Time course of the hepatic adaptation to TPN: interaction with glycogen depletion

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Chen, Sheng-Song, Carlos J. Torres-Sanchez, Nadeen Hosein, Yiqun Zhang, D. Brooks Lacy, and Owen P. McGuinness. Time course of the hepatic adaptation to TPN: interaction with glycogen depletion. Am J Physiol Endocrinol Metab 288: E163–E170, 2005. First published August 31, 2004; doi:10.1152/ajpendo.00192.2004.—In response to chronic (5 days) TPN, the liver becomes a major site of glucose disposal, removing ~45% (4.5 mg·kg⁻¹·min⁻¹) of exogenous glucose. Moreover, ~70% of glucose is not stored but released as lactate. We aimed to determine in chronically catheterized conscious dogs the time course of adaptation to TPN and the glycogen depletion impact on early time course. After an 18-h (n = 5) fast, TPN was infused into the inferior vena cava for 8 (n = 5) or 24 h (n = 6). A third group, of 42-h-fastened animals (n = 6), was infused with TPN for 8 h. TPN was infused at a rate designed to match the dog’s calculated basal energy and nitrogen requirements. NHGU (~2.3 ± 0.1 to 2.2 ± 0.7 to 3.9 ± 0.6 vs. ~1.7 ± 0.3 to 1.1 ± 0.5 to 2.9 ± 0.4 mg·kg⁻¹·min⁻¹, basal to 4 to 8 h, 18 vs. 42 h) and net hepatic lactate release (0.7 ± 0.3 to 0.6 ± 0.1 to 1.4 ± 0.2 vs. ~0.6 ± 0.1 to 0.1 ± 0.1 to 0.8 ± 0.1 mg·kg⁻¹·min⁻¹, basal to 4 to 8 h) increased progressively. Net hepatic glycogen repletion and tracer determined that glycogen syntheses were similar. After 24 h of TPN, NHGU (5.4 ± 0.6 mg·kg⁻¹·min⁻¹) and net hepatic lactate release (2.6 ± 0.4 mg·kg⁻¹·min⁻¹) increased further. In summary, 1) most hepatic adaptation to TPN occurs within 24 h after initiation of TPN, and 2) prior glucagon depletion does not augment hepatic adaptation rate.

In stressed states (trauma, injury, or infection), nutritional support can be provided via the parenteral (total parenteral nutrition; TPN) route. Prior studies suggest that, after 5 days of TPN infusion, the liver augments its capacity to take up glucose, removing ~45% of the exogenous glucose (5). Even more surprising is that this was accomplished in the absence of hyperglycemia (~120 mg/dl) with only mild hyperinsulinemia (~15 μU/ml) (5). To induce in the acute setting (<4 h) the approximate magnitude of net hepatic glucose uptake (NHGU) seen after chronic infusion of TPN (5 days), the arterial glucose levels would have to be increased to >200 mg/dl, and the arterial insulin levels would have to exceed 40 μU/ml (20). In addition, glucose would have to be infused into the portal vein. The hepatic response to a more prolonged (>4 h) glucose infusion has not been examined. More importantly, the duration over which TPN would have to be infused to initiate the adaptive response of the liver is not known.

Most patients in whom nutritional support is initiated are glycogen depleted. The impact of glycogen depletion on the adaptive response of the liver to TPN is unclear. The acute (2–2.5 h) regulation of NHGU in response to glucose delivery alone is not influenced by the fasting state (1, 3, 9). However, given that the adaptive response to TPN coincides with a marked augmentation of hepatic glycolysis in a setting of elevated glycogen content, the transition to elevated NHGU and glycolysis may necessitate glycogen repletion. Thus glycogen depletion may delay the overall adaptive response of the liver to glucose infusion. Moreover, the hepatic response to TPN, which contains glucose as well as other nutrients, may differ from that of glucose alone.

Aggressive maintenance of normoglycemia in stressed patients when TPN is initiated positively impacts clinical outcome (25). The liver plays an important role in glucose disposal and thus maintenance of normoglycemia during long-term TPN; its importance during the first 24 h of TPN is unknown. The aims of the study were 1) to determine the time course of the hepatic adaptation to TPN during the first 24 h of nutrient delivery, and 2) to determine whether the glycogen depletion state alters this time course.

