Glucose modulates hemodynamic, metabolic, and inflammatory responses to lipopolysaccharide in rabbits

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Glucose modulates hemodynamic, metabolic, and inflammatory responses to lipopolysaccharide in rabbits. J. Appl. Physiol. 83(5): 1566-1574, 1997.—Glucose is important for vascular and immunocompetent cell functions. We hypothesized that modifications in glucose metabolism (normal feeding, 24-h fasting, glucose loading) may influence the hemodynamic, metabolic, and inflammatory responses to lipopolysaccharide administration (LPS; 600 µg/kg iv) in rabbits. Aortic (ABFV), hepatic (HABFV), and portal vein blood flow velocities (PVBFV) (pulsed Doppler), plasma tumor necrosis factor (TNF) and nitrites were measured. Fasting depleted hepatic glucone glycogen content, and intraportal glucose load (2 g/kg) partially restored it. LPS induced a similar hypotension (−20%, P < 0.05) in three groups of animals. In fed animals, systemic vasoconstriction occurred with low ABFV and PVBFV (−40%, P < 0.05), together with lactacidemia and hyperglycemia. In fasted animals, ABFV and PVBFV were maintained, without metabolic acidosis or hyperglycemia. Glucose loading induced hemodynamic and metabolic patterns comparable to those observed in fed animals, although significantly more severe. TNF release was amplified fourfold by glucose loading, with no impact on nitrite levels. In conclusion, glucose metabolism interferes with hemodynamic, metabolic, and inflammatory responses to LPS.

Liver blood flow; tumor necrosis factor; metabolism.

IN HUMAN SEVERE SEPSIS, plasma glucose level is frequently elevated (1), but little attention has been paid to evaluate the impact of this elevation on cardiovascular patterns and immune response. Elevated plasma glucose concentration has been demonstrated to modify vascular reactivity. In diabetes mellitus, vascular resistances are increased (11) and vascular endothelium-dependent relaxation is impaired (27), suggesting a direct effect of glucose on endothelial functions and smooth muscle physiology. Glucose also is the major energetic substrate for immunocompetent cells like monocytes and macrophages. Glucose uptake has been shown to be tremendously increased in activated immunocompetent tissues and macrophages, especially in the liver (19). Glucose can regulate interleukin-1β (IL-1β) production by human endotoxin-stimulated monocytes in culture mainly via increased energetic availability (23).

Sepsis-induced hyperglycemia mainly results from enhanced hepatic glucose production (29). The liver is also an immunocompetent organ and could be considered a key organ during sepsis (24) because it contains the greatest proportion of resident macrophages and is a source of cytokines (5) and nitric oxide (NO) (3) during acute inflammation.

On the basis of this, we hypothesized that modifications in glucose balance induced by fasting might modify metabolic, inflammatory, and systemic/liver hemodynamic responses after lipopolysaccharide (LPS) injection in rabbits. Accordingly, we observed that, in normal glucose storage conditions (Fed), LPS induced a systemic vasoconstriction with a decreased portal venous blood flow, whereas in fasted animals (Fasted), LPS induced a peripheral vasodilatation with a maintained portal venous blood flow and a lesser increase in lactate. In glucose-loaded fasted animals (Fasted+Glc), the profile observed after LPS was similar to that in Fed animals. Tumor necrosis factor (TNF) release was dramatically amplified after glucose loading. Plasma NO variations (nitrites; NOx) did not parallel TNF release after LPS. These results suggest that the metabolic condition can markedly influence the cardiovascular modifications observed during LPS-induced inflammation.

MATERIALS AND METHODS

New Zealand White rabbits (Charles River), weighing 2.2 ± 0.2 (SD) kg, were studied in accordance with approved guidelines for the care and use of laboratory animals. The endotoxic shock model has been previously described (25) and was induced by an injection of 1,200 µg of a mixture of three types of LPS (Escherichia coli, Salmonella enteritidis, and Salmonella minnesota; 400 µg each; Sigma Chemical, St. Louis, MO) over 1 min through a marginal ear vein at the end of the surgical preparation.

Animal preparation. Anesthesia was induced by intravenous pentobarbital sodium (30 mg/kg) and was maintained by a continuous infusion (10 mg/h). Adequacy of anesthesia was assessed by monitoring heart rate and blood pressure responses to external noxious stimuli. After induction of anesthesia, animals were paralyzed (pancuronium bromide, 0.2 mg·kg$^\text{-1}·\text{h}^{-1}$), underwent tracheostomy, and were ventilated with 100% oxygen (Rodent Ventilator 638, Harvard Apparatus, Boston, MA). Tidal volume (7 ml/kg) at a respiratory rate of ~30 breaths/min was adjusted to maintain normocapnia. Apyrogen 0.9% saline solution with 0.2 meq/ml HCO$_3^-$ was continuously infused (4 ml·kg$^{-1}·\text{h}^{-1}$) throughout the experiment. The rectal temperature was maintained near 38°C by using a warming pad.

