Impact of Intraportal Nω-Nitro-L-Arginine Infusion on Hepatic Glucose Metabolism in Total Parenteral Nutrition-Adapted Dogs: Interaction With Infection

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During chronic total parenteral nutrition (TPN), liver glucose uptake and lactate release are markedly elevated. However, in the presence of an infection, hepatic glucose uptake and lactate release are reduced. Glucose delivery (the product of liver blood flow and inflowing glucose concentration) is a major determinant of liver glucose uptake. Hepatic blood flow is increased during infection, and increased nitric oxide (NO) biosynthesis is thought to contribute to the increase. Our aim was to determine if the increase in liver blood flow served to limit the infection-induced decrease in hepatic glucose uptake and metabolism. Chronically catheterized conscious dogs received TPN for 5 days at a rate designed to match daily basal energy requirements. On the third day of TPN administration, a sterile (SHAM) or *Escherichia coli* (*E. coli*)-containing (INF) fibrin clot was implanted in the peritoneal cavity. Forty-two hours later, somatostatin was infused with intraportal replacement of insulin (10 ± 2 *v* 23 ± 2 μU/mL, SHAM *v* INF, respectively) and glucagon (22 ± 4 *v* 90 ± 8 pg/mL) to match concentrations observed in sham and infected animals. Tracer and arteriovenous difference techniques were used to assess hepatic glucose metabolism. Following a 120-minute basal sampling period, sham and infected animals received either intraportal saline or Nω-nitro-L-arginine (L-NNA; 37 μg · kg^{-1} · min^{-1}) infusion for 180 minutes. Isoglycemia (120 mg/dL) was maintained with a variable glucose infusion. In the infected group L-NNA infusion decreased hepatic arterial blood flow (23.3 ± 0.7 to 8.6 ± 0.5 mL · kg^{-1} · min^{-1}), but not portal vein blood flow. Neither portal vein nor hepatic artery blood flow were altered by L-NNA infusion in the sham group. Hepatic glucose uptake and lactate metabolism were not altered by L-NNA infusion in either group. In summary, during infection, an increase in NO biosynthesis contributes to the increase in hepatic arterial blood flow, while it exerts no effect on hepatic glucose metabolism.

THE LIVER IS AN important site of glucose uptake during the fed state.[1] Its importance increases during chronic total parenteral nutrition (TPN).[2] The magnitude of liver glucose uptake is determined primarily by 3 variables: the inflowing glucose concentration to the liver, net fractional hepatic glucose extraction, and liver blood flow. In the normal setting, liver blood flow only modestly changes in response to feeding.[3] Glucose levels change as much as 2-fold, and net fractional hepatic glucose extraction can vary from 0 to as high as 20%.[4]

The liver's importance as a site of glucose uptake and metabolism is markedly altered by the presence of infection.[2] Infection decreases liver glucose uptake as well as liver glucose metabolism despite a marked increase in liver blood flow. Hepatic arterial blood flow, which normally comprises approximately 20% of total hepatic blood flow, can increase as much as 4-fold during infection.[2] Portal vein blood flow generally does not increase. An increase in nitric oxide (NO) production, a potent vasodilator, likely contributes to the increase in hepatic artery blood flow.[5-7] The importance of the increase in liver blood flow in regulating hepatic metabolism during infection is unknown.

In metabolically active tissues, glucose uptake and metabolism are influenced by tissue blood flow; when tissue blood flow decreases, tissue glucose uptake decreases. Specifically in skeletal muscle, insulin-stimulated glucose uptake is dependent upon muscle blood flow.[8] In fact, an impaired vasodilatory response in muscle may contribute to the insulin resistance observed in diabetes and obesity.[9] In the liver, the dependency of liver glucose uptake on liver blood flow has not been extensively examined. In the liver of normal animals, acetylcholine-mediated increase in liver blood flow augment liver glucose uptake.[10] Given the dependency of tissue glucose uptake on tissue blood flow, the increase in liver blood flow seen during infection may minimize the impairment in liver glucose uptake and metabolism.

Thus, the aim of this study was to determine if inhibition of the increase in NO biosynthesis seen during hypermetabolic infection would decrease liver blood flow and exacerbate the impairment in hepatic glucose uptake and metabolism. To detect a blood flow-dependent change in liver glucose metabolism, we studied the impact of inhibition of NO biosynthesis in normal and infected animals receiving TPN so changes in hepatic glucose uptake and metabolism could readily be detected.