METHODS

Animal preparation. Seventeen female nonpregnant mongrel dogs (22 ± 1 kg) were fed standard Kal-Kan meat (Vernon, CA) and Purina Lab Canine Diet no. 5006 (Purina Mills, St. Louis, MO) once daily and had free access to water. The composition of the diet on the basis of dry weight was 52% carbohydrate, 31% protein, 11% fat, and 6% fiber. Dogs were housed in a facility that met American Association for the Accreditation of Laboratory Animal Care International guidelines. The Vanderbilt University Medical Center Animal Care Committee approved the protocols. The health of the animals was determined before surgery and before TPN administration by a good appetite (i.e., consumed ≥75% of daily ration), normal stools, hematocrit >35%, and leukocyte count <18,000 mm⁻³.

Experimental preparation. Fourteen to seventeen days before initiation of TPN, a laparotomy was performed using sterile techniques with general anesthesia (15 mg/kg thiopental sodium iv for induction and 1.0% isoflurane as an inhalant during surgery) on healthy dogs. During the laparotomy, blood-sampling catheters (0.04-in. ID) were positioned in the portal and left common hepatic veins. Two infusion catheters (0.04-in. ID) for TPN were placed in the inferior vena cava (IVC), and the free ends were exteriorized and tunneled subcutaneously behind the left clavicle. Flow probes (Transonic Systems, Ithaca, NY) were positioned around the portal vein, hepatic artery, and right external iliac artery after the gastroduodenal vein was ligated. After an incision in the right inguinal regions, a sampling catheter (0.04-in. ID) was placed in the left common iliac vein, and the tip was positioned distal to the anastomosis with the IVC; another catheter was advanced from the right external iliac artery to the abdominal aorta.

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The catheters were filled with 0.9% NaCl (saline) containing heparin (200 U/ml). The free ends of the catheters and flow probes were exteriorized and placed in subcutaneous pockets. The dogs received penicillin G (500,000 U iv) in 1 liter of saline to minimize the possibility of infection. Fluixinamine (0.1 mg/kg; Fort Dodge Laboratory, Fort Dodge, IA) was injected intramuscularly immediately after wound closure for acute pain relief. Dogs also received penicillin G (600,000 U im) for 3 days after surgery.

Experimental protocol. The studies were performed on either 42- or 18-h-fasted dogs. The free ends of all catheters were exteriorized under local anesthesia (2% Lidocaine), and their contents were aspirated and flushed with saline. The free ends of the flow probes were also exteriorized and connected to a flow meter (Transonic Systems). The dog was placed in a Pavlov harness for the duration of the study. The study consisted of a 60-min equilibration period, a 30-min basal sampling period, 30 to 0 min) period, and an 8-h nutrition infusion period. During the 30-min basal sampling period, blood samples were taken from the four sampling catheters (femoral artery, portal vein, hepatic vein, and iliac vein) at approximately 30, 15, and 0 min. TPN was then infused via the IVC for 8 h, and blood samples were taken hourly for the first 4 h and every 30 min for the remaining 4 h. Primed and (22 and 27 µCi) constant infusions (0.4 and 0.3 µCi/min) of [3-3H]- and [U-14C]glucose (New England Nuclear, Wilmington, DE), respectively, were infused into the IVC 120 min after initiation of TPN. A third group of dogs (24-h TPN, n = 6) were infused with TPN starting at 12:00 PM the preceding day, after a 22-h fast. At 8:00 AM the next day, a primed [3-3H]-[U-14C]glucose infusion (rates identical to above) was initiated. Blood samples were obtained every 15 min between 10:00 AM and 12:00 noon (i.e., 22 and 24 h of TPN).

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 liver lobes and a muscle (adductor magnus et brevis) were freeze-clamped with Wallenburg clamps precooled in liquid nitrogen and stored at −70°C until analysis. The entire liver was removed and weighed.

Nutritional support. The dogs received TPN as the sole exogenous caloric source for 8 h. The TPN was designed to be isocaloric, based on predicted resting energy expenditure (21). The composition of the TPN included glucose, lipids, amino acids, saline (2.9 ml-kg⁻¹-h⁻¹), potassium phosphates (90 mg-kg⁻¹-day⁻¹), and a multi-vitamin supplement (MVI-12; Astra, Westborough, MA). Glucose (50% dextrose, Abbott) made up 75% of the nonprotein calories, and a fat and vitamin supplement (MVI-12; Astra, Westborough, MA). Glucose (50% dextrose, Abbott) made up 75% of the nonprotein calories, and a fat and vitamin supplement (MVI-12; Astra, Westborough, MA) contributed the remaining 25% of the energy requirements. Travalos (Baxter) was infused to supply basal nitrogen requirements (−12 g protein/day), calculated with the formula 1.5 × body wt⁰.⁶⁷ (in kg). Nutrition solutions were prepared under sterile conditions. TPN was infused with a calibrated infusion pump (Harvard Apparatus, Holliston, MA).