A 16-gauge catheter was inserted into the right carotid artery and connected to a pressure transducer (Abbott, North Chicago, IL) linked to a pressure monitor (CGR, Thomson Telco). A 20-MHz pulsed Doppler flow velocity probe [5.0 or 6.0 mm inner diameter (ID)] was positioned on the ascending aorta through a limited superior sternotomy. After a midline
20-MHz probes were placed around the proper hepatic artery (1.3 mm ID) and around the portal vein beneath its bifurcation (4.0 mm ID). The probes were connected to a multichannel directional pulsed Doppler flowmeter [Engineering Department of Baylor College of Medicine, Houston, TX (12)].

Hemodynamic parameters and calculations. Mean arterial pressure (MAP) and aortic, hepatic arterial, and portal venous blood flow velocities (ABVF, HABFV, and PVBVF, respectively; in cm/s) were continuously measured and recorded every 15 min on a paper graph monitor (Gould ES 1000). The use of conductance was preferred to resistance to assess peripheral vascular tone [aortic conductance (Gao)] because conductance best reflects in vivo the vascular tone modifications primarily due to changes in flow (17). Gao was calculated as the blood flow velocity to arterial pressure ratio (ABVF/MAP): Gao (cm s⁻¹ · mmHg⁻¹) = ABVF/MAP. Similarly, hepatic artery conductance (Gha) was calculated as HABFV/MAP.

Metabolic measurements. Plasma glucose and lactate levels (0.5-ml arterial blood samples collected in sodium fluoride/potassium oxalate-containing tubes) were measured by using a spectrophotometric technique (Ektachem C-700 Analyzer, Johnson & Johnson, Strasbourg, France). Arterial samples (0.3 ml heparinized blood) were collected at 30-min intervals for blood-gas analysis (Radiometer ABL-30, Copenhagen, Denmark).

TNF assay. In selected animals, blood samples were collected in pyrogen-free EDTA-containing glass tubes and centrifuged at 5°C. Plasma was stored at −40°C. Plasma TNF activity was measured by a specific in vitro cell cytotoxicity assay by using actinomycin-D-treated murine fibroblasts L-M cells (American Type Culture Collection, Rockville, MD). L-M cells were plated into 96-well microtiter plates at 7 × 10⁴ cells (150 µl) per well and incubated 24 h at 37°C in 5% CO₂. Fifty microliters of medium containing 10 mg/ml actinomycin-D were then added to all wells, and the cells were incubated for 2 h. Recombinant human TNF-α (sp act: 3 × 10⁶ U/mg) was diluted into medium standards. Two hundred microliters of standards or samples (4-fold diluted plasma) were pipetted in duplicate into the first column of wells, then serially diluted across the plate, and cells were incubated for 24 h. Cytotoxicity was detected by a tetrazolium dye technique. The plates were read at 570 nm on a microtiter plate reader (model 650, Dynatech Laboratories, Alexandria, VA) against n-propyl alcohol blanks. A standard curve relating cell cytotoxicity to doses of recombinant human TNF-α was used to quantify TNF activity in the samples. The sensitivity of this bioassay had a range of 0.1–0.2 U/ml, corresponding to 300–600 pg/ml, and the coefficient of variation was less than 15%.

To assess a potential gradient of TNF through the liver, plasma concentrations of TNF were measured simultaneously in the carotid artery ([TNF]a), and in portal ([TNF]pv) and hepatic ([TNF]ha) veins. Hepatic vein sampling was performed via a catheter retrogradely and manually pushed toward the intrahepatic portion of the hepatic vein. Sampling was performed slowly to limit contamination by peripheral venous blood. The total blood volume withdrawn for measurements (~10 ml) was exactly replaced by saline.

To evaluate a potential TNF release by the liver, TNF transhepatic gradient (THG_TNF) was calculated as

\[ \text{THG}_{\text{TNF}} = \frac{[\text{TNF}]_{\text{in}} - [\text{TNF}]_{\text{out}}}{[\text{TNF}]_{\text{out}}} \]

A negative THG_TNF reflects a net hepatic TNF release, and a positive THG_TNF reflects a net hepatic TNF uptake.