MATERIALS AND METHODS

Animal Preparation

Experiments were performed on 24 conscious female nonpregnant mongrel dogs (20.8 ± 0.5 kg). Before being studied, they received a diet consisting of KalKan meat (Vernon, CA) and Purina dog chow (St Louis, MO) once daily. The composition of the diet was 52% carbohydrate, 31% protein, 11% fat, and 6% fiber based on dry weight. The dogs were housed in a facility that met Association for Accreditation of

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Laboratory Animal Care International guidelines. The Vanderbilt University Animal Care Subcommittee approved the experimental protocols.

Experimental Preparation

Fourteen to seventeen days before a study, a laparotomy was performed under general anesthesia (isoflurane). Two infusion catheters (0.04-in internal diameter [ID]; Dow Corning, Helix Medical, Carpinteria, CA) were placed into the inferior vena cava for infusion of TPN. Silastic catheters (0.03-in ID) were placed into the splenic vein and a jejunal vein for intraportal infusion of insulin and glucagon and Nω-nitro-L-arginine (L-NNA). Sampling catheters (0.04-in ID) for blood sampling were inserted into the portal vein and the left common hepatic vein for blood sampling. Additional catheters (0.04-in ID) for blood sampling were inserted into the external iliac artery after an incision was made in the left inguinal area, and into the common femoral vein after an incision was made in the right inguinal region. The catheters were then filled with saline containing heparin (200 U/mL). Doppler flow probes were placed about the external iliac artery, portal vein, and hepatic artery after the gastroduodenal vein was ligated. The portal and hepatic vein sampling catheters and the Doppler flow probe leads were exteriorized and placed in a subcutaneous pocket in the abdominal area. The free ends of the inferior vena infusion catheters were exteriorized, tunneled subcutaneously, and placed under the skin between the clavicles. The femoral artery and iliac vein sampling catheters were placed under the skin in the inguinal region.

Two weeks after catheter implantation, all animals had (1) a good appetite (consuming the entire daily ration), (2) a normal stool, (3) a hematocrit above 35%, and (4) a leukocyte count less than 18,000 mm^{-3} before the study.

Nutritional Support

Fourteen days after catheter implantation, dogs were switched to TPN as the sole exogenous calorie source for a 5-day period. The nutritional support was designed to match their calculated basal energy requirements [144 + (62.2 $\times$ BW) kcal/d^{11}], in which BW is body weight in kilograms. Nitrogen (grams of protein/d) requirements were calculated according to the formula (1.5 $\times$ $BW^{0.67}$). This was met by the infusion of travasol (10%; Clintec Nutrition Division, Baxter Healthcare, Deerfield, IL). Twenty-five percent of the nonprotein energy requirements were derived from fat (20% intralipid, Fresenius Kabi Clayton, L.P., Clayton, NC) and 75% from dextrose (50%; Clintec Nutrition Division, Baxter Healthcare Corporation). The TPN also included potassium phosphate (90 mg potassium $\cdot$ kg^{-1} $\cdot$ d^{-1}) and sodium chloride (0.9%; 2.9 mL $\cdot$ kg^{-1} $\cdot$ d^{-1}), as well as a multivitamin infusion (MVI-12; Astra USA, Westborough, MA).

Experimental Design

TPN was given via a catheter placed into the inferior vena cava at the time of surgery. The dog was placed in a jacket (Alice King Chatham, Los Angeles, CA) containing a pocket into which a portable infusion pump (Dakmed Model AL-6-8L, Dakmed, Buffalo, NY) was placed. The TPN was given continuously over the 5-day period. TPN was prepared under sterile conditions and changed once daily. Dogs had free access to water. On the third day of TPN, the dogs were anesthetized with a short-acting anesthetic (15 mg $\cdot$ kg^{-1} thiamyl sodium) and a fibrin clot with (infected group, n = 12) or without (Sham group, n = 12) *Escherichia coli* (*E. coli*) (2 $\times$ 10^9 organisms/kg BW) added was placed into the peritoneal cavity using sterile techniques.[12] In addition to the TPN, dogs received 500 mL of saline resuscitation during the surgical procedure. Eighteen hours after clot implantation, additional saline was given to the sham (500 mL) and infected (1,000 mL) groups. Additional saline was given to the infected animals because they were more dehydrated than the sham animals. TPN was continued up to and including the day of the study (day 5).

