Impact of infection on glucose dependent liver glucose uptake
during TPN: interaction with insulin

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Abstract:

Chronic total parenteral nutrition (TPN) markedly augments net hepatic glucose uptake (NHGU). This adaptive increase is impaired by an infection despite accompanying hyperinsulinemia. In the non-adapted state NHGU is dependent upon the prevailing glucose levels. Our aims were to determine if the adaptation to TPN alters the glucose dependency of NHGU, if infection impairs this dependency and if insulin modulates the glucose dependency of NHGU during infection. Chronically catheterized dogs received TPN for 5 days. On day 3 of TPN dogs received either a bacterial fibrin clot to induce a non-lethal infection (INF, n=9) or a sterile fibrin clot (SHAM, n=6). Forty-two hours after clot implantation somatostatin was infused. In SHAM insulin and glucagon were infused to match the level seen in sham (9±1 µU/ml and 23±4 pg/ml). In infected animals insulin and glucagon were either infused to match the levels seen in infection (25±2 µU/ml and 101±15 pg/ml; INF-HI; n=5) or insulin was replaced to match the lower levels seen in SHAM (13±2 µU/ml), while glucagon was kept elevated (97±9 pg/ml; INF-LO; n=4). Then a 4-step (90 min each) hyperglycemic (120,150,200, 250 mg/dl) clamp was performed. NHGU (mg/kg/min) increased at each glucose step in SHAM (3.6±0.6 to 5.4±0.7 to 8.9±0.9 to 12.1±1.1); the slope of the relationship between glucose levels and NHGU (i.e. glucose dependency) was higher than that seen in non-adapted animals. Infection impaired glucose-dependent NHGU (mg/kg/min) in both INF-HI (1.3±0.4 to 2.9±0.5 to 5.5±1.0 to 7.7±1.6) and INF-LO (0.5±0.7 to 2.2±0.6 to 4.2±1.0 to 5.8±0.8). In summary: TPN augments glucose dependent NHGU, the presence of infection decreases glucose-dependent NHGU and the accompanying hyperinsulinemia associated with infection does not sustain the glucose dependency of NHGU.
Introduction

The liver is an important site of glucose uptake during the fed state (23) removing about 1/3 of an oral glucose load (17). The liver’s capacity to remove glucose is further enhanced during continuous nutritional support (3). The magnitude of liver glucose uptake (NHGU) is determined primarily by three variables: liver blood flow, inflowing glucose concentration to the liver and net fractional hepatic glucose extraction (23). Liver blood flow increases only modestly (↑20%) in response to mixed meal feeding (18). In contrast glucose concentration can change as much as 2-fold and the net fractional hepatic glucose extraction can vary from zero to 5% during a meal to as high as 15% during nutritional support. Thus, glucose concentration and net fractional hepatic glucose extraction are the regulated physiologic variables that primarily determine the magnitude of liver glucose uptake.

The glucose dependency of liver glucose uptake is influenced by the route of glucose delivery and the prevailing insulin levels. In the overnight fasted state a two-fold increase in glucose concentration in the presence of basal insulin level suppresses liver glucose production and switches the liver to be a modest consumer of glucose (~0.5 mg/kg/min). This is accompanied by a modest rise (1%) in net fractional hepatic glucose extraction (20). When combined with a physiologic rise in insulin (4 x basal) NHGU increases to ~2 mg/kg/min. NHGU is further enhanced up to ~4 mg/kg/min if the glucose is administered into the portal vein, which activates the portal signal (23).

It is unclear whether the liver will respond to the normal physiologic regulators of liver glucose uptake in the TPN-adapted state. With this adaptation liver glucose uptake
(~4 mg/kg/min) and fractional extraction (15%) are already high in the absence of significant hyperglycemia (120 mg/dl) or hyperinsulinemia (1.2 x basal). We previously observed that the adapted liver is poorly responsive to increases in insulin (6). In contrast to the non-adapted setting where a four fold increase in insulin increases liver glucose uptake more than 4-fold, in the TPN-adapted setting a four fold rise in insulin only enhanced liver glucose uptake by 25%. Whether, this augmentation in hepatic glucose uptake and fractional extraction seen during TPN would alter the dependency of liver glucose uptake on glucose concentration is unknown.

During hypermetabolic infection liver glucose uptake is markedly curtailed despite accompanying hyperinsulinemia. This impairment is seen both in response to an acute glucose load into a peripheral vein (15) as well as in the chronic TPN-adapted setting (14). The mechanism for this impairment is unknown, however, accompanying increases in glucagon, other stress hormones and cytokines and hepatic insulin resistance likely contribute. Despite this impairment NHGU remains greater than that which would be seen in the non-adapted setting. It is unclear if the infection-induced impairment in NHGU would alter the dependency of liver glucose uptake on the prevailing glucose levels. Moreover it is unclear if the accompanying hyperinsulinemia, which limits the fall in NHGU (5), would help to sustain the glucose dependency of NHGU. If NHGU is more dependent upon the prevailing glucose levels than on the compensatory hyperinsulinemia, then despite the underlying impairment in liver glucose uptake, treatment options (e.g. insulin infusion) that lower arterial glucose levels may not correct the already low liver glucose uptake.
Thus the aims of the study were to determine 1) if the glucose dependency of liver glucose uptake is augmented by TPN, 2) if infection impairs the glucose dependency of liver glucose uptake, and 3) if insulin can modulate the glucose dependency of liver glucose uptake during infection. To address these aims net hepatic glucose uptake was assessed in chronically catheterized normal and infected dogs receiving TPN.

**Methods**

*Animal preparation.* Fifteen female non-pregnant mongrel dogs were fed standard Kal-Kan meat (Vernon, CA) and Purina Lab Canine Diet #5006 (Purina Mills, St. Louis, MO) 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 American Association for the Accreditation of Laboratory Animal Care International Guidelines. The protocols were approved by the Vanderbilt University Medical Center Animal Care Committee. The health of the animals was determined before surgery and before TPN administration as having: a good appetite (i.e., consumed at least \(\frac{3}{4}\) of the daily ration), normal stools, hematocrit >35%, and leukocyte count < 18,000 mm\(^3\).