Liver fractional supply of TNF (U/ml) was calculated as

\[ \text{Arterial TNF fraction} = \frac{\dot{Q}_{ha}(\dot{Q}_{ha} + \dot{Q}_{pv})}{[\text{TNF}]_{a}} \]

\[ \text{Portal TNF fraction} = \frac{\dot{Q}_{pv}(\dot{Q}_{ha} + \dot{Q}_{pv})}{[\text{TNF}]_{pv}} \]

where hepatic artery and portal vein blood flow (Q̇ha and Q̇pv, respectively) were calculated as the direct product of the section (D²/4; in cm²) and the sectional velocity (V; in cm/s), where D corresponds to the inner diameter of the Doppler probe used, and [TNF] is TNF concentration. Thus TNF inflow corresponds to the sum of arterial and portal TNF fractions. The TNF outflow (from the liver) was the product of TNF concentration in the hepatic vein and total liver blood flow.

NOx/nitrate assay. NO release was assessed by the determination of plasma levels of NO derivatives NO₂ and NO₃ (i.e., NOx). Arterial and venous NOx plasma concentrations were determined by using an automated analyzer according to the method of Green (31) with minor modifications. The absorbance was spectrophotometrically measured at 546 nm. The data were analyzed in percentage of variations.

Study design. The modification of glucose availability was obtained by the following protocol. Three groups of animals were housed in individual cages in a controlled environment, with free water access. In the Fed group (n = 16), animals were normally fed with standard pellets (47.5% carbohydrates, 12% cellulose, 4% fat, 19% proteins). In the Fasted group (n = 11), animals were fasted for 24 h before initiation of experimental procedures. In a third group [Fasted+L-Glc; n = 11] and its control (Fasted+Glc control; n = 5), a 24-gauge catheter was inserted into an ileal vein to inject 8 ml of a 50% dextrose apyrogen solution over 30 min at the end of surgery (corresponding to 2 g Glc/kg). All groups except the latter one (Fasted+Glc control) received LPS. After a recovery period of 30 min after surgery, three consecutive hemodynamic measurements were performed over 30 min to assess the stability of the preparation. The observation period then covered 180 min after LPS or saline injection.

Hepatic glycogen content. To ensure that the protocol conditions had markedly influenced glucose metabolism, the hepatic glycogen content was measured in separate animals without LPS injection. Liver tissue samples (1.5–2.0 g) were obtained by the following protocol. Three groups of animals (n = 5) were normally fed with standard (47.5% carbohydrates, 12% cellulose, 4% fat, 19% proteins) pellets. After a 24-h desiccation at 30°C to determine dry-to-wet weight ratio (D/W%), whereas the other aliquot was freeze-dried by using liquid nitrogen and stored at −30°C for subsequent glycogen content measurement. After protein solubilization by using 40% KOH (100°C, 30 min) and overnight precipitation at 4°C by ethanol, the glycogen pellet was dissolved in 2N HCl and hydrolyzed to glucose (100°C, 3 h). Glucose content was then enzymatically determined (18), and the glycogen content was expressed as equivalent glucose per gram wet liver tissue weight (Glc/g wet wt).

Statistical analysis. Results are expressed as means ± SE (as %changes compared with reference value for hemodynamic parameters). Differences at time 0 between the different groups were tested by unpaired t-test. In glucose-supplied animals, paired t-tests were used to compare the acute modifications induced by glucose infusion. Comparisons over time between the groups after LPS or saline were performed by analysis of variance for repeated measures and one grouping factor. When the analysis was significant, comparisons of the mean values were tested by Scheffe’s test. Intragroup differences were tested by one-way analysis of variance for repeated measures. Differences with P < 0.05 were considered significant.
Table 1. Glycogen content and dry-to-wet ratio in liver samples from Fed and Fasted rabbit groups before and 30 min after end of intraportal infusion of 4 g glucose

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Glycogen, Glc/g wet liver tissue wt</th>
<th>D/W, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fed</td>
<td>404 ± 99</td>
<td>34 ± 2</td>
</tr>
<tr>
<td>Fasted</td>
<td>48 ± 24*</td>
<td>ND</td>
</tr>
<tr>
<td>Fasted + Glc</td>
<td>142 ± 40†</td>
<td>31 ± 1</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 3 rabbits/group. Glc, glucose; D/W, dry-to-wet wt ratio; Fed, normally fed rabbits; Fasted and Fasted + Glc: 24-h-fasted rabbits before and after infusion of 4 g glucose, respectively; ND, not done. *P < 0.05 compared with Fed (Mann-Whitney intergroup comparison). †P < 0.05 compared with Fasted group (Wilcoxon intragroup comparison).