Experimental Protocol

On day 5, 42 hours after clot implantation, the sampling catheters and free ends of the Transonic flow probes were removed from subcutaneous pockets under local anesthesia (2% lidocaine, Abbott Laboratories, North Chicago, IL). The dog was then placed into a Pavlov harness. Intravenous catheters (20 gauge, Angiocath, Becton Dickinson Vascular Access, Sandy, UT) were inserted percutaneously into the right and left cephalic veins. The chronic TPN was discontinued and replaced with a TPN solution of identical composition except that it did not contain glucose. An equal volume of saline was included instead of glucose. The modified TPN was infused for the duration of the experiment. The glucose component of the TPN was infused separately into the right cephalic vein at a variable rate as required to maintain isoglycemia (120 mg/dL). Experiments consisted of an equilibration period (-120 to -40 minutes), a basal sampling period (-40 to 0 minutes), and an experimental period (0 to 180 minutes). At t = -120 minutes, a bolus of glucose tracers (U-^{14}C: 24 μCi; 3-^{3}H: 50 μCi) was injected from left cephalic vein and then constant infusion of 3-[^{3}H]glucose (0.43 μCi/min) and [U-^{14}C]glucose (0.27 μCi/min) were given into the left cephalic vein and continued throughout the entire experiment. Somatostatin (0.8 $\mu g \cdot kg^{-1} \cdot min^{-1}$) was infused into a right cephalic vein and continued throughout the entire experiment. Insulin (400 *v* 1,000 $\mu U \cdot kg^{-1} \cdot min^{-1}$; sham *v* infected) and glucagon (0.1 *v* 2.5 ng $\cdot$ kg^{-1} $\cdot$ min^{-1}) were infused intraportally via the splenic and the jejunal vein catheters to match the levels of insulin and glucagon seen in these groups.[2] Two different protocols were instituted at 0 minute in the sham and infected groups. Animals either received L-NNA (37 $\mu g \cdot kg^{-1} \cdot min^{-1}$) or saline into the portal vein via the splenic vein and jejunal vein catheters (n = 6/group) for the duration of the experimental period. Glucose was administered into right cephalic vein in all as required to maintain isoglycemia (120 mg/dL) during the experimental period. Femoral artery, portal vein, hepatic vein, and femoral vein blood samples were taken every 20 minutes during the basal period and every 30 minutes during the experimental period. Artery and portal vein samples were taken simultaneously, approximtely 30 seconds before collection of the hepatic venous blood samples. This was done to compensate for the short transit time of glucose through the liver.

Processing of Blood Samples

Blood samples were drawn into syringes and transferred to chilled tubes containing potassium EDTA (15 mg). The collection and immediate processing of blood samples have been previously described.[12] Blood lactate, alanine, and glycerol were analyzed using the method of Lloyd et al[13] on a Monarch 2000 centrifugal analyzer (Instrumentation Laboratories, Lexington, MA). Plasma glucose was assayed immediately with a Beckman Glucose Analyzer II (Beckman Instruments). Plasma nonesterified fatty acids were determined spectrophotometrically (Wako Chemicals, Richmond, VA). Immunoreactive insulin[14] was assayed with a double antibody technique (Pharmacia Diagnostics, Piscataway, NJ; intra-assay coefficient of variation [CV] of 11%). Plasma (1 mL) treated with 500 kallikrein-inhibitor units of trasylol (Miles, Kankakee, IL) was assayed for immunoreactive glucagon[15] with a procedure similar to that for insulin (intra-assay CV of 8%). Plasma cortisol[16] was assayed with Clinical Assays Gamma Coat radioimmunoassay kit (intra-assay CV of 6%). Plasma nitrate and nitrate were measured in the infected group using the Griess reaction after conversion of nitrite to nitrate with nitrate reductase (Caymen Nitrite/Nitrate Assay; Alexis Corp, San Diego, CA). Plasma collected from blood samples that were immediately treated with EGTA and glutathione was assayed for epinephrine and norepinephrine with high-performance liquid chromatography (HPLC) techniques (CV of 14%)[17] as modified by Davis et al.[18]

Calculations

Net hepatic glucose uptake was calculated with the formula [($F_a \times A + F_p \times P$) – H] × HBF, in which H, A, and P are the blood glucose concentrations in the hepatic vein, femoral artery, and portal vein, respectively, and F_a and F_p represent the fractional contributions of the hepatic artery and portal vein, respectively, to total hepatic blood flow (HBF). Net fractional hepatic glucose extraction was calculated as the ratio of net hepatic glucose uptake and hepatic glucose load. Hepatic glucose load was calculated with the formula ($F_a \times A + F_p \times P$) × HBF. Plasma glucose concentrations were converted to whole blood concentrations with a correction factor of 0.73.[19] The above equations were used to calculate net hepatic lactate, alanine, nonesterified free fatty acid (NEFA), nitrate/nitrite and glycerol uptake, and fractional extraction as well. However, because the liver was a net producer (ie, negative uptake) of lactate, the lactate data are presented as positive values and denoted as net output. Plasma flow instead of blood flow was used to calculate net hepatic NEFA uptake. Blood flow was converted to plasma flow by multiplying by 1 — hematocrit ratio.