*Experimental preparation.* 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, Silastic catheters (0.03 in. ID) were placed in the splenic and jejunal veins, and the gastroduodenal vein was ligated. 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); the free ends were then exteriorized and tunneled.
subcutaneously behind the left clavicle. Flow probes (Transonic Systems, Ithaca, NY) were positioned about the portal vein, hepatic artery, and right external iliac artery. After an incision was made in the left inguinal region, 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. After an incision in the right inguinal region, a catheter was advanced from the right external iliac artery to the abdominal aorta.

All 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. Flunixamine (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.

Nutritional support. After allowing at least 14 days for recovery from surgery, each dog’s IVC catheters were exteriorized under local anesthesia (2% Lidocaine; Abbott, North Chicago, IL). TPN was infused into one of these catheters with an ambulatory infusion pump (Dakmed, Buffalo, NY). Dogs wore a jacket (Alice King Chatham, Los Angeles, CA) with two large pockets for the TPN bag and pump.

The dogs received TPN as the sole exogenous caloric source for 5 days. The TPN was designed to be isocaloric, based on predicted resting energy expenditure (25). The composition of the TPN included glucose, lipids, amino acids, saline (2.9 ml·kg\(^{-1}\)·min\(^{-1}\)), potassium phosphates (90 mg/kg/day), and a multivitamin supplement (MVI-12; Astra USA, Westborough, MA). Glucose (50% dextrose, Abbott) made up 75% of the nonprotein calories, and a fat emulsion (20% Intralipid; Baxter Healthcare, Deerfield, IL)
constituted the remaining 25% of the energy requirements. Travasol (Baxter) was infused to supply basal nitrogen requirements (~12 g protein/day), calculated with the formula 1.5 × body wt^{0.67} (in kg).

*Induction of infection.* A fibrin clot containing a nonlethal dose (2 × 10^9 organisms/kg body wt) of *Escherichia coli* was prepared. The dose of bacteria was determined by serial dilution followed by plating. Bacteria (ATTC #25922) were prepared by inoculation of 1 liter of trypticase soy broth (Becton Dickinson, Cockeysville, MD) and incubation overnight at 37° C. Bacteria were pelleted by centrifugation and then washed with and reconstituted in sterile saline. A 1% fibrinogen (10 ml/kg body wt; Sigma, St. Louis, MO) solution was filtered (0.45 µm diameter) under sterile conditions. Bacteria were mixed with the filtrate, and thrombin (1,000 U; Gentrac, Middleton, WI) was added to initiate clot formation.

On the third day of the 5-day TPN infusion, a second laparotomy was performed under anesthesia. An abdominal midline incision was made at a point caudal to that made during the first surgery; the infected clot was then implanted in the peritoneal cavity. Animals received 500 ml of saline during the laparotomy and 1000 ml the next day. During the course of the infection, animals were monitored closely. The same procedure was followed for Sham (n=6) dogs, except they received a sterile clot and 500 ml of saline on the subsequent day. Infected animals (n=9) were typically normotensive, hyperthermic, and tachycardic, with a characteristic increase in hepatic arterial blood flow (HABF) (14).

*Experimental protocol.* A study was performed 42 h after placing the clot, which was on the fifth day of the 5-day TPN infusion. The free ends of all catheters were
exteriorized under local anesthesia, 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, Ithaca, NY). 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, dextrose, and somatostatin (SRIF; Bachem, Torrance, CA). Primed (44 and 27 µCi) constant infusions (0.4 and 0.3 µCi/min) of [3-\(^3\)H]- and [U-\(^14\)C]glucose (New England Nuclear, Wilmington, DE), respectively, were begun at least 120 min before sampling.

In all animals a pancreatic clamp was performed in which SRIF was infused (0.8 µg/kg/min) to suppress endogenous insulin and glucagon secretion. In one group of infected animals insulin (porcine regular Iletin II; Eli Lilly, Indianapolis, IN) and glucagon (Eli Lilly) were replaced intraportally by infusion into the splenic and jejunal veins at rates of 1,000 µU/kg/min and 2.5 ng/kg/min, respectively, to simulate the levels seen during infection (INF-Hi; n=5). The other infected group received insulin at a rate designed to match the concentration seen in the SHAM group (400 µU/kg/min; INF-Lo; n=4) and received glucagon (2.5 ng/kg/min) to simulate the levels seen during infection. For the SHAM group (Sham; n=6) insulin (400 µU/kg/min), and glucagon (0.1 ng/kg/min) were infused to match the levels observed previously in sham animals (14).

All solutions were infused with calibrated syringe pumps (Harvard Apparatus, Holliston, MA). The glucose infusion rate (GIR) in the TPN was adjusted regularly to obtain the desired glucose levels, whereas the other components of the TPN were infused at the constant rate for the duration of the experiment.
The study had three periods: a tracer equilibration period (0-80 min), a basal sampling period (80-120 min, basal), and a 270 min experimental period. During the tracer equilibration and basal sampling periods the arterial glucose levels were clamped at 120 mg/dl. During the experimental period the arterial glucose levels were increased in a step-wise (90 min/step) manner from 120 to 150 to 200 to 250 mg/dl. Each experimental period step consisted of a 60-min glucose equilibration period and a 30-min steady-state sampling period during which substrate concentrations and balance were determined. Body temperature (Yellow Springs Instruments, Yellow Springs, OH), blood pressure, and heart rate (Micro-Med, Louisville, KY) were assessed during the study. Small blood samples (0.4 ml) were taken every 5-10 min and centrifuged immediately to measure arterial plasma glucose concentration with a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA). Saline was infused to replace the blood volume withdrawn by sampling. At least 80 min elapsed between initiation of the pancreatic clamp and blood sampling. Blood samples were obtained from the artery, portal vein, hepatic vein, and iliac vein every 20 min during the 40-min basal period and every 15 min during the 30-min glucose steady-state sampling period at each experimental period step. Blood flows and hematocrit were recorded at each sampling period. 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 were freeze-damped with Wallenburg clamps pre-cooled in liquid nitrogen and were stored at -70° C until analysis. The entire liver was removed rapidly and weighed.