Sample processing. Blood samples were placed in chilled tubes containing potassium EDTA (15 mg). The collection and immediate processing of blood samples have been described previously (6). Blood ¹⁴CO₂ was assessed in triplicate on arterial, portal vein, and hepatic vein samples (8). Blood samples were centrifuged at 3,000 rpm for 10 min. For the glucagon assay, 2 ml of plasma were added to 100 µl of trisylol (500 kallikrein inhibitor units; Miles, Kankakee, IL). Because of the very low glucagon levels seen during TPN, the samples were lyophilized and reconstituted with 1 ml of water before analysis. Plasma (0.5 ml) samples were deproteinized with Ba(OH)₂ and ZnSO₄ and exposed to anion and cation resin to remove charged intermediates (14) and to assess plasma [³H]- and [¹⁴C]glucose specific activity (SA). The remaining plasma was stored at −70°C for later analysis.

Analysis. Immunoactive insulin and glucagon were assayed using a double-antibody technique (Linco Research, St. Charles, MO) [intra-assay coefficients of variation (CV) of 11 and 10%, respectively], and cortisol was assayed with a Diagnostic Products RIA kit (Los Angeles, CA; CV 12%).

Analysis of gluconeogenic metabolites (lactate, alanine, and glycerc) in blood was performed on an automated centrifugal analyzer (Monarch 2000; Instrumentation Laboratory, Lexington, MA) by use of a modification of the method of Lloyd et al. (11). The concentration of nonesterified fatty acids (NEFA) was determined spectrophotometrically (Wako Chemicals, Richmond, VA). Tissue glucokinase (GK) and glucose-6-phosphatase (G-6-Pase) activities were analyzed on the quadruple lobe with the methods described by Barzilai and Rossetti (2). Total GK activity was calculated as the difference between activities at 100 and 0.5 mM glucose. G-6-Pase was measured at 10 mM glucose 6-phosphate (G-6-P). Protein content was assessed with the Biuret method. Tissue G-6-P, fructose 6-phosphate (F-6-P), and fructose 2,6-bisphosphate (F-2,6-P₂) were analyzed with fluorometric enzymatic methods (16, 26).

Calculations. The hepatic substrate load (Load In) was calculated as Aₛ × HABF + PVₛ × PVBF, where Aₛ and PVₛ represent the blood substrate concentrations in the iliac artery and portal vein, and HABF and PVBF represent blood flow in the hepatic artery and portal vein, respectively. Similarly, the substrate load leaving the liver (Load Out) was the product of HV × HBF, where HV and HBF represent the hepatic vein blood substrate concentration and total hepatic blood flow (HABF + PVBF) or plasma flow. Net hepatic substrate uptake was the difference between Load In and Load Out. Net hepatic substrate fractional extraction was calculated as the ratio of net hepatic substrate uptake and Load In. Net splanchic substrate uptake was calculated as (HABF + PVBF) × (Aₛ − Hᵥ), where Aₛ and Vᵥ represent the blood substrate concentrations in the iliac artery and hepatic vein, respectively. These equations were used to calculate net hepatic glucose, [¹⁴C]CO₂, lactate, alanine, glycerc, amino acid, and NEFA balances.

Unidirectional hepatic glucose uptake (HGU) was calculated as the ratio of hepatic [¹⁴C]glucose uptake to the corresponding inflowing [¹⁴C]glucose SA. Because the liver produced and consumed glucose simultaneously, hepatic glucose production (HGP) was the difference between unidirectional uptake and net uptake (HGP = HGU − NHGU). Similarly, net hindlimb glucose uptake was calculated with the formula (Aᵥ − Vᵥ) × ABF, where Aᵥ and Vᵥ represent blood glucose concentrations in the iliac artery and iliac vein, respectively, and ABF represents blood flow in the iliac artery. Plasma flow was calculated by multiplying blood flow by (1 − hematocrit).

Hepatic conversion of glucose to CO₂ (hepatic glucose oxidation) was calculated as the net hepatic [¹⁴C]CO₂ production rate divided by the hepatic [¹⁴C]glucose precursor SA. The hepatic [¹⁴C]glucose precursor SA was considered to be the [¹⁴C]glucose SA in the flowing blood to the liver. Hepatic glycogen storage was the difference between the NHGU and total net gluconeogenic precursor uptake (lactate, amino acids, and glycerc). Hepatic glycogen content in 18- and 42-h-fasted dogs was assumed to equal 35.5 and 18 mg/g liver, respectively (9).

