Infection impairs insulin-dependent hepatic glucose uptake during total parenteral nutrition

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Donmoyer, Christine M., Sheng-Song Chen, D. Brooks Lacy, David A. Pearson, Adrian Poole, Yiqun Zhang, and Owen P. McGuinness. Infection impairs insulin-dependent hepatic glucose uptake during total parenteral nutrition. Am J Physiol Endocrinol Metab 284: E574–E582, 2003. First published November 19, 2002; 10.1152/ajpendo.00035.2002.—Total parenteral nutrition (TPN) markedly augments hepatic net glycogenolysis (NHGU) and hepatic glucose uptake (HGCU). The hyperglycemia or hyperinsulinemia present during TPN is associated with continued hepatic glycogenolysis (14). Given the insulin-dependent hepatic glucose uptake (HGU) in the overnight-fasted setting, the liver is very responsive to insulin. In the presence of hyperglycemia (~200 mg/dl) a sixfold increase in insulin increased liver glucose uptake sixfold (30). This response to insulin occurs in isolated perfused liver and in vivo (30). It is also sensitive to insulin; hepatic insulin sensitivity (ED50) in a 42-h-fasted dog is 90 μU/ml (sinusoidal insulin levels). However, given the upregulation of liver glucose uptake by TPN, the liver’s response to insulin may be altered. Although insulin levels are relatively low in this adapted setting, the liver may not be responsive to further increases in insulin.

An infection impairs the liver’s adaptation. Both NHGU and net hepatic lactate release (NHLR) are decreased with infection (14). Consequently, peripheral tissues must directly metabolize a larger fraction of the exogenous glucose. However, the underlying peripheral insulin resistance characteristic of infection exacerbates the problem. As a consequence, a combination of hyperinsulinemia and hyperglycemia is required to facilitate glucose disposal. The mechanism for the impairment in NHGU with infection is unknown.

Given the hyperinsulinemia that accompanies infection, hepatic insulin resistance may be a contributing factor to the impairment in liver glucose uptake. Insulin resistance in peripheral tissues is widely recognized during infection (8, 10, 18), but the extent of involvement of the liver is controversial. Although hepatic glucose production (HGP) is elevated, it is not resolved whether or not infection impairs the ability of insulin to suppress HGP (9, 16). Liver glucose uptake is clearly enhanced ability of the liver to take up glucose and convert it to lactate allows the body to dispose of substantial quantities of glucose without marked hyperglycemia or hyperinsulinemia.

It is known whether the TPN-adapted liver is as responsive and sensitive to changes in insulin as the non-TPN-adapted liver. In the overnight-fasted setting, the liver is very responsive to insulin. In the presence of hyperglycemia (~200 mg/dl) a sixfold increase in insulin increased liver glucose uptake sixfold (20). It is also sensitive to insulin; hepatic insulin sensitivity (ED50) in a 42-h-fasted dog is 90 μU/ml (sinusoidal insulin levels). However, given the upregulation of liver glucose uptake by TPN, the liver’s response to insulin may be altered. Although insulin levels are relatively low in this adapted setting, the liver’s sensitivity to insulin may also have increased (30). If the mild hyperinsulinemia seen during TPN produces a near-maximal response to insulin, the liver may not be responsive to further increases in insulin.

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Impaired by infection; whether this is due to an impairment in insulin action has not been examined. Moreover, tight metabolic control with aggressive use of insulin profoundly improves the clinical outcome (25). Yet it is unclear whether the aggressive use of insulin corrects the impairment in NHGU or glycolysis, or diverts the glucose carbon to peripheral tissues.

The aims of the present study were to determine 1) whether NHGU in the TPN-adapted setting is responsive to increases in insulin, 2) whether the presence of an infection alters this response, and 3) whether additional insulin can reverse the infection-induced derangements in hepatic metabolism. Therefore, we established an insulin dose-response curve to evaluate the response of NHGU to changes in insulin in sham and infected animals receiving chronic TPN. Tracer and arteriovenous difference methods were used to assess unidirectional liver glucose uptake and production, NHLR, and net hindlimb substrate balance.

METHODS

Animal preparation. Ten female mongrel dogs (18–24 kg) were fed a standard meat (Pedigree; Kalkan, Vernon, CA) and chow (Purina Lab Canine no. 5006; Purina Mills, St. Louis, MO) diet once daily and had free access to water. The composition of the diet based on dry weight was 52% carbohydrate, 31% protein, 11% fat, and 6% fiber. Dogs were housed in a facility that met the guidelines of the Association for the Accreditation of Laboratory Animal Care International. The protocols were approved by the Vanderbilt University Medical Center Animal Care Committee. Good health of the animals was determined before surgery and before TPN administration as having a good appetite, i.e., consumed at least three-quarters of the daily ration, normal stools, hematocrit >0.35, and leukocyte count <18,000 mm$^{-3}$.