RESULTS

Impact of nutritional regimen on pre-LPS variables. Compared with normally fed animals, in Fasted animals liver glycogen store was drastically depleted (Table 1, P < 0.01) with lower pH, HCO₃⁻, and lactate levels (Table 2, P < 0.05), but glycemia and hemodynamic variables were similar. Thirty minutes after the end of glucose infusion in Fasted animals, hepatic glycogen content increased (Table 1, P < 0.05), with hyperglycemia (P < 0.01), lactate, and arterial Pco₂ (PaCO₂) increased (P < 0.05), leading to a pH decrease (Table 2, P < 0.05). ABFV and PVBFV also increased after glucose load, with no change in HABFV (Table 2).

Hemodynamic and metabolic parameters after LPS challenge. The LPS-induced hypotension was comparable in the three groups over the 180-min observation time but with different flow patterns (Figs. 1 and 2). In Fed animals, ABFV decreased after LPS injection to such a magnitude (−40%; P < 0.05) that Gao was also reduced (P < 0.05). PVBFV decreased in parallel with systemic flow (−40%; P < 0.05) after LPS, whereas HABFV remained unchanged after an initial transient increase. In Fasted animals, ABFV was unchanged after LPS, and Gao tended to increase, although the significance level was not reached. In these animals, compared with the Fed group, ABFV and Gao were significantly higher in relation to a systemic vasodilatation. As observed for systemic flow, PVBFV did not change, whereas the decrease in HABFV (−70%, P < 0.05) was more pronounced than in the Fed group, with a decreased Gha accounting for a local vasoconstriction.

Metabolic variables were also modified after LPS injection. Both glucose and lactate levels increased in Fed animals, ABFV decreased after LPS injection. Both glucose and lactate levels increased in

Table 2. Comparison of absolute baseline values for hemodynamic and metabolic parameters in Fed and Fasted rabbits

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>MAP, mmHg</th>
<th>ABFV, cm/s</th>
<th>Gao, cm/s · mmHg⁻¹</th>
<th>PVBFV, cm/s</th>
<th>HABFV, cm/s</th>
<th>Gha, cm/s · mmHg⁻¹</th>
<th>[La], mmol/l</th>
<th>[Glc], mmol/l</th>
<th>pH</th>
<th>PaCO₂, Torr</th>
<th>PaO₂, Torr</th>
<th>HCO₃⁻, mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fed</td>
<td>85 ± 3</td>
<td>16 ± 1</td>
<td>0.17 ± 0.03</td>
<td>16 ± 4</td>
<td>26 ± 2</td>
<td>0.29 ± 0.05</td>
<td>4.4 ± 0.9</td>
<td>7.5 ± 0.3</td>
<td>7.46 ± 0.02</td>
<td>38 ± 3</td>
<td>511 ± 24</td>
<td>269 ± 2.3</td>
</tr>
<tr>
<td>Fasted</td>
<td>83 ± 6</td>
<td>16 ± 2</td>
<td>0.23 ± 0.06</td>
<td>20 ± 5</td>
<td>32 ± 4</td>
<td>0.37 ± 0.07</td>
<td>1.8 ± 0.3†</td>
<td>7.0 ± 0.7</td>
<td>7.37 ± 0.03†</td>
<td>36 ± 2</td>
<td>457 ± 35</td>
<td>20.5 ± 2.4†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5 rabbits/group. MAP, mean arterial pressure; ABFV, PVBFV, and HABFV: ascending aorta, portal vein, and hepatic artery blood flow velocities, respectively; Gao and Gha, aortic and hepatic arterial conductances, respectively; [La] and [Glc], arterial lactate and glucose concentrations, respectively; pH, arterial Pco₂ (PaCO₂) and PaO₂ (PaO₂), respectively, and HCO₃⁻: blood-gas measurements. *P < 0.05; †P < 0.01 (unpaired t-test).
tate levels slightly increased (P < 0.05) but remained lower than in Fed animals (Fig. 3A).

In Fasted animals receiving a glucose load (i.e., Fasted+Glc), LPS challenge induced different patterns compared with the two other groups. For a similar hypotension, the decrease in ABFV from control (−60%, P < 0.05) was of larger magnitude than in the Fed group (P < 0.05) and, consequently, than in the Fasted group (P < 0.01). However, PVBFV and HABFV variations were comparable to those observed in the Fed group.

Figure 3B shows the plasma glucose and lactate variations, with or without LPS, in Fasted+Glc animals. Surprisingly, LPS injection did not influence the evolution of glycemia, whereas a higher lactate level after LPS (P < 0.01) was reached at 180 min. In the Fasted+Glc group, LPS decreased pH, with hypercapnia, and increased plasma lactate, but with unchanged HCO₃⁻ levels (Table 3), thus corresponding to a mixed metabolic and respiratory acidosis. After 180 min, these modifications were similar to those observed in the Fed group.