Net deposition of glycogen in liver was calculated by dividing hepatic [¹⁴C]glycogen accumulation (dpm/g liver) by the average inflowing [¹⁴C]glucose SA.

Statistics. All values for the basal period are the average of data obtained at ∼30, −15, and 0 min. Unless otherwise stated, data for the experimental period in the 8-h TPN groups are the mean of 7, 7.5, and 8 h. For the 24-h TPN group, the data collected between 22 and 24 h were averaged. Statistical comparisons were made with two-way ANOVA, followed by an F-test (SYSTAX, Evanston, IL), and one-way ANOVA when comparing within groups. P < 0.05 was regarded as significant.

RESULTS

Blood flow. As shown in Table 1, hepatic arterial, portal vein, and total hepatic blood flow did not change in either group during TPN infusion. Femoral artery blood flow (ml-kg⁻¹-h⁻¹) did not change from the basal period to 8 h of TPN infusion (11 ± 2 to 11 ± 5 vs. 9 ± 1 to 8 ± 1; 18 vs.
Table 1. Hepatic artery, portal vein, and total hepatic blood flow in dogs

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<th>Basal</th>
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<td>18 h</td>
<td>6.3±1.6</td>
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<td>42 h</td>
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<td>42 h</td>
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<td><strong>Total hepatic flow</strong></td>
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<td>42 h</td>
<td>30.4±3.7</td>
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<td>28.0±3.4</td>
<td>26.8±3.8</td>
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Values are means ± SE (blood flow units are ml·kg⁻¹·min⁻¹) of 42-h-fasted (n = 6) and 18-h-fasted (n = 5) dogs during the basal period and during 8 h of total parenteral nutrition (TPN) infusion.

42 h. In dogs receiving 24-h TPN, hepatic artery (6 ± 1 ml·kg⁻¹·min⁻¹), portal vein (22 ± 2 ml·kg⁻¹·min⁻¹), total hepatic blood flow (28 ± 2 ml·kg⁻¹·min⁻¹), and femoral artery blood flow (9 ± 1 ml·kg⁻¹·min⁻¹) were constant during the 2-h blood-sampling period.

**Hormone concentrations.** Arterial plasma insulin concentrations were similar during the basal period (6.8 ± 1.5 to 7.4 ± 1.3 μU/ml; 18 vs. 42 h) and increased to a similar extent in response to TPN infusion (Fig. 1). In both groups, the arterial plasma glucagon concentration was similar in the basal period (31 ± 2 vs. 33 ± 7 pg/ml; 18 vs. 42 h) and decreased (P < 0.05) progressively during TPN (12 ± 5 vs. 14 ± 7 pg/ml; 18 vs. 42 h). Portal vein glucagon levels demonstrated the same pattern (45 ± 8 to 20 ± 8 vs. 53 ± 21 to 21 ± 7 pg/ml; 18 vs. 42 h). In dogs receiving 24-h TPN, arterial plasma insulin concentrations were similar to those seen after 8 h of TPN (16.6 ± 2.7 μU/ml), and glucagon levels were 15 ± 3 and 15 ± 1 pg/ml in the artery and portal vein, respectively.

**Glucose metabolism.** The arterial plasma glucose concentrations (Fig. 1) were similar during the basal period (112 ± 6 vs. 108 ± 5 mg/dl; 18 vs. 42 h). They initially increased to a greater extent in 42-h TPN but after 8 h of TPN were similar (157 ± 15 to 146 ± 9 to 134 ± 7 vs. 188 ± 10 to 154 ± 16 to 138 ± 9 mg/dl; 1 to 4 to 8 h; 18 vs. 42 h). The arterial plasma glucose concentrations were lower after 24 h of TPN than those seen after 8 h of TPN (122 ± 5 mg/dl).

Net hepatic glucose output was lower for 42-h TPN (P < 0.05; Fig. 2) during the basal period (2.3 ± 0.1 vs. 1.7 ± 0.3 mg·kg⁻¹·min⁻¹). During TPN infusion, the liver rapidly switched to a net glucose consumer. A stable rate of NHGU was reached at 4 h (2.2 ± 0.7 vs. 1.3 ± 0.4 mg·kg⁻¹·min⁻¹),
after which NHGU gradually increased for the duration of the study. At 8 h, NHGU was increased by 211 ± 40 and 316 ± 86% in 18- and 42-h groups compared with NHGU seen at 4 h. The net fractional hepatic glucose extraction in the two groups shows a similar pattern as NHGU. Hepatic glucose oxidation was similar in the two groups (0.4 ± 0.1 vs. 0.2 ± 0.1 mg·kg⁻¹·min⁻¹; 18 vs. 42 h). After 24 h of TPN, NHGU (5.4 ± 0.6 mg·kg⁻¹·min⁻¹) and net fractional hepatic glucose extraction (0.21 ± 0.02) increased even further (ΔP < 0.05). Hepatic glucose oxidation was also increased (0.7 ± 0.3 mg·kg⁻¹·min⁻¹; ΔP < 0.05) after 24 of TPN.