Experimental preparation. As described previously (3), a laparotomy was performed under general anesthesia. Infusion catheters (Dow Corning, Midland, MI) were placed in the splenic and jejunal veins for intraportal hormone infusion and into the inferior vena cava (IVC) for TPN infusion. Sampling catheters were positioned in the portal vein, left common hepatic vein, left common iliac vein, and right external iliac artery. Flow probes (Transonic Systems, Ithaca, NY) were positioned around the portal vein, hepatic artery, and right external iliac artery.

All catheters were filled with 0.9% NaCl (saline) containing heparin (200 U/ml). The free ends of the catheters and flow probes were placed in subcutaneous pockets. Dogs received penicillin G (600,000 U) in saline intravenously during surgery. In addition, they received 1,000 mg of ampicillin (Principen; Bristol-Myers Squibb, Princeton, NJ) orally per day for 3 days. Flunixin (1 mg/kg body wt; Fort Dodge Laboratory, Fort Dodge, IA) was injected intramuscularly immediately after wound closure for acute pain relief.

Nutritional support. After ≥14 days were allowed for recovery from surgery, the IVC catheter was exteriorized under local anesthesia (2% Lidocaine; Abbott, North Chicago, IL). TPN was infused into one catheter continuously for 5 days using an ambulatory infusion pump (Dakmed, Buffalo, NY). The dog wore a jacket (Alice King Chatham, Los Angeles, CA) with two large pockets for the nutrition and pump. The TPN was designed to be isocaloric on the basis of predicted resting energy expenditure (kcal/day), calculated with the equation 144 + 62.2 × body weight (24). The TPN included glucose (75% of the nonprotein calories (NPC)), Intralipid (25% of NPC), Travalos, saline, potassium phosphate, and a multivitamin supplement, as described previously (14).

Induction of infection. On the 3rd day of TPN administration, a second laparotomy was performed under anesthesia. An abdominal midline incision was made at a point below that made during the previous surgery. Dogs were randomly assigned to two groups undergoing implantation of either a sterile (SHAM, n = 5) or bacterial (INF, n = 5) fibrin clot in the peritoneal cavity. TPN was continued, and animals received saline during the laparotomy (500 ml) as well as on the next day.

The fibrin clot was prepared by filtering (0.45 μm) a 1% fibrinogen (Sigma, St. Louis, MO) solution under sterile conditions. The bacterial clot contained a nonlethal dose (2 × 10$^9$ organisms/kg body wt) of Escherichia coli determined by serial dilution followed by plating. Bacteria (American Type Tissue Culture no. 25922) were prepared by inoculation of 1 liter of Trypticase soy broth (Becton Dickinson, Cockeysville, MD) and incubation overnight at 37°C. Bacteria were pelletted by centrifugation on the next day, washed, and resuspended in sterile saline before addition to the filtrate. To initiate clot formation, thrombin (1,000 U; Gentrac, Middleton, WI) was added to the filtrate.

Experimental protocol. A study was performed on the 5th day of TPN and 42 h after clot implantation. Free ends of all catheters were exteriorized under local anesthesia, and their contents were aspirated and flushed with saline. Leads from the flow probes were also exteriorized and connected to an Ultrasonic flowmeter. The dog was placed in a Pavlov harness for the duration of the study. Angiocaths (18 gauge; Abbott) were inserted into both cephalic veins for infusion of radioactive tracers, glucose, and somatostatin (SRIF; Bachem, Torrance, CA). Blood pressure and heart rate (Micro-Med, Louisville, KY) and rectal temperature (Yellow Springs Instruments, Yellow Springs, OH) were assessed during the study.

The chronic TPN solution was replaced with a TPN solution not containing glucose. The glucose was infused using a separate pump, allowing the infusion rate of glucose to be adjusted to maintain isoglycemia while not altering the infusion rate of the other TPN components. At ~120 min, priming doses (42 and 21 μCi of $[3-{ }^2H]$ and $[U-{ }^{13}C] $ glucose (New England Nuclear, Wilmington, DE) were injected. Both tracers were mixed with the infused dextrose (1.6 and 0.8 μCi/ml), allowing the glucose infusion rate to be varied while changes in arterial glucose specific activity (SA) were minimized. In some studies, additional tracer was infused at a progressively decreasing rate to account for the decrease in endogenous glucose production when the insulin infusion rate was increased.