The rates of total glucose appearance (R_a) and utilization (R_d) were calculated with a 2-compartmental model as described by Mari et al.[20] Whole body endogenous glucose production was calculated as the difference between R_a and the exogenous glucose infusion rate.

Hemodynamic Measurements

Hepatic artery and portal vein blood flow were continuously monitored using Transonic flow probes (Transonic Systems, Ithaca, NY). Mean arterial blood pressure and heart rate (HR) were measured at each sampling time point (Digi-Med Blood Pressure Analyzer, Model 190; Micro-Med, Louisville, KY). In the infected + L-NNA group, portal vein and hepatic vein pressures were also measured at each sampling time point.

Statistics

Hepatic blood flow and substrate flux are expressed on a per kilogram body weight basis. Statistical comparisons were made with analysis of variance (ANOVA) (Systat for Windows; Systat, Evanston, IL). A univariate post hoc F test was used when a significant F ratio was found. Statistical significance was accepted at $P < .05$.

RESULTS

Hemodynamic Data

During the basal period, HR and body temperature were significantly increased during infection, while mean arterial blood pressure was normal (Table 1).

In both groups receiving L-NNA infusion, mean arterial blood pressure increased. By the last 90 minutes of the experimental period, it increased by 9 ± 1 and 14 ± 3 mm Hg (sham and infected; $P < .05$), while HR decreased by 18 ± 9 and 31 ± 6 beats · min^{-1} during infusion of L-NNA ($P < .05$). Hepatic vein and portal vein pressures in the infected group did not change during infusion of L-NNA (data not presented). Total arterial plasma nitrite and nitrate concentration were not altered by L-NNA infusion in the infected group (17.1 ± 1.3 to 16.3 ± 0.6 μmol/L). However, net hepatic nitrite + nitrate release was completely suppressed by L-NNA infusion (87 ± 34 to -6 ± 33 nmol · kg^{-1} · min^{-1}).

Hepatic Blood Flow

Hepatic arterial blood flow was increased in the infected group (9.0 ± 0.9 and 23.3 ± 0.7 mL · kg · $^{-1}$ · min^{-1} in sham and infected groups). Thirty minutes after initiation of L-NNA infusion in the infected group, hepatic arterial blood flow started to decrease significantly (Fig 1). It reached a minimum of 8.6 ± 0.5 mL · kg · $^{-1}$ · min^{-1} at 120 minutes and remained significantly below baseline values during the subsequent 60 minutes. L-NNA infusion in the sham group did not alter hepatic arterial blood flow.

Portal vein blood flow was similar in sham control and infected control groups during the basal period (22.1 ± 2.3 and 21.7 ± 0.4 mL · kg · $^{-1}$ · min^{-1}). Infusion of L-NNA did not alter portal vein blood flow in either group. Infection increased total hepatic blood flow (45.0 ± 0.5 *v* 31.1 ± 2.0 mL · kg^{-1} · min^{-1}; infected *v* sham) because of the increase in hepatic arterial blood flow. Total hepatic blood flow decreased during L-NNA infusion because of the decrease in hepatic artery blood flow in the infected group.

Table 1. HR, MAP, and BT During the Basal Period in Sham and Infected Chronically Catheterized Conscious Dogs Receiving Chronic TPN

	Sham	Infection
HR (beats/min)	89 ± 8	132 ± 8*
MAP (mm Hg)	92.7 ± 5.6	97.9 *v* ± 7.0
BT (°C)	38.4 ± 0.2	39.3 ± 0.4*

NOTE. Data are means ± SE of 12 dogs in each group.

Abbreviations: HR, heart rate; MAP, mean arterial pressure; BT, body temperature; TPN, total parenteral nutrition.

*Significantly different from sham group.

Hormone Levels

Arterial plasma insulin concentrations were 10 ± 2 μU/mL in the sham (n = 12) and 23 ± 2 μU/mL in the infected (n = 12) groups (Fig 2). They were not significantly altered by saline or L-NNA infusion. Arterial plasma glucagon concentrations were higher in the infected group (22 ± 4 *v* 90 ± 8 pg/mL; sham *v* infected; n = 12/group). However glucagon concentration did not significantly change from the baseline concentration in any group during the experimental period. Baseline arterial plasma cortisol concentrations were 2.9 ± 0.5 and 3.2 ± 0.7 μg/dL in sham and infected groups, respectively. Arterial plasma epinephrine concentrations were 122 ± 28 and 124 ± 22 pg/mL, and arterial plasma norepinephrine concentrations were 187 ± 54 and 207 ± 24 pg/mL in sham and infected groups, respectively. All of the hormones were not significantly altered by the infusion of L-NNA or saline.