Inflammation after LPS injection. Baseline TNF levels were detectable but low and were similar in all groups (Fig. 4). However, THG_TNF suggested a prominent participation of the liver in circulating TNF only in Fed animals (Fig. 5).

In Fed and Fasted groups, LPS injection increased arterial plasma TNF levels to a similar extent, with a 1-h early peak, followed by a gradual decrease to control values over 3 h. Compared with results in the two other groups, pre-LPS TNF levels were similar in the Fasted+Glc group but dramatically increased after LPS (Fig. 4). The peak value was fourfold greater (P < 0.01) than those observed in the other groups, with a similar concentration decay. No significant difference in plasma TNF levels was observed according to the sampling sites (arterial, portal, and hepatic venous). After LPS, the hepatic production of TNF was undetectable in all groups.

Arterial NOx concentrations were lower (P < 0.05) in normally Fed (15.0 ± 2.1 µmol/l) than in Fasted (32.4 ± 12.5 µmol/l) and in Fasted+Glc groups (54.0 ± 12.1 µmol/l). However, 180 min after LPS, NOx significantly increased in the Fed group to 46.1 ± 13.9 µmol/l (+300%, P < 0.05, Fig. 6). In the two remaining groups, 180 min after LPS, NOx levels did not change and were comparable to those in the Fed group. NOx concentrations and kinetics in portal and hepatic veins did not differ from those observed in the arterial site.

DISCUSSION

In this in vivo study, a glucose regimen significantly modified the hemodynamic, metabolic, and inflammatory responses to LPS. For a similar hypotension, and despite opposite changes in liver flows in Fed and Fasted animals, amplitude and kinetics of TNF release and plasma NOx levels were similar. However, in Fasted+Glc animals, TNF release was explosive and associated with a marked systemic vasoconstriction. These results apparently contradict the usual observation of a reduced mortality rate with glucose load or supply in severe experimental sepsis (7, 13). However, the type of fluid resuscitation performed was not detailed, and the observed improvement in hemodynamics and survival rate was mainly related to the correction of the hypoglycemia accompanying a severe sepsis. In our study, hypoglycemia never occurred, even in the Fasted group.

It is difficult to definitively conclude that fasting or reduction in glucose supply limits the severity of septic shock in this short-term (<4 h) model with a low spontaneous mortality. However, admitted criteria for sepsis severity, such as high levels of lactate, increased PaO₂/FiO₂, acidosis (4), low systemic and liver blood flows, and high TNF release (28), were significantly improved by fasting and deteriorated with glucose load.
Critique of the model. To ensure reproducible results, animals were anesthetized, and this per se might have impaired cardiovascular (29) and metabolic (22) responses to sepsis. Animals were ventilated with 100% inspiratory O2 fraction to prevent any risk of hypoxemia because it is frequently done clinically. Fluid-loading and/or vasoactive drugs were not administered because catecholamines, especially epinephrine, interfere with glucose metabolism. One of the limitations of the Doppler technique used is that variations in vessel diameter could not be measured. However, this technique using Doppler velocity measurements and diameter of the probe used for calculated flows is adequate in such small vessels during similar conditions (12, 25).

This unresuscitated model of shock after a bolus injection of LPS allowed us to evaluate relatively selectively the impact of glucose metabolism. Because of the long surgical preparation with careful dissection before LPS injection, control values might have been influenced by surgical stresses. A nonspecific inflammation might account for the detectable plasma TNF level, and also for elevated lactate levels without lactic acidosis or hypoxia, seen in Fed animals. This hyperlactatemia was not observed in 24-h Fasted animals, a duration that appeared sufficient to deplete liver glycogen stores. Consequently, a relative energetic deficit may have stimulated, first, gluconeogenesis with lactate uptake, and, second, fatty acid oxidation, leading to an overproduction of ketone bodies. In Fasted animals, the observed metabolic acidosis with normal lactate concentrations before LPS is consistent with this hypothesis.

The protocol conditions should also be discussed. Fasting concerned substrates other than glucose alone, such as proteins and lipids, potentially interfering with the observed results. Because the data obtained in the Fasted + Glc group resembled those observed in the Fed group, we concluded that at least glucose metabolism modifications could explain the results. Metabolic data such as PaCO2 and lactate and glycogen kinetics were in accordance with this hypothesis.