SRIF (0.8 μg·kg$^{-1}$·min$^{-1}$) was infused into a peripheral vein to suppress endogenous pancreatic hormone secretion. Insulin (Regular Iletin II; Eli Lilly, Indianapolis, IN) and glucagon (Eli Lilly) were replaced by intraportal infusion via the splenic and jejunal veins. In the SHAM group, glucagon was infused at a rate of 0.1 ng·kg$^{-1}$·min$^{-1}$, whereas the INF group received a glucagon infusion rate of 2.5 ng·kg$^{-1}$·min$^{-1}$ to match the higher concentration seen during infection (3).

Both groups received identical insulin doses in three sequential 120-min periods: Lo-Ins (400 μU·kg$^{-1}$·min$^{-1}$; 120–0 min), Mid-Ins (1,000 μU·kg$^{-1}$·min$^{-1}$; 0–120 min), and Hi-Ins (2,000 μU·kg$^{-1}$·min$^{-1}$; 120–240 min). All infusions were performed with calibrated syringe pumps (Harvard Apparatus, Holliston, MA).

Blood samples from the iliac artery, portal vein, hepatic vein, and iliac vein were drawn every 20 min (e.g., ~40, ~20,
and 0 min during Lo-Ins) during the final 40 min for each of the three insulin infusion periods. Small blood samples were obtained every 5 min to measure arterial glucose concentration. Blood flows and hematocrit were recorded at each sampling point. Saline was infused to replace blood volume withdrawn by sampling. At the end of the study, the animals were killed with an overdose of pentobarbital sodium (Veterinary Lab, Lenexa, KS). Tissue samples from each of the seven lobes and the adductor magnus et brevis muscle were freeze-clamped with Wallenburg clamps precooled in liquid nitrogen and stored at −70°C. The entire liver was removed and weighed.

Sample processing and analysis. Blood samples were collected and processed on the day of the study as previously described (12). Whole blood perchloric acid extracts were analyzed for lactate, alanine, and glyceraldehyde on an automated centrifugal analyzer (Monarch 2000; Instrumentation Laboratory, Lexington, MA) (11). Whole blood amino acids were assayed using HPLC techniques (26). Blood glutamine content was measured with a Technicon Autoanalyzer II (Bran Luebbe, Buffalo Grove, IL), adapted from Bernt and Bergmeyer (19), and nonesterified fatty acid (NEFA; Wako Chemicals, Richmond, VA) concentrations. Plasma collected from whole blood treated with EGTA and glutathione was analyzed for epinephrine and norepinephrine with HPLC techniques [coefficients of variation 11 and 6%, respectively] (7). Hepatic glycogen content was determined using the enzymatic method of Chan and Exton (2).

Calculations. The substrate (glucose, lactate, amino acids, glyceraldehyde, and NEFA) and hormone (insulin) load entering the liver was calculated as the sum of the loads in the hepatic artery and portal vein, \( A_i \times HABF + (P_i - P_B) \times PBPF \), where \( A_i \) and \( P_i \) represent substrate concentrations in the artery and portal vein and HABF and PBPF represent blood flows in the hepatic artery and portal vein. Similarly, the substrate load leaving the liver equaled \( H_e \times THBF \), in which \( H_e \) and \( THBF \) represent the hepatic vein substrate concentration and total hepatic blood flow (HABF + PBPF). The net hepatic substrate uptake rate was calculated as the difference between the entering and exiting substrate loads. In cases where the liver was a net substrate producer, output was denoted as a positive value. Similarly, net hindlimb and gut substrate uptake rates were calculated using the equations (As – Ps) / H1003 / H11003 instead of blood flows. The factor 0.8 in the equation accounts for the ratio of hepatic arterial blood flow (HABF) to perfusion (PPF), where As and Ps represent substrate concentrations in the arterial and portal veins, respectively. Portal venous blood flows were comparable and were not altered with insulin infusion (18 ± 2, 18 ± 2, and 19 ± 2 vs. 18 ± 4, 19 ± 4, and 18 ± 4 ml·kg⁻¹·min⁻¹ in SHAM vs. INF, respectively).