Hepatic Glucose Metabolism

Arterial plasma glucose concentrations were clamped at approximately 120 mg/dL in all 4 groups during both the basal and experimental periods (Fig 1). The rates of glucose infusion, appearance, disappearance, and clearance were not altered by L-NNA or saline infusion in either group (Table 2).

During the basal period, net hepatic glucose uptake was greater in the sham group (3.3 ± 0.3 mg · kg^{-1} · min^{-1}; n = 12) than in the infected group (1.9 ± 0.3 mg · kg^{-1} · min^{-1}; n = 12). During the basal period, net fractional hepatic glucose extraction was higher, as well in the sham group (0.13 ± 0.01 *v* 0.05 ± 0.01; sham *v* infected).

In both the sham and infected groups, infusion of L-NNA did not alter net hepatic glucose uptake when compared with their respective control groups (Fig 3). Upon the initiation of L-NNA

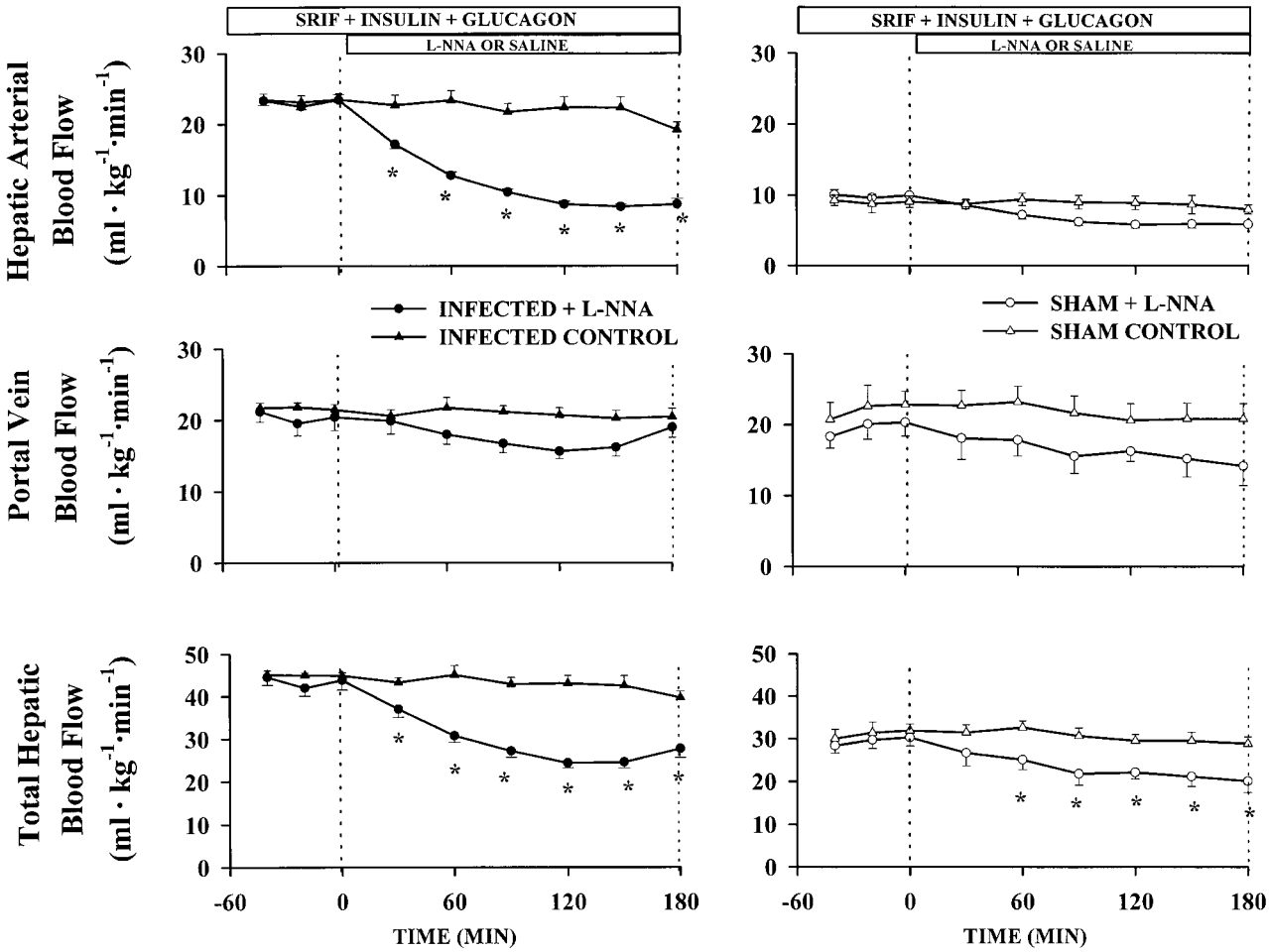

Fig 1. Hepatic artery, portal vein, and total hepatic blood flow in sham and infected animals receiving saline (sham control; infected control) or L-NNA (sham + L-NNA; infected + L-NNA) into the portal vein during a pancreatic clamp (somatostatin [SRIF], insulin, and glucagon). Data are expressed as mean ± SEM. *Significantly different from control group.

