 \times 10^8$ CFU and $P < 0.001$ for $2 \times 10^9$ CFU) and adiponectin ($P = 0.032$ for $5 \times 10^8$ CFU and $P < 0.001$ for $2 \times 10^9$ CFU) after 24 h (Fig. 3, C and D).

Multiway ANOVA analysis showed a significant effect of time and inoculum dose on IL-6, adiponectin, and resistin secretion ($P < 0.001$ each), whereas the effect on MCP-1 and visfatin was not significant.

**Infection with S. aureus reduces lipolysis in differentiated 3T3-L1 cells**

As demonstrated by Oil-red-O staining, differentiated 3T3-L1 cells infected with *S. aureus* had larger lipid droplets than uninfected control cells (Fig. 4A). Image analysis of at least three independent photographs per experimental group showed a significantly larger lipid droplet size in adipocytes infected with $5 \times 10^8$ CFU/well than in uninfected controls both at 24 and 48 h after infection ($P = 0.03$ and $P = 0.02$, Fig. 4B). Similarly, the intracellular triacylglycerol content per total protein was significantly higher in *S. aureus*-infected cells 24 h after inoculation ($P < 0.01$).

Additionally, supernatants of differentiated cells infected with $1 \times 10^8$ CFU, $5 \times 10^8$ CFU, or $2 \times 10^9$ CFU 24 or 48 h before were tested for glycerol content to investigate the effect of bacterial infection on lipolysis. Cells infected with $5 \times 10^8$ CFU or $2 \times 10^9$ CFU had significantly less glycerol in their supernatants both at 24 and 48 h after infection ($P < 0.001$ after 24 and 48 h; Fig. 4D).
reflecting a reduced rate of lipolysis. Multiway ANOVA analysis confirmed a significant effect of time and inoculum dose on supernatant glycerol content ($P < 0.001$).

Insulin receptor expression and insulin signaling is not affected in *S. aureus*-infected differentiated 3T3-L1 cells

Western blots were used to study the expression of insulin receptors in differentiated 3T3-L1 cells infected with $5 \times 10^8$ CFU *S. aureus*. We were unable to detect differences in insulin receptor expression between infected and uninfected cells at 2, 5, and 22 h after infection (Fig. 5). Additionally, no differences in p38 MAPK, ERK, JNK, and Akt expression between infected and uninfected cells were found (not shown). Similarly, no differences in the phosphorylation (P) of insulin receptors (IR), Akt (P-Akt), JNK (P-JNK), and ERK (P-ERK) between cells infected with $5 \times 10^8$ CFU *S. aureus* for 24 h and controls were
observed after stimulation with 10, 50, and 100 nM insulin for 5–60 min (Figs. 6 and Fig. 7), reflecting undisturbed insulin signaling in infected cells. Both insulin and incubation with \textit{S. aureus} lead to a dose-dependent increase in phosphorylation of p38 MAPK, resulting in a significantly higher expression of P-p38 MAPK in insulin-stimulated cells infected with \textit{S. aureus} after stimulation for 30 and 60 min. Relative expression of P-p38 MAPK in \textit{S. aureus}-infected cells was 1.91-fold higher after stimulation with 10 nM insulin for 30 min (\(P = 0.005\)), 1.82-fold higher after stimulation with 50 nM insulin (\(P = 0.002\)), and 2.28-fold higher after stimulation with 100 nM insulin (\(P = 0.031\)) (Fig. 7). Additionally, we observed a dose-dependent down-regulation of IRS in \textit{S. aureus}-infected cells stimulated with insulin. Relative expression of IRS in \textit{S. aureus}-infected cells was 0.66-fold compared with uninfected cells after stimulation with 10 nM insulin for 30 min (\(P < \text{H}11005\) n.s.), decreased to 0.46-fold after stimulation with 50 nM insulin (\(P < \text{H}11005\) 0.049), and decreased 0.43-fold after stimulation with 100 nM insulin (\(P < \text{H}11005\) 0.008) (Fig. 7).