Hormones. Insulin infusion of insulin achieved comparable circulating insulin concentrations in SHAM and INF at each insulin dose (Fig. 1). Arterial plasma insulin concentrations in the Lo-Ins, Mid-Ins, and Hi-Ins periods (SHAM: 8 ± 1, 9 ± 1, and 9 ± 2 ml·kg⁻¹·min⁻¹, respectively) were significantly greater with infection (INF: 21 ± 4, 21 ± 3, and 23 ± 2 ml·kg⁻¹·min⁻¹, respectively). Portal venous blood flows were comparable and were not altered with insulin infusion (18 ± 2, 18 ± 2, and 19 ± 2 vs. 18 ± 4, 19 ± 4, and 18 ± 4 ml·kg⁻¹·min⁻¹ in SHAM vs. INF, respectively).

### Results

**Baseline variables.** Body temperature and heart rate were elevated in INF, but mean arterial pressure, body weight, and liver weight were comparable to those of SHAM (Table 1). Plasma glucose levels were 108 ± 6 vs. 118 ± 5 mg/dl before initiation of the somatostatin infusion. Hepatic arterial blood flows in Lo-Ins, Mid-Ins, and Hi-Ins periods (SHAM: 8 ± 1, 9 ± 1, and 9 ± 2 ml·kg⁻¹·min⁻¹, respectively) were significantly greater with infection (INF: 21 ± 4, 21 ± 3, and 23 ± 4 ml·kg⁻¹·min⁻¹, respectively). Portal venous blood flows were comparable and were not altered with insulin infusion (18 ± 2, 18 ± 2, and 19 ± 2 vs. 18 ± 4, 19 ± 4, and 18 ± 4 ml·kg⁻¹·min⁻¹ in SHAM vs. INF, respectively).

### Table 1. Baseline variables in SHAM and INF groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>SHAM</th>
<th>INF</th>
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<tbody>
<tr>
<td>n</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Body temperature, °C</td>
<td>38.4 ± 0.2</td>
<td>40.0 ± 0.3*</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>21.9 ± 1.0</td>
<td>21.4 ± 1.0</td>
</tr>
<tr>
<td>Liver weight, g</td>
<td>810 ± 60</td>
<td>855 ± 41</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>114 ± 6</td>
<td>105 ± 3</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>86 ± 9</td>
<td>122 ± 12*</td>
</tr>
<tr>
<td>Cortisol, µg/ml</td>
<td>2.2 ± 0.3</td>
<td>3.3 ± 0.5</td>
</tr>
<tr>
<td>Norepinephrine, pg/ml</td>
<td>179 ± 41</td>
<td>439 ± 204 (n = 3)</td>
</tr>
<tr>
<td>Epinephrine, pg/ml</td>
<td>123 ± 44</td>
<td>264 ± 180 (n = 3)</td>
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</tbody>
</table>

Data are expressed as means ± SE. SHAM, sham operated; INF, infected. *Significantly different from SHAM.
Arterial plasma insulin (A) and glucagon (B) concentrations in sham (SHAM, n = 5) and infected (INF, n = 5) groups during low (0–120 min), middle (120–240 min), and high (240–360 min)-dose insulin infusions.

Fig. 1. Arterial plasma insulin (A) and glucagon (B) concentrations in sham (SHAM, n = 5) and infected (INF, n = 5) groups during low (0–120 min), middle (120–240 min), and high (240–360 min)-dose insulin infusions.

Hepatic glucose metabolism. The exogenous glucose infusion rate (GIR; Table 2) was varied to maintain the arterial plasma glucose concentration near 120 mg/dl (Fig. 2). As expected, GIR increased as the insulin infusion rate was increased, and infection impaired this increase.

Table 2. Glucose metabolic parameters at 3 insulin doses

<table>
<thead>
<tr>
<th></th>
<th>Lo-Ins</th>
<th>Mid-Ins</th>
<th>Hi-Ins</th>
<th>Lo-Ins</th>
<th>Mid-Ins</th>
<th>Hi-Ins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose infusion rate</td>
<td>8.1 ± 0.8</td>
<td>16.0 ± 2.3</td>
<td>26.4 ± 1.9</td>
<td>5.8 ± 0.6</td>
<td>12.5 ± 0.9</td>
<td>20.2 ± 1.4</td>
</tr>
<tr>
<td>Unidirectional hepatic glucose uptake</td>
<td>3.4 ± 0.4</td>
<td>3.5 ± 0.3</td>
<td>4.4 ± 0.8</td>
<td>2.5 ± 0.5</td>
<td>3.3 ± 0.4</td>
<td>3.4 ± 0.3</td>
</tr>
<tr>
<td>Hepatic glucose production</td>
<td>−0.3 ± 0.4</td>
<td>−0.8 ± 0.3</td>
<td>−0.6 ± 1.0</td>
<td>1.5 ± 0.3*</td>
<td>0.7 ± 0.2*</td>
<td>0.6 ± 0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed as mg·kg⁻¹·min⁻¹. Lo-Ins, Mid-Ins, and Hi-Ins, low, medium, and high doses of insulin. *Significantly different from SHAM at corresponding insulin dose.