infusion into the portal vein, net fractional hepatic glucose extraction increased in sham + L-NNA groups (0.12 ± 0.02 to 0.16 ± 0.02, basal and experiment period, respectively, $P < .05$). However, in the infected + L-NNA group, net fractional hepatic glucose extraction increased transiently as compared with the infected control group, reaching a maximum between 60 and 120 minutes; after which, it returned to a value that was above the basal value, but was not significantly different from the saline control group.

Hepatic Substrate Balance

Arterial lactate concentrations were similar in the infected and sham groups during the basal period (Fig 4). However, the release of lactate in the infected group was decreased (23.6 ± 2.2 *v* 8.3 ± 1.3 μmol/L · kg^{-1} · min^{-1}; sham *v* infected; n = 12). Arterial lactate concentration and net hepatic lactate output were not altered during the infusion of L-NNA.

Blood alanine concentration was lower in the infected group (469 ± 54 *v* 207 ± 23 μmol/L; $P < .05$; n = 12), while both net fractional hepatic alanine extraction (0.10 ± 0.02 *v* 0.26 ± 0.02; $P < .05$) and net hepatic alanine uptake (1.3 ± 0.2 *v* 2.7 ± 0.3 μmol/L · kg^{-1} · min^{-1}; $P < .05$) were increased as compared with the sham group. However, blood alanine concentration and net hepatic alanine uptake were unaltered by L-NNA infusion in either group (Table 3).

Arterial glycerol concentrations and net hepatic glycerol uptake were similar in sham control groups and infected control group (Table 4). They were not altered by the infusion of L-NNA. Plasma NEFA concentrations and net hepatic NEFA uptake were similar in sham control and infected control groups. Plasma NEFA concentrations, net hepatic NEFA uptake, and net fractional hepatic NEFA extractions were not altered in either group during infusion of L-NNA or saline.

Hind Limb Glucose Metabolism

Femoral vein blood flow decreased similarly during both L-NNA and saline infusion in the infected groups (161 ± 9 to 125 ± 8 and 179 ± 26 to 146 ± 21 mL/min; + L-NNA and saline; $P < .05$). Limb glucose uptake remained unaltered (12 ± 2 to 13 ± 1 and 12 ± 2 to 13 ± 2 mg/min), because of an increase in net fractional limb glucose extraction (0.09 ± 0.01 to 0.12 ± 0.01 and 0.08 ± 0.01 to 0.12 ± 0.01; $P < .05$).