*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 (12). Blood $^{14}$CO$_2$ was assessed in triplicate on arterial, portal vein, and hepatic vein samples (22). Blood samples were centrifuged at 3,000 rpm for 10 min. For the glucagon assay, 1 ml of plasma was added to 50 µl of Trasylol (500 kallikrein inhibitor units; Miles, Kankakee, IL). To assess plasma $[^3]$H- and $[^14]$C-glucose specific activity (SA), plasma (0.5 ml) sample were deproteinized with Ba(OH)$_2$ and ZnSO$_4$ (24) and charged intermediates were removed (13). The remaining plasma was stored at -70º C for later analyses.

**Analysis.** Immunoreactive insulin and glucagon were assayed using a double antibody technique [(19) intra-assay coefficient of variation (CV) 11% and 10%, respectively], and cortisol was assayed with Diagnostic Products (Los Angeles, CA) RIA Kit (CV 12%) (7) HPLC methods were used to assess plasma epinephrine and norepinephrine (CV 15% and 12% respectively) (8).

Analysis of lactate, alanine, and glycerol in perchloric acid extracts of blood were performed on an automated centrifugal analyzer (Monarch 2000; Instrumentation Laboratory, Lexington, MA) using a modification of the method of Lloyd et al (11). The plasma concentration of nonesterified fatty acids (NEFA) was determined spectrophotometrically (Wako Chemicals, Richmond, VA).

**Calculations.** The hepatic substrate load (Load In) was calculated as $A_s \times HABF + PV_s \times PVBF$, where $A_s$ and $PV_s$ represent the blood or plasma 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 $HV_s \times HBF$, in which $HV_s$ and HBF represent the hepatic vein substrate concentration and total hepatic blood (HABF + PVBF) or plasma flow.
[blood flow × (1-hematocrit)]. 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.

These equations were used to calculate net hepatic glucose, $^{14}\text{CO}_2$, lactate, alanine, glycerol, and NEFA balances. Plasma glucose was converted to blood glucose by a correction factor of 0.73. Unidirectional hepatic glucose uptake (HGU) was calculated as the ratio of hepatic $[^3\text{H}]$-glucose uptake and the corresponding $[^3\text{H}]$-glucose inflowing glucose SA. In cases where the liver was a net producer of substrate (i.e., negative uptake), these data were presented as positive values and denoted as net output. The liver can simultaneously produce and consume glucose. Thus, hepatic glucose production (HGP) was calculated as the difference between unidirectional uptake and net uptake (HGP = HGU - NHGU). Net hindlimb glucose uptake was calculated with the formula $(A_g - V_g) \times ABF$, where $A_g$ and $V_g$ represent glucose concentrations in the iliac artery and iliac vein and ABF represents blood flow in the iliac artery. Net non-hepatic glucose uptake was the difference between exogenous GIR and NHGU. Net intestinal glucose uptake was calculated with the formula $(A_g - PV_g) \times PVBF$, where $PV_g$ represents the glucose concentration in the portal vein.

Hepatic conversion of glucose to CO$_2$ (hepatic glucose oxidation) was calculated as the ratio of net hepatic $^{14}\text{CO}_2$ production rate and the hepatic $[^1\text{C}]$-glucose precursor SA. The hepatic $[^1\text{C}]$-glucose precursor SA was considered to be the $[^1\text{C}]$-glucose SA in the flowing blood to the liver. Net hepatic glucose storage was the portion of NHGU not accounted for by glucose oxidation and NHLR.
Net deposition of glycogen in liver derived from plasma glucose was calculated by dividing hepatic \[^{14}\text{C} \text{-glucose accumulation (dpm/kg/min)}\] by the average inflowing \[^{14}\text{C} \text{-glucose SA}\]. This is an underestimate of glycogen synthesis from glucose 6P to the extent that glucose 6P specific activity is diluted by non-plasma glucose sources of glucose 6P derived from gluconeogenesis or glycogen turnover.

**Enzyme analysis.** Hepatic glycogen content was determined using the enzymatic method of Chan and Exton(2). Tissue glucokinase (GK) and glucose-6-phosphatase (G6Pase) activities were analyzed on the quadrate lobe with the methods described by Barzilai and Rossetti(1). Total GK activity was calculated as the difference between activities at 100 and 0.5 mM glucose. G6Pase was measured at 10 mM glucose-6-phosphate (G6P). Protein content was assessed with the Biuret Method. Glycogen synthase (GS) activity was assessed in the presence (total) and absence (active) of 6.6 mM Glucose 6 phosphate (G6P) (26). The ratio of these two activities (-G6P/+G6P) is an estimate of the activation state of the enzyme. Glycogen phosphorylase activity was assessed in the presence of 7.5 mM AMP (total) or 0.75 mM caffeine (active) (9). Tissue G6P, and fructose-2,6-bisphosphate (F2,6P\(_2\)) were analyzed using fluorometric enzymatic methods (16;28).

**Statistics.** All mean and SE values reported for the basal period are the means of the 40-min period using three time points. For the experimental periods, the mean of the last 30 min of each glucose step was used. Statistical comparisons were made with two-way ANOVA vs. change from basal for infected groups followed by an *F* test (SYSTAT, Evanston, IL), and one-way ANOVA when comparing Sham and infected groups. *P* < 0.05 was regarded as significant.
Results

Hemodynamic Parameters. Body temperature, heart rate, mean arterial pressure, and blood flow (hepatic artery, portal vein and iliac artery) were measured in SHAM, INF-Hi and INF-Lo groups (Table 1). Infected groups showed characteristic increases in body temperature, heart rate and hepatic arterial blood flow. Portal vein and iliac arterial blood flow was not different between the three groups during the basal period. Blood flow did not change over time in any group during experimental period (data not shown).