During the basal period, the arterial blood lactate concentration was higher in the 18-h group (9.1 ± 0.9 vs. 3.8 ± 0.4 mg/dl; ΔP < 0.05; Fig. 3). In the 42-h group, arterial blood lactate concentration levels increased after initiation of TPN to the concentrations seen in the 18-h group (8.7 ± 1.2 vs. 8.6 ± 1.2 mg/dl). During the basal period, the liver was a consumer of lactate the 42-h group and a net producer of lactate in the 18-h group (0.6 ± 0.3 vs. −0.6 ± 0.1 mg·kg⁻¹·min⁻¹). The rate of lactate release in the 18-h group gradually increased after initiation of TPN. However, in the 42-h group, with the onset of TPN, a brisk but transient increase in lactate release occurred. After 3 h, net hepatic lactate release increased progressively for the duration of the study in the 42-h group, reaching rates seen in the 18-h group (1.1 ± 0.2 vs. 0.8 ± 0.1 mg·kg⁻¹·min⁻¹). At 4 h, a significant fraction of NHGU could be accounted for by lactate release (37 ± 11%) in the 18-h group, whereas only 6 ± 8% of NHGU could be accounted for as lactate release in the 42-h group. However, by 8 h, this percentage increased in the 42-h group such that 30 ± 3% of NHGU could be accounted for as lactate release, which was similar to that seen in the 18-h group (38 ± 6%). After 24 h of TPN, net hepatic lactate release was 2.6 ± 0.4 mg·kg⁻¹·min⁻¹, which accounted for 47 ± 4% of the NHGU.

The time course of NHGU (i.e., glucose phosphorylation) and glycolysis (i.e., net hepatic lactate release + glucose oxidation) in the 18-h fasted group as well as data from our previous work in animals infused for 5 days (5) is presented (see Fig. 6). The peak rate of glucose phosphorylation occurs within 24 h after initiation of TPN and may subside somewhat by 5 days (120 h), whereas the rate of glycolysis increases beyond the rate seen at 24 h.

Arterial blood alanine concentrations (411 ± 44 vs. 282 ± 28 μM; 18 vs. 42 h; Fig. 4) and net hepatic alanine uptake (4.1 ± 0.5 vs. 2.9 ± 0.4 μmol·kg⁻¹·min⁻¹) in the basal period before TPN infusion were lower (ΔP < 0.05) in the 42-h group. In response to TPN infusion, arterial blood alanine levels gradually increased in the 42-h group, whereas they fell modestly in the 18-h group. By 8 h of TPN, both groups had similar arterial blood alanine concentrations (404 ± 34 vs. 391 ± 40 μM). Net fractional hepatic alanine extraction was similar in the basal period (0.28 ± 0.04 vs. 0.31 ± 0.02), remained unaltered for the first 4 h of TPN infusion, and then gradually decreased for the duration of the study in both groups. As a consequence, net hepatic alanine uptake gradually fell in both groups (2.4 ± 0.5 vs. 1.7 ± 1.2 μmol·kg⁻¹·min⁻¹; ΔP < 0.05). Arterial blood alanine levels increased further after 24 h of TPN (505 ± 76 μM), and net hepatic fractional alanine extraction fell further (0.12 ± 0.02). Consequently, net hepatic alanine uptake was not different from that seen after 8 h of TPN (1.8 ± 0.2 μmol·kg⁻¹·min⁻¹) and net fractional hepatic

*Fig. 3. Arterial blood lactate concentrations and net hepatic lactate release in 42- and 18-h-fasted dogs during a basal period and during a constant infusion of TPN. Data are expressed as means ± SE.*

*Fig. 4. Arterial blood alanine concentrations, net hepatic alanine uptake, and net fractional hepatic alanine extraction in 42- and 18-h-fasted dogs during a basal period and during a constant infusion of TPN. Data are expressed as means ± SE.*
glycerol extraction (0.62 ± 0.02 to 0.64 ± 0.03 vs. 0.61 ± 0.01 to 0.58 ± 0.02) did not change and were similar in both groups. After 24 h of TPN, arterial NEFA levels (75 ± 8 μM), net hepatic NEFA uptake (1.2 ± 0.1 μmol·kg⁻¹·min⁻¹), and net hepatic fractional NEFA extraction (0.58 ± 0.04) were similar to that seen after 8 h of TPN.