The glucose load administered via the portal route, although large, was similar to the doses used for oral glucose tolerance tests in clinical practice (75-g oral glucose intake, i.e., 1–1.5 g/kg), and in the same range as those used to test glycogen synthesis (21). The portal route was chosen to control the quantity delivered to the liver.

Table 3. Absolute values of hemodynamic and metabolic parameters in Fasted rabbits during pre- and post-glucose-infusion conditions

<table>
<thead>
<tr>
<th>Condition</th>
<th>MAP, mmHg</th>
<th>ABFV, cm/s</th>
<th>Gao, cm·s⁻¹·mmHg⁻¹</th>
<th>PVBFV, cm/s</th>
<th>HABFV, cm/s</th>
<th>Gha, cm·s⁻¹·mmHg⁻¹</th>
<th>[La], mmol/l</th>
<th>[Glc], mmol/l</th>
<th>pH</th>
<th>PaCO₂, Torr</th>
<th>PaO₂, Torr</th>
<th>HCO₃⁻, mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-glucose infusion</td>
<td>81±3</td>
<td>18±2</td>
<td>0.21±0.04</td>
<td>15±3</td>
<td>16±3</td>
<td>0.21±0.03</td>
<td>1.9±0.2</td>
<td>6.9±0.3</td>
<td>7.36±0.02</td>
<td>35±2</td>
<td>469±18</td>
<td>21.6±0.9</td>
</tr>
<tr>
<td>Post-glucose infusion</td>
<td>84±2</td>
<td>21±20*</td>
<td>0.24±0.03</td>
<td>23±5*</td>
<td>17±3</td>
<td>0.20±0.03</td>
<td>3.0±0.5*</td>
<td>33.9±1.7†</td>
<td>7.31±0.01†</td>
<td>41±2*</td>
<td>395±28</td>
<td>20.5±0.8</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 10 rabbits/condition. Pre-glucose infusion, baseline. *P < 0.05; †P < 0.01 (paired t-test).
the liver and mimic oral glucose loading. The intraportal injection would therefore have induced similar consequences to those for the oral route, especially considering hormonal release (14). Hyperglycemia induced by this glucose load was “unphysiological” for the peak level reached, but it rapidly decreased to values comparable to those of the Fed group during the observation period. In Fasted animals, glucose loading led to a rapid hepatic glycogen formation within 30 min, consistent with the literature (20). This synthesis was accompanied by an increased glycogenolysis, as suggested by the increase in lactate and CO₂ production (at a constant ventilation), with an increased ABFV and portal vein blood flow that might be interpreted as an equivalent of postprandial hyperemia.

Hemodynamic modifications. In the absence of any fluid resuscitation, LPS injection induced a similar hypotension in the three groups. In Fed animals, however, a low ABFV and systemic vasoconstriction with a decreased PVBFV were observed. The portal vein drains the splanchnic area and thus depends on systemic hemodynamics because autoregulation efficiency is negligible in this territory (25). The trend for HABFV was similar in Fed and Fasted + Glc animals: after a transient increase in HABFV in accordance with the hepatic arterial buffer response (16), the final level was close to control or slightly decreased in Fasted + Glc animals. In Fasted animals, HABFV was not maintained, but decreased after LPS. Because PVBFV was at least maintained, the observed hepatic arterial vasoconstriction was unlikely to result from the arterial buffer response but rather was a multifactorial consequence of LPS administration.

Similar hemodynamic patterns have been described in different models of septic shock in absence of any fluid resuscitation (6, 8). Among the possible mechanisms involved, glucose metabolism and/or elevated plasma glucose concentration may play a role because a pronounced vasoconstriction was observed in acutely glucose-loaded animals, whereas a vasodilation was present after 24-h fasting. Although we did not measure insulin levels, one could reasonably assume that insulin released in response to a high plasma glucose level would have negligible hemodynamic effects because a vasodilatation rather than a vasoconstriction would be expected (2).

Other mechanisms could account for the observed effects of changes in glucose metabolism in this study. In nonseptic animals, glucose can impair endothelium-dependent relaxation in vitro (27), in relation to synthesis of vasoconstricting prostanoids and increased endothelium-generated oxygen-reactive species (ORS). In our study conditions, it is tempting to relate glycemia and systemic conductance: in the Fasted group, the absence of hyperglycemia after LPS was associated

Fig. 4. Arterial (A), portal (B), and hepatic venous (C) kinetics of plasma tumor necrosis factor (TNF) in LPS-treated rabbits in Fed, Fasted, and Fasted + Glc groups. Values are means ± SE. Symbols are defined as in Fig. 1. **P < 0.01 for intergroup comparisons (2-way ANOVA for repeated measures).