Hormone concentrations. Arterial plasma insulin concentration was elevated in the INF-Hi group (9±1 vs. 25±2 µU/ml; SHAM vs. INF-Hi, p<0.05). The insulin concentration in INF-Lo group was reduced to 13±2µU/ml (Fig. 1). In SHAM and INF-Hi groups, insulin levels remained constant as glucose levels were increased. In INF-Lo, however, insulin levels did not remain constant. While insulin levels remain significantly below that seen in INF-Hi, somatostatin was only partially effective in suppressing endogenous insulin secretion in INF-Lo. As expected the arterial plasma glucagon concentration was higher (p<0.05) in the infected groups (101±15pg/ml, and 97±9pg/ml, INF-Hi and INF-Lo, respectively) compared with SHAM group (23±4pg/ml) and remained elevated for the duration of the experiment. Cortisol, epinephrine, and nonepinephrine did not significantly change overtime (Data not presented).

Whole body glucose metabolism. The arterial plasma glucose concentration (Fig. 1) was similar during the basal period (118±2, 122±2 and 125±1mg/dl; SHAM, INF-Hi and INF-Lo; respectively). Following the basal period the arterial glucose concentration was increased in a stepwise manner to ~150, 200 and 250 mg/dl in all groups by increasing the glucose infusion rate (GIR; Fig. 2). The GIR in SHAM and INF-Hi groups
were similar at each glucose level \((p=0.099; \text{SHAM vs. INF-Hi})\). In INF-Lo the GIR was consistently lower than in INF-Hi at each glucose level \((p<0.05)\).

**Hepatic glucose kinetics.** In each group hepatic glucose load \((HGL)\) increased as glucose concentration was increased (Fig. 2). At each glucose step HGL was lower \((p<0.05)\) in the SHAM than in both INF-Hi and INF-Lo groups. NHGU was greater in SHAM at each glucose level than in both INF-Hi and INF-Lo groups \((p<0.05, \text{SHAM vs. INF-Groups}), \text{Fig 3}\)\). Net fractional hepatic glucose extraction \((\text{NFHGE})\) was markedly decreased by infection \((p<0.05)\). While NFHGE increased progressively as arterial blood glucose concentration was increased in all groups; it appeared to saturate at high arterial glucose concentrations in the infected group (Fig 3). Both NHGU and NFHGE were not different between INF-Hi and INF-LO as glucose levels were increased. The glucose dependency of NHGU as evidenced by the slope of the relationship between NHGU and arterial glucose levels was \((p<0.05)\) decreased \((6.5\pm0.4 \text{ vs. } 4.2\pm0.3 \text{ ml/kg/min; SHAM vs. INF-Lo})\) and the compensatory hyperinsulinemia did not alter this dependency \((4.0\pm0.2; \text{INF-Hi})\).

The dependency of NHGU on HGL is presented in Fig. 4. Although NHGU increased in all three groups with increases in the hepatic glucose load, NHGU in SHAM group was greatest at each glucose level \((p<0.05)\). Moreover the disparity between the SHAM and infected groups increased as the glucose loads were increased. The glucose load dependency of NHGU as evidenced by the slope of the relationship between NHGU and hepatic glucose load was markedly \((p<0.05)\) decreased \((0.28\pm0.01 \text{ vs. } 0.14\pm0.02; \text{SHAM vs. INF-Lo})\) and the compensatory hyperinsulinemia did not alter this dependency \((0.12\pm0.04; \text{INF-Hi})\)
Unidirectional HGU in the basal period was 4.1±0.5, 1.1±0.2 and 2.1±0.6 mg/kg/min in SHAM, INF-Hi and INF-Lo groups. HGU increased as the arterial glucose concentration was increased in all three groups (SHAM: Δ1.5±0.5, Δ4.6±0.8, Δ7.1±1.0mg/kg/min; INF-Hi: Δ0.6±0.3, Δ3.4±0.6, Δ6.2±1.0mg/kg/min; INF-Lo: Δ1.7±0.3, Δ1.4±1.0, Δ5.3±1.2 mg/kg/min). Basal hepatic glucose production (HGP) was similar in SHAM and INF-Hi groups (0.5±0.2, 0.4±0.4 mg/kg/min, respectively). However, it was elevated (1.7±0.8 mg/kg/min; p=0.05) in INF-Lo group. HGP did not significantly change in all three groups as arterial glucose levels increased (Δ-0.4±0.5, Δ-0.7±0.7, Δ-1.2±0.8; Δ-1.1±0.7, Δ-0.4±0.4, Δ1.01±0.9; Δ-0.1±0.5, Δ-2.6±0.9, Δ-0.03±1.4 mg/kg/min; SHAM, INF-Hi and INF-Lo; respectively).

Hepatic substrate kinetics. The basal arterial lactate concentrations were similar in INF-Hi (7.3±0.8, 5.9±0.8, and 6.6±0.4 mg/dl; SHAM, INF-Hi and INF-Lo; respectively). The arterial blood lactate concentrations rose in a similar manner in the three groups as the glucose concentration was increased. Net hepatic lactate release (NHLR) in the SHAM group was greater (2.4±0.4 mg/kg/min; p<0.05) than in both INF-Hi and INF-Lo groups (0.5±0.3 and 0.9±0.3mg/kg/min). The fraction of NHGU converted to lactate was decreased by 67% and 54% in INF-Hi and INF-Lo groups. NHLR did not increase as arterial blood glucose concentration and NHGU increased in each group (Fig. 5). Consequently, the fraction of NHGU converted to lactate did not increase as arterial glucose levels-rose in all three groups.