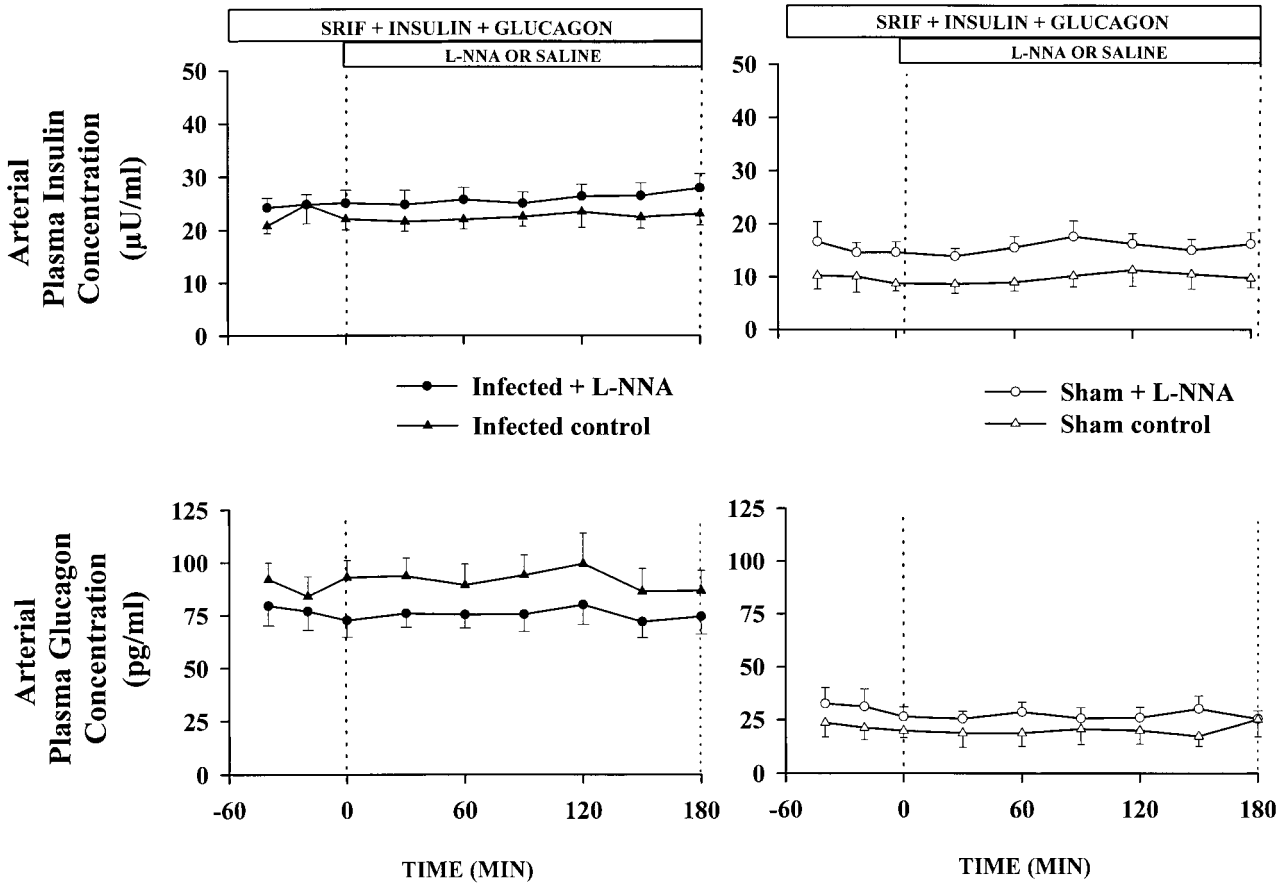

Fig 2. Arterial plasma insulin and glucagon concentrations in sham and infected animals receiving saline (sham control; infected control) or L-NNA (sham + L-NNA; infected + L-NNA) into the portal vein during a pancreatic clamp (somatostatin [SRIF], insulin, and glucagon). Data are expressed as mean ± SEM.

In the sham groups, femoral vein blood flow was unchanged during L-NNA (199 ± 36 to 209 ± 39 mL/min) and saline (203 ± 37 to 162 ± 12 mL/min) infusion. Limb glucose uptake (11 ± 3 to 10 ± 2 and 15 ± 6 to 21 ± 9 mg/min) and limb glucose extraction (0.07 ± 0.01 to 0.08 ± 0.01 and 0.11 ± 0.05 to 0.13 ± 0.04) remained unaltered.

DISCUSSION

In the present study, we examined the effect of L-NNA infusion on glucose metabolism in noninfected and infected chronically catheterized conscious dogs receiving TPN. L-NNA infusion reversed the infection-induced increase in he-

Table 2. Exogenous Glucose Infusion Rates and Tracer-Determined Whole Body Glucose Appearance, Disappearance, and Clearance Rates in Chronically Catheterized Conscious Dogs Receiving Chronic TPN During Basal and Experimental Period in Sham Control and Sham + L-NNA Groups and in Infected Control and Infected + L-NNA Groups

	Sham Control		Sham + L-NNA	
Parameter	Basal Period	Experimental Period	Basal Period	Experimental Period
Glucose infusion rate	8.0 ± 1.0	8.2 ± 1.1	8.4 ± 0.8	8.4 ± 0.8
Glucose appearance	9.3 ± 0.9	9.6 ± 1.4	9.1 ± 1.3	9.1 ± 1.2
Glucose disappearance	9.2 ± 1.0	9.6 ± 1.4	9.1 ± 1.3	9.1 ± 1.3
Glucose clearance	8.0 ± 0.9	8.5 ± 1.4	7.6 ± 0.8	7.5 ± 0.9
	Infected Control		Infected + L-NNA	
Glucose infusion rate	10.9 ± 0.7	10.7 ± 0.9	11.7 ± 0.5	10.2 ± 0.4
Glucose appearance	13.2 ± 2.1	13.8 ± 2.4	13.2 ± 1.4	11.9 ± 0.5
Glucose disappearance	13.3 ± 2.0	13.8 ± 2.4	13.1 ± 0.5	11.9 ± 0.5
Glucose clearance	11.3 ± 1.9	11.9 ± 2.3	10.8 ± 0.4	9.7 ± 0.4

NOTE. Data are means ± SE of 6 dogs in each group.