In the Lo-Ins period, NHGU was dramatically reduced with infection (3.5 ± 0.2 vs. 1.1 ± 0.5 mg·kg⁻¹·min⁻¹, SHAM vs. INF, P < 0.05; Fig. 3). NHGU increased (P < 0.05 from basal) in both groups in the Mid-Ins and Hi-Ins periods. In SHAM, NHGU increased to 4.2 ± 0.4 and 4.6 ± 0.5 mg·kg⁻¹·min⁻¹, whereas NHGU in INF increased to 2.6 ± 0.5 and 2.8 ± 0.7 mg·kg⁻¹·min⁻¹. HFE was also significantly decreased in INF in Lo-Ins (0.16 ± 0.01 vs. 0.03 ± 0.01, SHAM vs. INF, P < 0.05). HFE increased in SHAM (to 0.19 ± 0.01 and 0.20 ± 0.02) as well as in INF (to 0.08 ± 0.02 and 0.08 ± 0.02) as the insulin concentration was increased.

In one sham dog and one infected dog, an additional experimental period was allowed before the Lo-Ins period, in which the insulin infusion rate was halved (200 μU·kg⁻¹·min⁻¹). In the sham dog, with arterial and sinusoidal insulin concentrations equaling 4 and 19 μU/ml, NHGU (1.9 mg·kg⁻¹·min⁻¹) and HFE (0.09) were reduced relative to the Lo-Ins period. In the infected dog, with arterial and sinusoidal insulin concentrations of 5 and 14 μU/ml, NHGU was completely suppressed (−0.3 mg·kg⁻¹·min⁻¹), and the liver was, in fact, releasing glucose.

Unidirectional HGU and HGP are shown in Table 2. In SHAM, changes in HGU and HGP were not significantly different between insulin periods. In INF, however, HGP decreased from 1.5 ± 0.3 to 0.7 ± 0.2 mg·kg⁻¹·min⁻¹ (Lo-Ins to Mid-Ins), whereas HGU increased significantly (2.5 ± 0.5 to 3.3 ± 0.4 mg·kg⁻¹·min⁻¹). There were no differences in either variable between Mid-Ins and Hi-Ins periods. The inflowing [³H]glucose SA (entering the liver) was 8,568 ± 564, 9,242 ± 302, and 9,687 ± 300 dpm/mg in SHAM (n = 4) and 9,797 ± 2,474, 9,358 ± 1,580, and 9,171 ± 1,348 dpm/mg in INF (n = 4) in Lo-Ins, Mid-Ins, and Hi-Ins periods, respectively.

Glucose disposition. Arterial blood lactate concentrations were similar in the Lo-Ins period (978 ± 92 vs. 813 ± 210 μM, SHAM vs. INF) and did not change with...
in insulin did not enhance lactate release even though they enhanced NHGU.

Based on mass balance of carbohydrate (glucose, lactate, and gluconeogenic precursors), the net hepatic consumption of carbohydrate increased as insulin was increased in both groups (2.2 ± 0.2 to 3.2 ± 0.4 to 3.8 ± 0.5 vs. 0.4 ± 0.3 to 2.5 ± 0.4 to 2.8 ± 0.3 mg glucose·kg⁻¹·min⁻¹, SHAM vs. INF). Increases in insulin had no effect on hepatic oxidation of plasma glucose in either group (0.3 ± 0.1, 0.4 ± 0.1, and 0.3 ± 0.1 mg·kg⁻¹·min⁻¹ vs. 0.4 ± 0.0, 0.4 ± 0.1, and 0.4 ± 0.1 mg·kg⁻¹·min⁻¹, SHAM vs. INF; n = 4). As expected, terminal hepatic glycogen content was substantially higher in SHAM (83 ± 9 vs. 36 ± 6 mg glycogen/g liver, n = 4 vs. n = 3, P < 0.05). Tracer-determined hepatic glycogen synthesis rates were 1.2 ± 0.4 and 0.5 ± 0.1 mg·kg⁻¹·min⁻¹ in SHAM and INF, respectively [not significant (NS), P = 0.07].