During the basal period the arterial alanine concentration was decreased with infection (p<0.05, SHAM group vs. INF- groups) (Table 2). Arterial alanine concentration increased (p<0.05) in all groups in response to increases in arterial glucose
concentration. Net hepatic alanine uptake (NHAU) was lower (P<0.05) in the SHAM group than in both infected groups during the basal period. NHAU increased in SHAM and INF-Lo as arterial glucose levels increased. Net fractional hepatic alanine extraction was increased in the infected groups (p<0.05), but was not altered by hyperglycemia.

Arterial glycerol concentration and hepatic glycerol uptake were not altered by infection (Table 2). Lowering of insulin concentration in INF-Lo did not alter arterial glycerol concentration or net hepatic glycerol uptake. There were no significant changes in glycerol metabolism in the experimental period for all three groups.

Arterial plasma NEFA concentrations in the basal period were similar in all groups and were unaltered during hyperglycemia. However, net hepatic NEFA uptake was greater in both INF-Hi and INF-Lo groups than in SHAM group in the basal and hyperglycemia periods. When arterial glucose levels were increased, net hepatic NEFA uptake did not change.

Basal hepatic glucose oxidation rate was similar in the three groups (0.3±0.1, 0.5±0.1, 0.4±0.1 mg/kg/min, SHAM, INF-Hi and INF-Lo, respectively). Hepatic glucose oxidation increased to a similar extent as arterial glucose levels increased in the three groups. NHLR and glucose oxidation could account for the majority of hepatic glucose disposition during the basal period in all groups. However as HGL increased glucose storage [NHGU-(NHLR+glucose oxidation)] was the primary fate of the additional glucose disposed of by the liver (Fig. 6), shown as the area between the NHGU and NHRL + CO₂. Moreover the net rate of glucose storage was substantially greater (p<0.05) in the SHAM group than in both infected groups at all elevated glucose loads.
The glucose was primarily stored as glycogen, as evidenced by substantial tracer incorporation into liver glycogen (Table 3).

Liver glycogen content was significantly higher in the SHAM group than in the infected groups. The glucose-6-phosphate concentration, glucose-6-phosphatase and glucokinase activities in liver in SHAM, INF-Hi and INF-Lo were not significantly different (Table 3). However the glycogen synthase activity and activity ratio as well as fructose 2,6 bis-phosphate were decreased in the infected groups. Glycogen phosphorylase activity was increased (p<0.05) in the infected groups.

Non-hepatic, hindlimb and intestine glucose metabolism. (Fig 7) Basal non-hepatic glucose uptake (Non-HGU) was higher in INF-Hi (8.1±1.1mg/kg/min; p<0.05) than in both the SHAM and INF-Lo groups (5.1±0.7 and 4.6±0.9 mg/kg/min). Following the stepwise increase in arterial plasma glucose concentration, non-HGU rose in all three groups. The glucose dependency of Non-HGU as evidenced by the slope of the relationship between NHGU and arterial glucose levels was decreased (p<0.05) by infection (6.4±1.5 vs. 1.6±0.7 ml/kg/min; SHAM vs. INF-Lo) and the compensatory hyperinsulinemia did not alter this dependency (2.8±0.9 ml/kg/min; INF-Hi). Because of the greater glucose dependency in the SHAM group than in the INF-Hi group at high glucose levels Non-HGU was not significantly different in the SHAM and INF-Hi groups. Net hindlimb glucose uptake was not significantly different between SHAM, INF-Hi and INF-Lo (Table 4). At a glucose level of 120 mg/dl net fractional hind limb glucose extraction was lower (p=0.05) in INF-Lo as compared to INF-Hi. Net intestinal glucose uptake (Table 4) was not altered by infection and did not increase as glucose levels increased.


Discussion

The arterial glucose concentration is an important determinant of liver glucose uptake following an acute glucose load. During chronic TPN the liver adapts to become an even more efficient consumer of glucose. However infection impairs the TPN induced adaptive response. Our studies demonstrate that in addition to enhancing NHGU, TPN augments the glucose dependency of NHGU. Moreover this TPN induced enhancement in glucose-dependent hepatic glucose disposal is markedly impaired by infection. Our studies also suggest that, the compensatory hyperinsulinemia seen during infection did not ameliorate the infection induced decrease in glucose-dependent NHGU. Surprisingly, despite the high rate of glucose conversion to lactate in the TPN-adapted liver, the additional glucose taken up by the liver in response to hyperglycemia was not released as lactate but was primarily stored as glycogen.

TPN enhances glucose-dependent liver glucose uptake. As we have seen previously liver glucose uptake was markedly increased by TPN in the presence of mild elevations of glucose and insulin (3). In the present study as glucose levels were increased liver glucose uptake increased in proportion to the rise in glucose levels. A rise in the arterial glucose levels from 120 to 250 mg/dl increased NHGU from 4.5 to 11.5 mg/kg/min. This glucose-dependent response in the TPN adapted setting is in marked contrast to the non-adapted setting where mild hyperglycemia (120 mg/dl) and hyperinsulinemia (15 µU/ml) would not initiate NHGU (20). Moreover even in the presence of elevated insulin levels a doubling of glucose load (Figure 4; shaded area) in the presence of hyperinsulinemia (~30 µU/ml) would only increase NHGU from 0.5 to 4 mg/kg/min (21).
Infection impaired glucose-dependent NHGU. As we have seen previously, infection impairs liver glucose uptake despite accompanying hyperinsulinemia and mild hyperglycemia (14). NHGU was decreased by ~50% at each glucose concentration in the infected groups. However not only was NHGU decreased but the dependency of NHGU on arterial glucose levels was decreased by 35%. The extent of the defect in the responsiveness of the liver to take up glucose during infection is even more apparent if one examines the relationship between hepatic glucose load and NHGU (Fig 4). The slope of this relationship was decreased by more than 50%. Interestingly, in contrast to the normal animal where both net fractional hepatic glucose extraction and NHGU rose as glucose was increased, net fractional hepatic glucose extraction tended to saturate at high glucose levels in the infected animal thus limiting the rise in NHGU (Fig 3).