Arterial NEFA levels decreased markedly during TPN (758 ± 274 to 223 ± 43 vs. 962 ± 55 to 281 ± 53 μM). Because net fractional hepatic NEFA extraction did not change, net hepatic NEFA uptake decreased in both groups (2.9 ± 0.9 to 0.8 ± 0.1 vs. 3.5 ± 0.9 to 0.9 ± 0.3 μmol·kg⁻¹·min⁻¹). After 24 h of TPN, arterial NEFA levels (280 ± 23 μM), net hepatic NEFA uptake (1.0 ± 0.2 μmol·kg⁻¹·min⁻¹), and net hepatic fractional NEFA extraction (0.17 ± 0.04) were similar to those seen after 8 h of TPN.

The net retention of glucose carbon in the liver increased at a similar rate in both groups (Fig 5). Because of the differing baseline glycogen content, terminal liver glycogen content (81 ± 13 vs. 54 ± 5 mg/g liver) was higher in the 18-h group. ¹⁴C-determined glycogen synthesis (22 ± 6 vs. 23 ± 4 mg/g liver) was similar and could account for 52 ± 9 and 86 ± 10% of NHGU. Hepatic glycogen content was 87 ± 6 mg/g after 24 h of TPN, and ¹⁴C-determined glycogen synthesis could account for only 27 ± 7% of NHGU. Hepatic glycogen content rapidly increased during the first 24 h of TPN but did not increase further compared with the levels seen previously (5) after 5 days of TPN (Fig. 6).

Analysis of liver biopsies taken at the end of each study indicated that the tissue levels of glucose, G-6-P, F-6-P, and F-2,6-P₂ as well as GK and G-6-Pase activities (Table 2) were not dependent on the duration of fasting or the duration of TPN infusion.

**Intestinal metabolism.** The intestine was a net consumer of glucose during the basal period, and consumption gradually increased during TPN infusion in both groups (Table 3). The intestine was a net producer of lactate in the 42- but not the 18-h group. On initiation of TPN, net intestinal lactate output decreased in the 42-h group to match that seen in the 18-h group and changed minimally thereafter. Net intestinal alanine release was greater in the 18-h group and gradually decreased to match the rate seen in the 42-h group, which did not change during TPN infusion. Net intestinal glucose, lactate, and alanine output after 24 h of TPN was similar to that seen at 8 h

**DISCUSSION**

The liver undergoes a profound adaptation to TPN; it becomes a major site of glucose disposal during chronic (>5 days) nutritional support (13). Interestingly, the majority (75%) of the glucose carbon removed by the liver is released as lactate, and the route of nutrient delivery does not influence the overall adaptive response (5). The present results indicate that this adaptive response to increase hepatic glucose phosphorylation occurs rapidly and is complete within 24 h. Moreover, prior glycogen depletion does not significantly modify the time course of the overall adaptive response.

The early response to TPN after an 18-h fast (1–4 h) is similar to that seen with glucose infusion alone. The liver rapidly switched from a net glucose producer to a net glucose consumer (1.1 mg·kg⁻¹·min⁻¹) in the presence of mild hyperglycemia and hyperinsulinemia. NHGU is comparable to
previous studies in which only glucose was infused in the presence of comparable glucose and insulin levels; it was $-1.5$ mg·kg$^{-1}·min^{-1}$ (19, 20). Thus the inclusion of Intralipid and amino acids in the TPN did not significantly affect the acute response of the liver. This is consistent with previous studies in which delivery of amino acids into a peripheral vein did not alter liver glucose uptake (17). Although Intralipid infusion can impair NHGU, generally much higher doses have been used and are associated with normal or elevated concentrations of nonesterified fatty acids (4, 18, 23). In contrast, in our studies, nonesterified fatty acids were markedly suppressed during TPN.