Metabolites. Arterial plasma NEFA concentrations were similar in SHAM and INF at each insulin dose and declined as insulin was increased (369 ± 64 to 225 ± 37 to 184 ± 30 μM in SHAM and 316 ± 48 to 210 ± 30 to 172 ± 23 μM in INF). Net hepatic NEFA uptake rates were also comparable and fell in response to increases in insulin (0.6 ± 0.3 to −0.3 ± 0.4 to 0.1 ± 0.2 μmol·kg⁻¹·min⁻¹ and 0.9 ± 0.2 to 0.5 ± 0.2 to 0.1 ± 0.1 μmol·kg⁻¹·min⁻¹). Neither arterial plasma glycerol concentrations (54 ± 10, 53 ± 8, and 49 ± 6 μM vs. 63 ± 7, 59 ± 8, and 56 ± 10 μM, SHAM vs. INF) nor net hepatic glycerol uptake rates (1.0 ± 0.2, 0.9 ± 0.2, and 0.9 ± 0.2 μmol·kg⁻¹·min⁻¹ vs. 1.4 ± 0.2, 1.5 ±

higher insulin doses (Fig. 4). NHLR rates were also comparable in the basal period and did not increase as insulin was raised in SHAM (1.6 ± 0.2 to 1.4 ± 0.1 to 1.2 ± 0.2 mg·kg⁻¹·min⁻¹) and in INF (1.3 ± 0.4 to 1.0 ± 0.3 to 0.6 ± 0.5 mg·kg⁻¹·min⁻¹). Thus the proportion of NHGU released as lactate declined as insulin was increased (46 ± 5, 33 ± 1, and 28 ± 4% in SHAM and 119 ± 25, 37 ± 7, and 14 ± 14% in INF). The proportion of hepatic [¹⁴C]glucose uptake released as [¹⁴C]lactate in SHAM was similar (51 ± 9%) to the percentage of NHGU released as lactate in the Lo-Ins period (n = 3); data for the other insulin periods, however, were not available. The proportion of hepatic [¹⁴C]glucose uptake released as [¹⁴C]lactate in INF decreased as the insulin concentration was elevated (70 ± 7, 30 ± 19, and 20 ± 24%; n = 4). Thus increases
0.1, and 1.4 ± 0.2 μmol·kg⁻¹·min⁻¹) changed significantly with increasing insulin concentrations.

Arterial gluconeogenic amino acid (GAA) concentrations and hepatic GAA uptake rates are shown in Table 3. Infection significantly reduced arterial alanine and glycine concentrations, although net hepatic alanine and glycine uptake rates were not reduced, due in part to a higher HFE of the two GAs (P < 0.05). Arterial serine and threonine concentrations were not altered by infection. However, the HFE of these amino acids was increased (P < 0.05); consequently, net hepatic serine and threonine uptake was significantly increased by infection. In contrast to the other GAs, the liver was a net producer of glutamine at basal levels of insulin. Increases in insulin significantly reduced arterial GAA concentrations in both groups. However, net hepatic GAA uptake was not altered by insulin, because HFE of these amino acids increased. Total gluconeogenic precursor uptake (Total GAA + glycerol) was increased by infection (3.5 ± 0.8 to 3.9 ± 0.6 to 4.1 ± 0.7 vs. 7.2 ± 1.3 to 7.5 ± 1.3 to 5.9 ± 0.5 μmol·kg⁻¹·min⁻¹, SHAM vs. INF). Thus infection increased the net uptake of gluconeogenic precursors by the liver, and the infusion of insulin did not significantly decrease net hepatic gluconeogenic precursor uptake.

**Gut substrate balance.** Glucose uptake by the gut was 0.8 ± 0.1, 0.6 ± 0.1, and 0.9 ± 0.2 mg·kg⁻¹·min⁻¹ in SHAM and 0.8 ± 0.2, 0.9 ± 0.2, and 1.2 ± 0.3 mg·kg⁻¹·min⁻¹ in INF. Gut glucose oxidation (0.2 ± 0.1 vs. 0.2 ± 0.1 mg·kg⁻¹·min⁻¹, SHAM vs. INF, Lo-Ins) did not change with insulin (data not shown). The gut was a net consumer of glutamine (1.6 ± 0.6, 1.5 ± 0.5, and 1.1 ± 0.3 μmol·kg⁻¹·min⁻¹ vs. 1.4 ± 0.5, 1.0 ± 0.4, and 0.7 ± 0.2 μmol·kg⁻¹·min⁻¹, SHAM vs. INF).