Hyperinsulinemia accompanying infection did little to improve glucose-dependent liver glucose uptake. In our previous study (5), the compensatory rise in insulin helped to limit the fall in NHGU (Δ1.4±0.5 mg/kg/min) during infection in the presence of mild hyperglycemia (120 mg/dl). In this study, NHGU tended to be lower (Δ0.8 mg/kg/min) when insulin was suppressed, however this was not significant. In part this may be due to the already low NHGU in INF-HI, which could not be suppressed further by lowering insulin levels. The slope of the relationship between NHGU and either glucose levels or glucose load was identical in the two infected groups. This suggests that additional insulin does little to correct the infection induced impairment in glucose-dependent NHGU. There are however two caveats. First, in INF-Lo at the highest glucose levels insulin levels rose indicating that somatostatin was not completely effective in suppressing endogenous insulin secretion. Second, the limited number of
animals in the INF-Lo group prevented us from having the statistical power to detect less than a 50% change in glucose dependent liver glucose uptake when insulin was suppressed in the infected setting. The break-through in insulin secretion occurred at only the highest glucose level. Despite this the insulin levels remained lower than that seen in INF-Hi. Even when we calculated the slope of the relationship between NHGU and glucose load after excluding the highest glucose load (0.22±0.06 vs.0.15±0.03; INF-Lo vs. INF-Hi ) there was no detectable insulin dependent effect. Thus, the failure of somatostatin to completely suppress insulin secretion did not prevent us from detecting an insulin-dependent effect. Consistent with this, liver biopsies taken at the end of the study indicated that insulin was unable to modulate glucokinase, glucose-6-phosphatase or glycogen synthase or phosphorylase activities. The small number of animals in the INF-LO group prevented us from detecting less than a 50% change in glucose dependent NHGU in the infected group. However given the already low NHGU during infection less than a 50% change in glucose dependent liver glucose uptake would have little impact on hepatic or whole body glucose disposal.

Impairment in glycogen synthesis is the mechanism for the infection induced decrease in NHGU. While the major metabolic fate of glucose within the liver in the TPN adapted state is diversion to glycolysis and subsequent release as lactate, acute increases in arterial glucose levels did not increase net hepatic lactate release. The primary metabolic fate was glycogen. This was confirmed by gradual rise net hepatic glucose storage (Fig. 7) and significant incorporation of $^{3}$H glucose into glycogen. A likely explanation for this is a glucose dependent increase in glycogen synthase activity (4). Infection decreased both glucose-dependent NHGU and glycogen synthesis (Fig. 7).
This impairment was confirmed by both a decrease in net glycogen storage and $^3$H glucose incorporation into glycogen. The later will tend to overestimate the impairment in total glycogen synthesis in the infected groups to the extent indirect glycogen synthesis is higher in the infected group. However net glycogen storage was also suppressed and it takes into account differences in gluconeogenic precursor uptake. Analysis of liver biopsies obtained at the end of the study indicated that the impairment in glycogen synthesis was not due to differences in either glucokinase or glucose 6 phosphatase. However both total glycogen synthase and its activity ratio were markedly decreased and there was a corresponding rise in glycogen phosphorylase. Thus the ratio of the active glycogen synthase and phosphorylase fell by over 90%. Moreover the fall in hepatic glycolysis seen during infection is likely explained by an 80% reduction in fructose 2,6 bis-phosphate levels during infection. The mechanism for the enzymatic alterations is likely due to hepatic insulin resistance and the accompanying hyperglucagonemia of infection.

The compensatory hyperinsulinemia serves to overcome the infection induced impairment in liver glucose uptake by diverting the exogenous glucose to peripheral (i.e. non-hepatic) tissues. As we have seen previously at basal glucose levels (120 mg/dl), non-hepatic glucose uptake was enhanced with infection (INS-HI) relative to the SHAM group. (5). Non-hepatic glucose uptake was decreased in INF-Lo to the rate seen in the SHAM group. Interestingly glucose-dependent non-hepatic glucose uptake (i.e. slope of the relationship between glucose levels and Non-HGU) was impaired by infection (p<0.05). The additional insulin seen during infection raised non hepatic glucose uptake, however, it did not augment the glucose dependency of non hepatic glucose uptake. The
failure of insulin to increase glucose dependent glucose uptake is consistent with insulin resistance in the periphery during infection (5;10). As we have seen previously (6) at low insulin levels detecting a decrease in insulin mediated peripheral glucose uptake is difficult, because infection enhances insulin-independent glucose uptake. It is only when insulin levels are increased that the insulin resistance is apparent.

As a consequence of the pronounced effects of infection on hepatic metabolism and its resistance to the effects of insulin, the compensatory hyperinsulinemia seen during infection primarily corrects hyperglycemia by diverting exogenous glucose away from the liver and to peripheral tissues. For any given GIR the compensatory hyperinsulinemia lowers the glucose level required to dispose of the exogenous glucose. While the infection induced decrease in NHGU at any given glucose level is limited by the rise in insulin, the consequent lowering of the glucose levels further diminishes the contribution of the liver to whole body glucose disposal. For example in the infected groups to dispose of 15 mg/kg/min of exogenous glucose the arterial glucose levels would have to be 200 and 250 mg/dl at the high and low insulin concentrations, respectively. However NHGU decreases as glucose levels decrease despite the rise in insulin levels. Thus the proportion of the infused glucose (GIR) taken up by hepatic tissues will decrease as the insulin concentration is increased.

In summary, in the TPN adapted setting net hepatic glucose uptake is directly dependent on the hepatic glucose load over a wide range of glucose levels. The presence of infection decreases the glucose dependency of net hepatic glucose uptake, and the accompanying hyperinsulinemia does not help to sustain this glucose dependency. Rather the additional insulin minimizes hyperglycemia and diverts the exogenous glucose to
peripheral tissues. Despite the marked elevation in hepatic lactate release during TPN, a glucose dependent increase in NHGU does not enhance hepatic glycolysis. The primary metabolic fate is glycogen synthesis.