Surprisingly, the adaptive response of the liver was manifested as early as 4.5 h after initiation of TPN infusion. For the first 4 h, NHGU was stable. However, NHGU gradually increased after 4.5 h, despite a gradual fall in glucose and insulin concentrations. The enhanced ability of the liver to remove glucose was reflected in the gradual rise in net hepatic fractional extraction of glucose. In the 18-h group, net hepatic fractional extraction of glucose doubled between 4 and 8 h (0.06 ± 0.01 to 0.14 ± 0.02; 4 to 8 h). A number of studies have examined the response of the liver to glucose infusion given into either a portal or peripheral vein and did not detect a progressive rise in NHGU. This may be because most investigators did not infuse glucose for more than 4 h. Another explanation is that investigators held glucagon and insulin constant by accompanying infusion of somatostatin with basal replacement of glucagon and a variable infusion rate of insulin (18, 22). The accompanying fall in glucagon in the present study may have facilitated the augmentation of NHGU. Whereas previous work has suggested that a rise in glucagon will acutely impair NHGU (10), the role of sustained decreases in glucagon on this process has not been examined. Additional possibilities are that the decrease in nonesterified fatty acids and the robust early increase in insulin may also contribute (7). Irrespective of the mechanism, the onset of the adaptive response occurs very quickly.

The adaptive response of NHGU is essentially complete after 24 of TPN. Net hepatic fractional glucose extraction was 0.14 at 8 h and increased to 0.21 after 24 h. If we compare this to our previous observations, where TPN was infused for 5 days, net hepatic fractional extraction of glucose did not increase further (0.15) and in fact was decreased compared with that seen at 24 h (5). Consequently, NHGU (i.e., glucose phosphorylation) may be higher after 24 h than after 5 days (Fig. 6). With the caveat that this is a comparison with historical controls, it suggests that other factors limit the maintenance of the very high NHGU and net hepatic fractional extraction observed at 24 h. The mechanism for the gradual increase in the capacity of the liver to extract glucose is not known. Analysis of liver biopsies taken at 8 and 24 h was unable to detect changes in either glucokinase or glucose-6-phosphatase activity or tissue intermediates that could explain the increased capacity between 8 and 24 h. AS will be discussed below, the failure to sustain the very high fractional extraction of glucose after 24 h may be influenced by a shift in the metabolic fate of the glucose.

Hepatic glycolytic flux (i.e., lactate release + glucose oxidation) gradually increased as the duration of TPN increased (Fig. 6). The primary fate of glucose taken up by the liver during the first 8 h of TPN infusion was glycogen. However, there was a gradual activation of glycolysis as NHGU increased. In 18-h-fasted dogs after 4 and 8 h of TPN, approximately one-third of NHGU could be accounted for as lactate release. However, by 24 h nearly one-half of the glucose was diverted to lactate. After 5 days, more than three-fourths of NHGU was converted to lactate (5). The adaptation to TPN manifested as a relatively rapid increase in NHGU had already begun after 4.5 h and was followed by a somewhat delayed

Table 2. Enzyme and substrate analysis of liver from 18-h-fasted dogs receiving 8- or 24-h TPN infusion and 42-h-fasted dogs receiving 8-h TPN infusion

<table>
<thead>
<tr>
<th>Duration of Prior Fasting</th>
<th>Duration of TPN</th>
<th>GK, mU/mg protein</th>
<th>G-6-Pase, mU/mg protein</th>
<th>F-2,6-P$_2$, mmol/g</th>
<th>Glucose, μmol/g</th>
<th>G-6-P, μmol/g</th>
<th>F-6-P, μmol/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 h</td>
<td>8 h</td>
<td>10.5±1.5</td>
<td>42±6</td>
<td>52±8</td>
<td>5.5±0.8</td>
<td>0.41±0.04</td>
<td>0.24±0.03</td>
</tr>
<tr>
<td>42 h</td>
<td>8 h</td>
<td>8.8±0.7</td>
<td>40±3</td>
<td>42±2</td>
<td>5.5±0.8</td>
<td>0.48±0.13</td>
<td>0.24±0.05</td>
</tr>
<tr>
<td>18 h</td>
<td>24 h</td>
<td>10.8±0.8</td>
<td>34±2</td>
<td>54±5</td>
<td>5.9±1.1</td>
<td>0.42±0.03</td>
<td>0.24±0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE. GK, glucokinase; G-6-Pase, glucose-6-phosphatase; G-6-P, glucose 6-phosphate; F-6-P, fructose 6-phosphate; F-2,6-P$_2$, fructose 2,6-bisphosphate.