Fig. 5. Evolution of transhepatic gradient of TNF after intravenous LPS injection (T0) in Fed (open bars), Fasted (hatched bars), and Fasted + Glc rabbits (solid bars). Values are means ± SE. *P < 0.05 for intergroup comparisons (Mann-Whitney).

Fig. 6. Evolution of plasma nitrates (NOx) after intravenous LPS injection (T0) in Fed, Fasted, and Fasted + Glc rabbits. Values are means ± SE. Symbols are defined as in Fig. 1. †P < 0.05 for intragroup comparisons (Wilcoxon). *P < 0.05 for intergroup comparisons (Mann-Whitney).
with a systemic vasodilatation, whereas in the two other groups, particularly in Fasted+Glc animals, the increased glycemia after LPS paralleled the amplitude of systemic vasconstriction, an observation consistent with the ex vivo effect of glucose on blood vessels. Second, it has been shown that high extracellular glucose concentration impairs the endothelial release of NO, which partly mediates the alteration of the vascular smooth muscle cell Na⁺-K⁺-adenosinetriphosphatase pump, responsible for an impairment of vascular relaxation (11). Plasma NOx are thought to be a reliable index of the NO generation in vivo during sepsis (10) and might reflect the balance between production and consumption of NO. A deficit in NO release during hyperglycemic situations was not observed in the present study because plasma NOx levels did not differ among the three groups after LPS. However, animals from the Fed group had a significantly lower baseline concentration of NOx than in the Fasted groups, possibly because of an interaction with ORS (15). One could speculate that the Fed animals produced higher levels of ORS in relation to a modification of glucose metabolism because of surgical preparation and stresses, explaining lower NOx control values. Interestingly, after LPS in Fasted animals, NOx levels increased to a larger magnitude to reach a plateau within 30 min, followed by a 1-h peak, and a fall thereafter. TNF concentrations were observed, with a rapid increase within 30 min, followed by a 1-h peak, and a fall during the following 2 h. The most striking result concerned the large increase in TNF release observed in Fasted animals receiving intraportal glucose because TNF peak was fourfold higher and remained significantly increased compared with the two other groups. The comparison of the TNF response in the two Fasted groups would support an activation of this TNF release in connection with glucose metabolism. After glucose loading, hepatic glycogen stores were partially restored, and plasma glucose levels were higher than in the fasted condition and this glucose availability would be responsible for this higher amplitude of the immunoinflammatory response. During sepsis, tissue glucose uptake is enhanced, especially in immunocompetent organs or cells such as macrophages and granulocytes that increase ex vivo glucose incorporation after LPS stimulation (19). Moreover, the amplitude of IL-1β release ex vivo by human endotoxin-stimulated monocytes is positively related to the glucose concentration in the culture medium (23). These findings are in accordance with our observations in vivo, suggesting a potential modulation of the immunoinflammatory response by carbohydrates.

We failed to demonstrate in this study a particular implication of the liver in the TNF release. No differences among arterial, portal, and hepatic venous TNF levels were observed after LPS, possibly because the marked stimulation of the entire immune system might have masked a specific contribution of the liver, an organ that both produces and clears TNF (5). However, Fed animals had a THG T7NF level that indicated a net hepatic release of TNF in Fed animals, one might speculate that this hepatic production was less efficient in Fasted animals, possibly in relation to energy-sparing mechanisms during a situation of reduced hepatic glycogen stores.

Inflammatory response. Classic kinetics of arterial TNF concentrations were observed, with a rapid increase within 30 min, followed by a 1-h peak, and a fall during the following 2 h. The most striking result concerned the large increase in TNF release observed in Fasted animals receiving intraportal glucose because TNF peak was fourfold higher and remained significantly increased compared with the two other groups. The comparison of the TNF response in the two Fasted groups would support an activation of this TNF release in connection with glucose metabolism. After glucose loading, hepatic glycogen stores were partially restored, and plasma glucose levels were higher than in the fasted condition and this glucose availability would be responsible for this higher amplitude of the immunoinflammatory response. During sepsis, tissue glucose uptake is enhanced, especially in immunocompetent organs or cells such as macrophages and granulocytes that increase ex vivo glucose incorporation after LPS stimulation (19). Moreover, the amplitude of IL-1β release ex vivo by human endotoxin-stimulated monocytes is positively related to the glucose concentration in the culture medium (23). These findings are in accordance with our observations in vivo, suggesting a potential modulation of the immunoinflammatory response by carbohydrates.