**Significance:**

A recent clinical trial indicates that aggressive normalization of glucose levels in hospitalized ICU patients decreases morbidity and mortality (27). Aggressive glucose normalization necessitates the infusion of insulin and consequent correction of hyperglycemia. Our studies suggest that in the TPN supported setting the infection induced hyperglycemia is sustained by the failure of the liver to adapt to TPN. The decreased contribution of the liver to whole body glucose disposal necessitates that peripheral tissues play an even bigger role in disposing of exogenous glucose. However in the infected setting the accompanying insulin resistance in peripheral tissues further aggravates the hyperglycemia and raises insulin requirements. Clearly the compensatory hyperinsulinemia seen during infection serves to normalize glucose levels. However it does so not by markedly reversing the infection induced impairment in liver glucose uptake, but by enhancing peripheral glucose uptake. Thus, while aggressive glycemic management corrects hyperglycemia it does not correct the underlying disturbances in hepatic metabolism.
Table 1. Body and liver weight, body temperature and basal hemodynamic parameters, in SHAM, INF-Hi, and INF-Lo groups receiving TPN for 5 days.

<table>
<thead>
<tr>
<th></th>
<th>SHAM</th>
<th>INF-Hi</th>
<th>INF-Lo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>21±1</td>
<td>22±2</td>
<td>22±1</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>890±91</td>
<td>1083±129</td>
<td>808±37</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>38.6±0.2</td>
<td>39.3±0.2*</td>
<td>39.2±0.6</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>125±3</td>
<td>121±16</td>
<td>110±9</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>110±16</td>
<td>122±8</td>
<td>134±8</td>
</tr>
<tr>
<td>Hepatic arterial blood flow (ml/kg/min)</td>
<td>9.0±1.0</td>
<td>28.7±5.2*</td>
<td>20.3±4.6*</td>
</tr>
<tr>
<td>Portal vein blood flow (ml/kg/min)</td>
<td>18.0±1.1</td>
<td>23.4±1.6</td>
<td>21.1±3.2</td>
</tr>
<tr>
<td>Iliac arterial blood flow (ml/kg/min)</td>
<td>10.0±1.0</td>
<td>7.0±1.0</td>
<td>8.9±0.7</td>
</tr>
</tbody>
</table>

Data are expressed as means ±SEM. INF-Hi, infected + high insulin; INF-Lo, infected + reduced insulin; TPN, total parenteral nutrition. *Statistically different from SHAM group.
Table 2. Arterial plasma metabolite concentrations, hepatic uptake, fractional hepatic extraction (F.E.), and hepatic glucose oxidation rate (CO\textsubscript{2}) in Sham, INF-Hi, and INF-Lo groups during the basal period and three steps of the experimental period.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Sham</th>
<th>INF-Hi</th>
<th>INF-Lo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Step I</td>
<td>Step II</td>
</tr>
<tr>
<td><strong>Concentration</strong></td>
<td>487±23</td>
<td>557±47\textsuperscript{*}</td>
<td>643±64\textsuperscript{*}</td>
</tr>
<tr>
<td><strong>Uptake</strong></td>
<td>0.8±0.2</td>
<td>0.9±0.4</td>
<td>0.8±0.3</td>
</tr>
<tr>
<td><strong>F. E.</strong></td>
<td>0.06±0.02</td>
<td>0.06±0.03</td>
<td>0.04±0.01</td>
</tr>
<tr>
<td><strong>Concentration</strong></td>
<td>67±14</td>
<td>62±11</td>
<td>59±10</td>
</tr>
<tr>
<td><strong>Uptake</strong></td>
<td>1.3±0.3</td>
<td>1.3±0.2</td>
<td>1.3±0.3</td>
</tr>
<tr>
<td><strong>F. E.</strong></td>
<td>0.67±0.03</td>
<td>0.72±0.04</td>
<td>0.70±0.03</td>
</tr>
<tr>
<td><strong>Concentration</strong></td>
<td>298±26</td>
<td>255±28</td>
<td>233±33</td>
</tr>
<tr>
<td><strong>Uptake</strong></td>
<td>0.3±0.2</td>
<td>0.2±0.2</td>
<td>0.1±0.2</td>
</tr>
<tr>
<td><strong>F. E.</strong></td>
<td>0.06±0.02</td>
<td>0.04±0.03</td>
<td>0.02±0.03</td>
</tr>
<tr>
<td><strong>Hepatic glucose oxidation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as means ±SEM. INF-Hi, infected + high insulin; INF-Lo, infected + reduced insulin; Concentrations are in µM; uptake rates are in µmol/kg/min; glucose oxidation rates are in mg/kg/min. *Statistically different from Sham group. +Statistically different from basal period in each group.
Table 3. Enzyme and substrate analysis of the liver in Sham, INF-Hi, and INF-Lo groups.