Table 3. Net intestinal glucose, lactate, and alanine output

<table>
<thead>
<tr>
<th>Glucose, mg·kg$^{-1}·min^{-1}$</th>
<th>TPN Infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>1 h</td>
</tr>
<tr>
<td>18 h</td>
<td>-0.2±0.2</td>
</tr>
<tr>
<td>42 h</td>
<td>-0.6±0.1</td>
</tr>
<tr>
<td>Lactate, mg·kg$^{-1}·min^{-1}$</td>
<td></td>
</tr>
<tr>
<td>18 h</td>
<td>-0.04±0.04</td>
</tr>
<tr>
<td>42 h</td>
<td>0.22±0.02</td>
</tr>
<tr>
<td>Alanine, μmol·kg$^{-1}·min^{-1}$</td>
<td></td>
</tr>
<tr>
<td>18 h</td>
<td>1.4±0.3</td>
</tr>
<tr>
<td>42 h</td>
<td>0.9±0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE of 24-h-fasted (n = 6) and 18-h-fasted (n = 5) dogs during the basal period and during 8 h of TPN infusion.
diversion of glucose carbon to lactate. Presumably, this delay in the activation of glycolysis was due to the initial diversion of NHGU to glycogen. The mechanism for the gradual activation of glycolysis could not be explained by an increase in fructose 2,6-bisphosphate, a potent regulator of glycolysis.

Prior fasting did not alter the overall adaptive response; however, the metabolic handling of the glucose carbon did differ. As expected, 42-h-fasted dogs were lactate consumers and had less glycogen than 18-h-fasted dogs. However, in both groups, upon initiation of TPN the liver rapidly switched to a glucose consumer and lactate producer. The rate of hepatic glycogen accumulation during the 8 h of TPN was identical in 18- and 42-h groups. While NHGU tended to be lower in the 42-h group, net hepatic lactate release was even lower. Because glycogen accretion was similar, the efficiency of the diversion of plasma glucose to glycogen was higher in the 42-h group. Consistent with this, between 4 and 8 h a greater percentage of the [14C]glucose phosphorylated was incorporated into glycogen in the 42-h group (52 vs. 87%, 18 vs. 42 h). This percentage decreased even further (27%) after 24 h of TPN as the glycogen stores increased. This also paralleled the progressive increase in the percentage of net glucose carbon phosphorylated that was diverted to lactate as glycogen stores increased. The glycogen content of the 18- and 42-h-fasted dog is comparable to that of the 12- and 36-h-fasted human (12). In both dog (9) and humans (3), fasting does not blunt NHGU during a brief (2–2.5 h) glucose infusion. The enhanced diversion of glucose to glycogen in the glycogen-depleted state has been observed with duodenal glucose infusion in the dog (9). The gradual decrement in glycogen mass and the duration of TPN increased may force the liver to be more reliant on glycolytic capacity in determining total glucose phosphorylation. A limiting glycolytic capacity in turn may explain why, after 5 days of TPN, NHGU is actually not increased further. In fact, it may be less than that seen after 24 h of TPN, because glycogen synthesis is no longer a significant metabolic fate.

An interesting correlation can be made between NHGU and hepatic handling of alanine. Net hepatic alanine fractional extraction fell rapidly after 5 h, coincident with the abrupt rise in net hepatic fractional glucose extraction. By 8 h it had fallen from 0.31 ± 0.03 to 0.19 ± 0.03 and by 24 h to 0.12 ± 0.02 (4 to 8 to 24 h). In fact, there is a strong negative correlation between NHGU and net hepatic alanine fractional extraction (r = 0.8). Because hyperinsulinemia is known to enhance the fractional extraction of alanine, this decrease is likely not attributable to insulin. The fall in net hepatic alanine fractional extraction is likely determined by the observed fall in glucagon during TPN. Previous studies have examined the sustained effects of glucagon deficiency; the glucagon-dependent changes in net hepatic fractional extraction of alanine are gradual even after abrupt changes in glucagon (15, 24). In fact, after 5 days of TPN, net hepatic alanine fractional extraction is very low (0.08 ± 0.02) (13). Although the time course of the fall in alanine fractional extraction and the increase in NHGU is only correlative, it does suggest that basal levels of glucagon may exert a tonic inhibitory effect on NHGU (10).

In summary, the adaptation of the liver is rapid in onset, starting ~5 h after initiation of nutritional support. By 24 h, the liver is a highly efficient glucose-consuming organ. This increase likely reflects the peak efficiency of the liver, as NHGU does not increase further if the TPN is continued for an additional 5 days. Surprisingly, prior glycogen depletion does little to modify the overall adaptive response to TPN. The implication is that while the adaptive response of the liver is important in minimizing the accompanying hyperglycemia during long-term TPN, it is equally important when TPN is first initiated. Thus difficulties in maintaining normoglycemia when TPN is initiated may be due in part to a failure of the liver to adapt.

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