**Hindlimb substrate balance.** As expected in response to elevations in insulin, net hindlimb glucose uptake (LGU) increased substantially in both groups, from 11 ± 4 to 21 ± 3 to 39 ± 7 mg/min in SHAM and from 6 ± 1 to 15 ± 4 to 27 ± 5 mg/min in INF (Fig. 5). LGU was not significantly different between groups at comparable insulin doses. Net hindlimb fractional extraction of glucose also increased in response to insulin (0.07 ± 0.02 to 0.19 ± 0.05 to 0.34 ± 0.07 in SHAM and 0.05 ± 0.01 to 0.13 ± 0.02 to 0.23 ± 0.03 in INF). In the Hi-Ins period, net hindlimb fractional extraction of glucose was significantly decreased with infection. Net hindlimb lactate uptake was lower in INF relative to SHAM in the Lo-Ins period (4.5 ± 1.3 vs. 0.9 ± 0.4 mg/min, P < 0.05); it tended to fall when insulin was increased (to 2.6 ± 1.2 and 0.1 ± 1.4 mg/min in SHAM and to 0.8 ± 0.6 and 0.3 ± 0.6 mg/min in INF), although the changes were not significantly different.

**DISCUSSION**

The liver consumes ~45% of the glucose infused in chronic TPN, and peripheral tissues consume the remaining 55%. The presence of an infection sharply decreases NHGU; consequently, peripheral glucose uptake increases to sustain whole body glucose uptake. We previously demonstrated that the compensatory rise in insulin limits the fall in NHGU and increases

Table 3. Blood gluconeogenic amino acid concentrations, net hepatic amino acid uptake rates, and net hepatic fractional amino acid extraction in SHAM and INF groups at 3 insulin doses

<table>
<thead>
<tr>
<th></th>
<th>SHAM</th>
<th>INF</th>
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<tbody>
<tr>
<td></td>
<td>Lo-Ins</td>
<td>Mid-Ins</td>
</tr>
<tr>
<td>Alanine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration</td>
<td>480 ± 58</td>
<td>424 ± 70†</td>
</tr>
<tr>
<td>Uptake</td>
<td>1.3 ± 0.8</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td>Extraction</td>
<td>0.12 ± 0.03</td>
<td>0.13 ± 0.01</td>
</tr>
<tr>
<td>Glycine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration</td>
<td>926 ± 256</td>
<td>845 ± 252†</td>
</tr>
<tr>
<td>Uptake</td>
<td>−1.7 ± 0.7</td>
<td>−1.1 ± 0.5</td>
</tr>
<tr>
<td>Extraction</td>
<td>0.07 ± 0.02</td>
<td>0.09 ± 0.02</td>
</tr>
<tr>
<td>Serine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration</td>
<td>206 ± 34</td>
<td>194 ± 33</td>
</tr>
<tr>
<td>Uptake</td>
<td>0.6 ± 0.1</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>Extraction</td>
<td>0.12 ± 0.04</td>
<td>0.15 ± 0.02</td>
</tr>
<tr>
<td>Threonine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration</td>
<td>255 ± 41</td>
<td>228 ± 44†</td>
</tr>
<tr>
<td>Uptake</td>
<td>−0.1 ± 0.2</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>Extraction</td>
<td>−0.01 ± 0.01</td>
<td>0.02 ± 0.02</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE; concentrations are in μM, uptake rates in μg·kg⁻¹·min⁻¹; n = 5 for each group. Negative values (e.g., glutamine) indicate hepatic release. *Significantly different from SHAM at corresponding insulin dose; †significantly different from Lo-Ins period.
peripheral glucose uptake during infection (3). Our study demonstrates that both hepatic insulin sensitivity (ED50) and responsiveness are enhanced in the TPN-adapted state (i.e., SHAM). We also demonstrate that infection impairs both the responsiveness and the sensitivity of the liver to insulin. Although the liver is reliant on the compensatory hyperinsulinemia to sustain liver glucose uptake during an infection, NHGU does not improve further with even higher doses of insulin. Moreover, the modest benefit of improving NHGU gained by the additional insulin predisposes the liver to accumulate glycogen rather than to improve hepatic glycolysis.