**Discussion**

Severe bacterial infections (\textit{e.g.} sepsis) and systemic inflammatory response syndrome are commonly associated with metabolic alterations such as hyperglycemia, insulin resistance, and elevated serum levels of free fatty acids (18), and vice versa, metabolic diseases are characterized by a chronic and low-grade state of inflammation (19). The role of adipose tissue in this setting has only recently received more attention. Adipose tissue does serve as not only an organ dedicated to energy and lipid storage but also as an immunological organ expressing functional TLR and secreting several cytokines and adipokines (reviewed in Ref. 9). TLR-2 and TLR-4 agonists, for example, are able to induce IL-6 secretion in cultured adipocytes but inhibit the release of resistin (16). So far, adipocyte infections with viral (adenovirus, cytomegalovirus, influenza, respiratory syncytial virus) (20, 21) and classical intracellular pathogens (chlamydia) (21) have been described. Here we studied the alterations in murine adipocyte-like differentiated 3T3-L1 cells caused by a clinically relevant pathogen (\textit{S. aureus}) associated with invasive infections (\textit{e.g.} bacteremia and deep soft tissue...
infections). Although generally considered an extracellular pathogen, *S. aureus* is able to infect a variety of cell lines. However, no direct infection of adipocytes or adipogenic differentiated cells has been demonstrated so far.

The present study provides the proof of principle that adipocyte-like differentiated 3T3-L1 cells can be infected by *S. aureus* dose dependently for up to 5 d. Low bacterial inocula did not affect cell viability because an inoculum-dose dependent persistence of *S. aureus* over several days was not associated with significant cell lysis. Intracellular survival of *S. aureus* was reduced when cells were cultivated under hyperglycemic conditions, whereas different insulin concentrations did not influence bacterial survival. It can be speculated that elevated glucose levels frequently observed during systemic infections might facilitate the clearance of intracellular bacteria. Interestingly, some TNF effects on adipocytes have been reported to be glucose dependent (22). Thus, aggressive management of hyperglycemia (23) during infections by insulin therapy in intensive care patients might affect clinical outcome in Gram-positive infections.

Similar to what has been reported for specific TLR agonists (16, 24), we observed an inoculum dose-dependent production of IL-6 and MCP-1 (CC chemokine ligand 2), highlighting the potential role of adipocytes in the host immune response to bacterial infections. At the same time, infection with *S. aureus* profoundly altered the secretion of adipokines by differentiated adipocytes. Although the release of the proinflammatory and prodiabetic adipokine visfatin was induced, infection with *S. aureus* significantly reduced the constitutive release of the antiinflammatory and antidiabetic adipokine adiponectin and the proinflammatory and prodiabetic adipokine resistin. Taken together, infection by *S. aureus* leads to a uniform proinflammatory secretion profile of cytokines and chemokines, whereas metabolically active and immunomodulating adipokines are regulated in a complex manner. The increase of visfatin and the decrease of adiponectin might result in insulin resistance as a net effect, whereas the decrease of resistin more likely improves insulin sensitivity.

Only few *in vivo* data on systemic adipokine levels during infections have been reported so far. Decreased adiponectin levels have been described in chronic hepatitis B infection (25) and in critically ill patients (26). Additionally, adiponectin has been demonstrated to be critically involved in the immune response to polymicrobial sepsis and infection with *Listeria* in mouse models (14, 27).

We also set out to investigate whether some of the metabolic changes observed during bacterial infections *in vivo* could be correlated to alterations observed in adipocytes after *in vitro* infection. Although inflammation and infection are frequently associated with increased insulin resistance, we could not detect any changes in insulin receptor (IR) expression on adipocytes after infection with *S. aureus*. Similarly, we could not detect any changes in P-IR, P-Akt, P-ERK, and P-JNK. We observed an increased phosphorylation of P-p38 MAPK only in *S. aureus*-infected cells and a trend toward lower expression of IRS. The physiological relevance of a higher p38 MAPK phosphorylation remains unclear and has to be clarified in future studies. Similarly, we cannot explain the mechanism behind our observation of a lower IRS expression. Overall, the present data suggest an undisturbed insulin signaling despite infection with *S. aureus*.