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>INF-Hi</th>
<th>INF-Lo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tracer determined glycogen synthesis (mg/g liver)</td>
<td>24.5±7.7</td>
<td>21.1±0.3 *</td>
<td>9.0±1.8*#</td>
</tr>
<tr>
<td>Glycogen (mg/g liver)</td>
<td>92.9±8.4</td>
<td>51.5±9.1*</td>
<td>32.4±5.6*</td>
</tr>
<tr>
<td>G-6-P (nmol/g liver)</td>
<td>594±171</td>
<td>544±111</td>
<td>438±92</td>
</tr>
<tr>
<td>G-6-Pase (nU/mg protein)</td>
<td>53±9</td>
<td>46±4</td>
<td>46±5</td>
</tr>
<tr>
<td>GK (µU/mg protein)</td>
<td>11±2</td>
<td>9±1</td>
<td>9±1</td>
</tr>
<tr>
<td>Glycogen phosphorylase (mU/mg protein)</td>
<td>0.27±0.05</td>
<td>0.66±0.09*</td>
<td>0.63±0.04*</td>
</tr>
<tr>
<td>Glycogen phosphorylase activity ratio</td>
<td>0.44±0.04</td>
<td>0.59±0.08</td>
<td>0.49±0.03</td>
</tr>
<tr>
<td>Glycogen synthase (mU/mg protein)</td>
<td>0.09±0.01</td>
<td>0.05±0.01*</td>
<td>0.05±0.01*</td>
</tr>
<tr>
<td>Glycogen synthase Activity ratio</td>
<td>0.21±0.04</td>
<td>0.14±0.03*</td>
<td>0.12±0.01*</td>
</tr>
<tr>
<td>Fructose 2,6 P_{2} (nmol/g liver)</td>
<td>40.06±11.22</td>
<td>10.45±1.75*</td>
<td>13.87±2.13*</td>
</tr>
</tbody>
</table>

Data are expressed as means ±SEM. INF-Hi, infected + high insulin; INF-Lo, infected + reduced insulin; G-6-P, glucose-6-phosphate; G-6-Pase, glucose-6-phosphatase; GK, glucokinase. * p<0.05 as compared to sham. # p<0.05 as compared to INF-Hi.
Table 4. Net hindlimb and intestinal glucose uptake and net hindlimb glucose fractional hepatic extraction (F.E.), in Sham, INF-Hi, and INF-Lo groups during the basal period and three steps of the experimental period.

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>INF-Hi</th>
<th>INF-Lo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Step I</td>
<td>Step II</td>
</tr>
<tr>
<td><strong>Hindlimb</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uptake</td>
<td>0.46±0.10</td>
<td>0.55±0.38</td>
<td>0.65±0.19</td>
</tr>
<tr>
<td>F. E.</td>
<td>0.05±0.01</td>
<td>0.05±0.01</td>
<td>0.05±0.01</td>
</tr>
<tr>
<td><strong>Intestinal</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uptake</td>
<td>0.86±0.21</td>
<td>0.85±0.21</td>
<td>1.08±0.30</td>
</tr>
</tbody>
</table>

Data are expressed as means ±SEM. INF-Hi, infected + high insulin; INF-Lo, infected + reduced insulin; uptake rates are in mg/kg/min. # Statistically different from INF-Lo group.
Figure Legends:

1. Arterial plasma glucose, insulin and glucagon concentrations in sham (SHAM, n=6) and infected chronically catheterized conscious dogs receiving either a low insulin infusion rate (INF-Lo; n=4) or an elevated insulin infusion (INF-Hi; n=5) during a 4 step hyperglycemic clamp. Data are expressed as means ±SEM.

2. The relationship between both the glucose infusion rate and hepatic glucose load and the arterial plasma glucose levels in sham (SHAM, n=6) and infected chronically catheterized conscious dogs receiving either a low insulin infusion rate (INF-Lo; n=4) or an elevated insulin infusion (INF-Hi; n=5). Data are expressed as means ±SEM.

3. The relationship between both net hepatic glucose uptake and net fractional hepatic glucose extraction and the arterial plasma glucose levels in sham (SHAM, n=6) and infected chronically catheterized conscious dogs receiving either a low insulin infusion rate (INF-Lo; n=4) or an elevated insulin infusion (INF-Hi; n=5). Shaded areas are the data from a non-adapted dog (Myers et. al.) Data are expressed as means ±SEM.
4. The relationship between both net hepatic glucose uptake and net fractional hepatic glucose extraction and hepatic glucose load in sham (SHAM, n=6) and infected chronically catheterized conscious dogs receiving either a low insulin infusion rate (INF-Lo n=4) or an elevated insulin infusion (INF-Hi; n=5). Shaded area represents data from the studies of Myers et al (20) in the non-adapted dog. In the non adapted dog net hepatic glucose uptake was 1.5±0.8, 3.6±2.0 and 6.3±3.9 mg/kg/min at the following glucose loads 50±6, 77±10 and 94±10 mg/kg/min, respectively. Data are expressed as means ±SEM.

5. The relationship between arterial blood lactate concentration and net hepatic lactate release and the arterial plasma glucose levels in sham (SHAM, n=6) and infected chronically catheterized conscious dogs receiving either a low insulin infusion rate (INF-Lo; n=4) or an elevated insulin infusion (INF-Hi; n=5). Data are expressed as means ±SEM.

6. The relationship between arterial glucose levels and both net hepatic glucose uptake and the sum of hepatic glucose oxidation and lactate release in sham (SHAM, n=6) and infected chronically catheterized conscious dogs receiving either a low insulin infusion rate (INF-Lo n=4) or an elevated insulin infusion (INF-Hi; n=5). Data are expressed as means ±SEM.

7. The relationship between non-hepatic glucose uptake and the arterial plasma glucose levels in sham (SHAM, n=6) and infected chronically catheterized conscious dogs receiving either a low insulin infusion rate (INF-Lo n=4) or an elevated insulin infusion (INF-Hi; n=5). Data are expressed as means ±SEM.
Net Hepatic Glucose Uptake (mg/kg/min)

- INF-Hi
- Sham
- INF-Lo

Arterial Plasma Glucose Concentration (mg/dl)

Net Fractional Hepatic Glucose Extraction

- INF-Hi
- Sham
- INF-Lo
Net Hepatic Glucose Uptake (mg/kg/min)

Hepatic Glucose Load (mg/kg/min)

Net Fractional Hepatic Glucose Extraction

INF-Hi
Sham
INF-Lo
Glucose Oxidation + Lactate Release
Net Hepatic Glucose Uptake

Arterial Plasma Glucose Concentration (mg/dl)
Non-hepatic Glucose Uptake (mg/kg/min) vs. Arterial Plasma Glucose Concentration (mg/dl)

INF-Hi
Sham
INF-Lo
References