We failed to demonstrate in this study a particular implication of the liver in the TNF release. No differences among arterial, portal, and hepatic venous TNF levels were observed after LPS, possibly because the marked stimulation of the entire immune system might have masked a specific contribution of the liver, an organ that both produces and clears TNF (5). However, Fed animals had a THG T7NF level that indicated a net hepatic release of TNF, which was not observed in Fasted animals. Whatever the stimuli responsible for a net hepatic release of TNF in Fed animals, one might speculate that this hepatic production was less efficient in Fasted animals, possibly in relation to energy-sparing mechanisms during a situation of reduced hepatic glycogen stores.

Metabolic response. LPS-induced variations in plasma glucose should be analyzed in conjunction with lactate levels and PaCO₂ (Table 4). In the Fed group, the increases in glucose and lactate levels were associated with a hypercapnia, generating a mixed acidosis. In the Fasted group, glucose levels and PaCO₂ did not change after LPS. The Fasted+Glc group had similar patterns to those in Fed animals. Glycogenolysis in the Fed group would be activated after LPS (26), leading to an overproduction of lactate and CO₂, implying a sufficient intrahepatocyte storage of glycogen. As observed after 24-h fasting, hepatic glycogen was largely decreased, reducing the efficiency of glycogenolysis, consistent with stable and physiological glucose and PaCO₂ levels after LPS. The pathophysiological mechanisms responsible for the observed hyperlactatemia need further investigations to differentiate an overproduction from an impairment of hepatic metabolism of lactate.

Table 4. Arterial blood-gas measurements in Fed, Fasted, Fasted+Glc groups at T0 and 180 min after intravenous LPS injection

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>n</th>
<th>pH</th>
<th>PaCO₂</th>
<th>PaO₂</th>
<th>HCO₃⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fed T0</td>
<td>10</td>
<td>7.46 ± 0.02</td>
<td>38 ± 3</td>
<td>511 ± 24</td>
<td>26.9 ± 2.3</td>
</tr>
<tr>
<td>180 min</td>
<td>7.22 ± 0.06†</td>
<td>48 ± 7</td>
<td>452 ± 40</td>
<td>18.8 ± 1.7†</td>
<td></td>
</tr>
<tr>
<td>Fasted T0</td>
<td>5</td>
<td>7.37 ± 0.03*</td>
<td>36 ± 4</td>
<td>457 ± 35</td>
<td>20.5 ± 2.4*</td>
</tr>
<tr>
<td>180 min</td>
<td>7.31 ± 0.03*</td>
<td>36 ± 4</td>
<td>460 ± 20</td>
<td>18.6 ± 2.0</td>
<td></td>
</tr>
<tr>
<td>Fasted+Glc T0</td>
<td>5</td>
<td>7.40 ± 0.02</td>
<td>34 ± 3</td>
<td>478 ± 26</td>
<td>19.5 ± 0.9*</td>
</tr>
<tr>
<td>180 min</td>
<td>7.20 ± 0.02†</td>
<td>49 ± 4</td>
<td>398 ± 38</td>
<td>18.5 ± 1.3†</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rabbits. T0, time 0. LPS, lipopolysaccharide (600 µg/kg). *P < 0.05 compared with Fed group [intergroup analysis of variance (ANOVA)]. †P < 0.05 compared with T0 (intragroup ANOVA).
During sepsis, a relative insulin resistance–impairing peripheral glucose uptake (26) has been shown, in addition to a hyperglycogenolysis. Interestingly, the kinetics of plasma glucose after intraportal glucose load did not differ between endotoxic and normal animals, suggesting, at least in this model, that peripheral glucose uptake is not influenced at the early phase of endotoxic shock. However, lactate kinetics differed: during the first 60 min, plasma lactate levels increased similarly in both groups, but lactate levels further increased only in endotoxic animals, suggesting additional mechanisms for this increase. The initial phase may result from activation of glycolysis in relation to substrate overload. The difference in the second phase might be related to mechanisms induced by LPS injection such as an overstimulation of glycolgenolysis (although glyceria and $\text{Ca}^{2+}$ were identical in both septic and control groups) and/or an impairment of lactate uptake.

In conclusion, glucose metabolic status seems to influence systemic and hepatic hemodynamic, inflammatory, and metabolic responses to endotoxin. This concept may have clinical implications for a discussion in both groups, but lactate levels further increased only in endotoxic animals, suggesting additional mechanisms for this increase. The initial phase may result from activation of glycolysis in relation to substrate overload. The difference in the second phase might be related to mechanisms induced by LPS injection such as an overstimulation of glycolgenolysis (although glyceria and $\text{Ca}^{2+}$ were identical in both septic and control groups) and/or an impairment of lactate uptake.

REFERENCES