In contrast, infection with *S. aureus* was associated with changes in lipid metabolism. Significantly larger lipid droplets and higher intracellular triacylglycerol concen-

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**FIG. 5.** IR expression in *S. aureus*-infected adipocyte-like differentiated 3T3-L1 cells. A, IR in whole-cell lysates prepared 2, 5, and 22 h after infection from adipocytes infected with 5 × 10⁶ CFU/well and uninfected controls was analyzed by Western blot and normalized to β-actin. B, Densitometric quantification (relative expression normalized to β-actin) revealed no differences in insulin receptor quantities in infected and uninfected cells.

**FIG. 6.** IR signaling in *S. aureus*-infected adipocyte-like differentiated 3T3-L1 cells. Whole-cell lysates were prepared from differentiated 3T3-L1 cells infected with 5 × 10⁶ CFU/well and uninfected controls after incubation in serum-free medium for 4 h and stimulation with 50 nM insulin for 5, 10, or 30 min. Expression of ERK, p38 MAPK, IR, Akt, IRS, and the levels of P-ERK, P-p38 MAPK, P-IR, and P-Akt after infection with *S. aureus* for 24 h were analyzed by Western blot and compared with uninfected controls. No significant differences in the phosphorylation of ERK, IR, and Akt were detected. However, a trend toward increased phosphorylation of p38 MAPK and toward lower IRS expression was observed.
trations were observed after infection, and these changes may at least in part be attributed to a reduced rate of lipolysis. These results seem to contradict findings in humans in which adipocyte lipolysis is increased in infectious diseases (28). Furthermore, a challenge of rodents with either lipopolysaccharide or lipoteichoic acid (cell wall components of Gram negative and Gram positive bacteria, respectively) leads to increased lipolysis (29, 30). However, these studies were mostly performed in vivo in which effects in individual cells/tissues cannot be analyzed. In addition, primary adipocytes (which may contain small numbers of macrophages or other immune cells) have been used. Increased cytokine and catecholamine levels in vivo during inflammatory diseases may be highly relevant factors that increase lipolysis. Indeed, both inflammatory cytokines [e.g. TNF (31), IL-1β (32)] and catecholamines (33) are able to increase lipolysis in adipocytes. It has been shown recently that adipose tissue macrophages play an important role in adipose tissue remodeling and lipolysis during adipose tissue inflammation (34). These cytokines may also disturb insulin signaling and antilipolytic effects of insulin, thereby further increasing basal lipolysis of adipocytes.

Regarding adipocyte physiology, the present data have to be interpreted with caution because hormonally differentiated 3T3-L1 cells infected with $5 \times 10^6$ CFU/well and uninfected controls after incubation in serum-free medium for 4 h and stimulation with 10, 50, and 100 nM insulin for 5–60 min. Expression of JNK, ERK, p38 MAPK, IR, Akt, IRS, and the levels of P-JNK, P-ERK, P-p38 MAPK, P-IR, and P-Akt after infection with S. aureus for 24 h was analyzed by Western blot, normalized to β-actin or total levels of the specific protein, and compared with uninfected controls. Densitometric quantification was used to analyze up-regulation after infection with S. aureus (relative expression after insulin stimulation in infected over relative expression in uninfected cells). Results are given as the mean of three to five independent experiments ± SE (>1 higher levels in infected cells, < 1 lower levels in infected cells); data indicating a significant difference (P < 0.05) are presented in bold.

### Summary

The present study provides the proof of principle that adipocyte-like fibroblasts can be infected by S. aureus dose...
dependently. Intracellular survival of *Staphylococcus aureus* is glucose dependent but not insulin dependent, and IR expression or IR signaling is not altered. Infection increases MCP-1, visfatin, and IL-6 secretion, whereas resistin and adiponectin secretion is decreased. Infected adipocytes have higher intracellular triacylglycerol concentrations and larger lipid droplets due to a decreased lipolysis.

**Conclusions**

Our findings shed light on the role of adipocytes in bacterial infections and in the host immune response. In particular, the adipokine release by adipocytes is strongly modulated during *S. aureus* infection and may have implications for metabolic and immunological changes observed during infections. The data provide a novel cellular and molecular basis for a better understanding of infection-related proinflammatory and prodiabetic disturbances. However, the relevance of these observations during an infection in humans still needs to be elucidated and has to be investigated in future studies.

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