Chronic TPN enhanced the responsiveness and sensitivity (i.e., ↓ ED50) of NHGU to insulin. As we have reported previously (22), NHGU in sham TPN-adapted dogs was substantially higher than rates achieved in normal 42-h-fasted dogs (3.5 vs. 0.55 mg·kg\(^{-1}\)·min\(^{-1}\)) despite lower insulin and glucose levels (sinusoidal insulin, 35 vs. 51 μU/mL; arterial plasma glucose, 120 vs. 240 mg/dL). Moreover, NHGU with chronic TPN increased to 4.6 mg·kg\(^{-1}\)·min\(^{-1}\) at the higher insulin level (163 μU/mL), which corresponded to an HFE of 0.20. In contrast, in the 42-h-fasted dog, NHGU and HFE were only 3.0 mg·kg\(^{-1}\)·min\(^{-1}\) and 0.04, respectively, after ~4 h of combined hyperglycemia (200 mg/dL) and hyperinsulinemia (191 μU/mL) (20). TPN also enhances the sensitivity of NHGU to insulin. In the TPN-adapted state, when the sinusoidal insulin concentration was halved (19 μU/mL) in a SHAM dog, NHGU fell by ~50%. We estimate hepatic insulin sensitivity (ED50) to be below a sinusoidal insulin concentration of 25 μU/mL in TPN-adapted animals, which is substantially lower than that seen in the 42-h-fasted dog (~90 μU/mL) (20). Thus chronic TPN administration enhances both the sensitivity and the responsiveness of the liver to insulin.

Infection impairs both the sensitivity and the responsiveness of NHGU to insulin in the TPN-adapted setting. At the low sinusoidal insulin concentration (29 μU/mL), NHGU was 70% lower in INF than in SHAM. When insulin was raised to the levels normally seen during infection, NHGU increased to 2.6 mg·kg\(^{-1}\)·min\(^{-1}\). However, when the sinusoidal insulin concentration was raised above 66 μU/mL, NHGU was not significantly elevated, suggesting that a near-maximal response was attained. Compared with SHAM, NHGU (2.8 mg·kg\(^{-1}\)·min\(^{-1}\)) and HFE (0.08) were 38 and 60% lower, respectively, in INF at the highest insulin dose studied. Moreover, we observed that NHGU was completely suppressed at a sinusoidal insulin level of 14 μU/mL. Hepatic insulin sensitivity (ED50) was estimated to be at a sinusoidal insulin level of ~40 μU/mL for infected animals receiving TPN. There are no other reports of the impact of infection on the sensitivity of liver glucose uptake to insulin. There are, however, conflicting results as to infection’s impact on the sensitivity of HGP to insulin. Lang et al. (9) found no impairment in the suppression of tracer-determined HGP by insulin in conscious septic rats. McLane et al. (16) observed that insulin’s suppression of HGP was impaired in anesthetized septic dogs. In the latter study, in contrast to our study, glucagon, a potent regulator of HGP and HGU, was not controlled. Thus our studies demonstrate for the first time that infection reduces both the sensitivity and the responsiveness of NHGU to insulin.

One factor contributing to the infection-induced decrease in NHGU at low, but not at high, insulin levels was an elevated rate of HGP. The magnitude of NHGU is determined by the balance between HGU and HGP, and infection enhances both the sensitivity and the responsiveness of NHGU to insulin.
Peripheral insulin resistance, predominantly in skeletal muscle, is a well-known characteristic of critical illness (10). Figure 5 shows that nonhepatic tissues were responsible for the majority of the increase in whole body glucose uptake in response to insulin. In the Lo-Ins period, net nonhepatic glucose uptake rates were equivalent in SHAM and INF animals. This is not surprising. An increase in insulin-independent glucose uptake during inflammatory stress could offset any decrease in insulin-mediated glucose disposal. This would be most evident at low insulin concentrations, where insulin-mediated glucose uptake is a small fraction of the total glucose uptake (17). A corresponding impairment in LGU was not detected at any insulin dose because of a large variability in iliac arterial blood flow. However, a variable that is less sensitive to variation in blood flow, fractional extraction of glucose across the hindlimb, was reduced in INF at the highest insulin dose.

In summary, our in vivo studies demonstrate that, following the adaptation to TPN, 1) the liver is more sensitive and responsive to insulin than in a normal animal, 2) the presence of an infection reduces both the responsiveness and the sensitivity of NHGU to insulin, and 3) despite marked augmentation of NHLR in the TPN-adapted state, increases in insulin do not correct the infection-induced impairment in glycolysis. These results suggest that, although an increase in arterial insulin commonly seen during infection will minimize the infection-induced impairment in liver glucose uptake, insulin did not reverse the inhibition of glycolysis. Additional insulin will not enhance NHGU further. The implication of this study is that, although the administration of insulin will correct the hyperglycemia induced by an infection or critical illness, insulin will not overcome the defects in hepatic metabolism. Any beneficial effects of insulin on liver glucose uptake do not lead to a corresponding rise in glycolysis and in fact favor glucose storage, initially in the form of glycogen. The long-term consequence of sustained hepatic storage of carbohydrate is unclear.

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