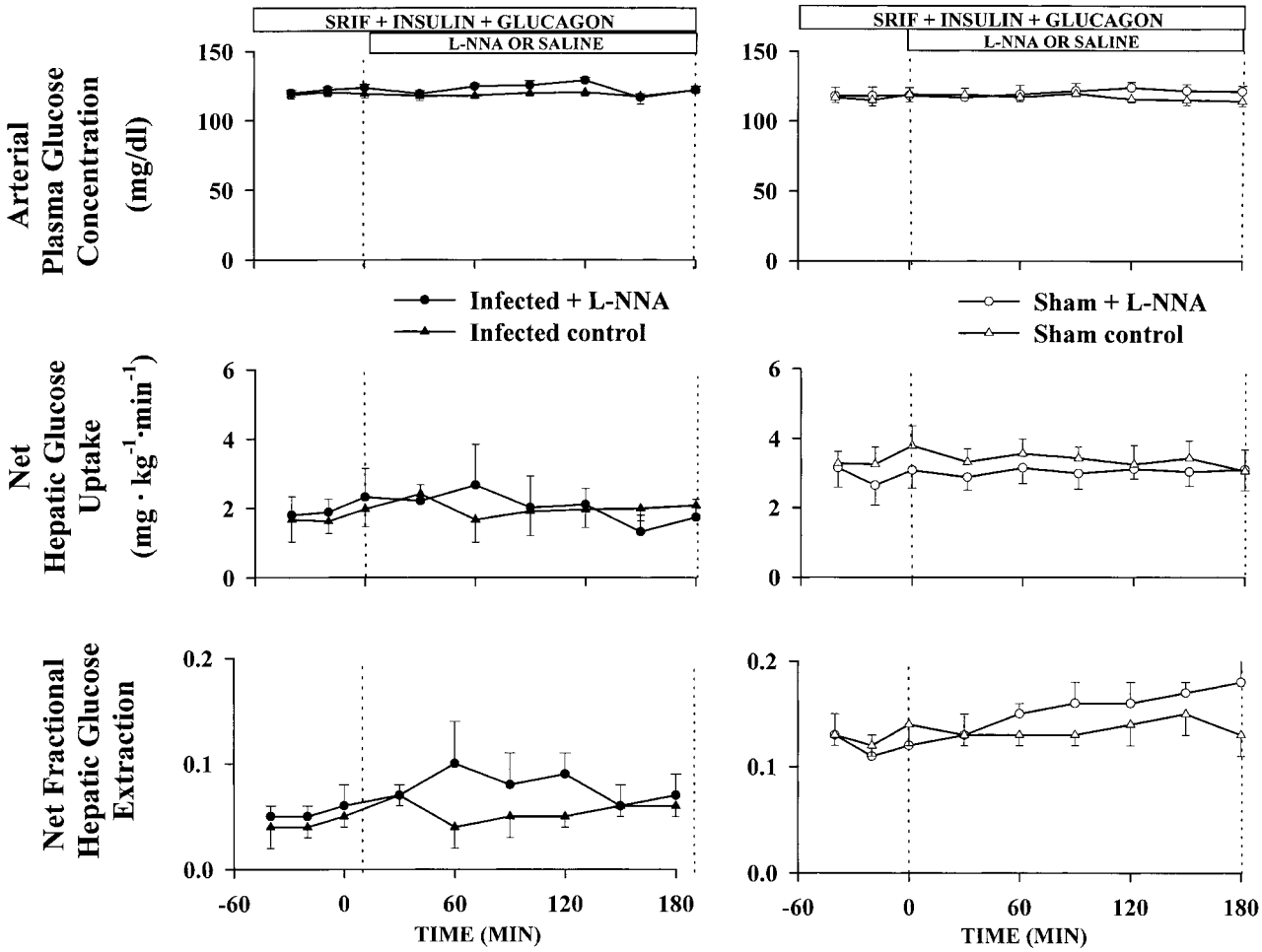

Fig 3. Arterial plasma glucose concentration, net hepatic glucose uptake, and net hepatic fractional extraction of glucose in sham and infected animals receiving saline (sham control; infected control) or L-NNA (sham + L-NNA; infected + L-NNA) into the portal vein during a pancreatic clamp (somatostatin [SRIF], insulin, and glucagon). Data are expressed as mean ± SEM.

patic artery blood flow. However, despite the marked decrease in liver blood flow during L-NNA infusion, liver glucose uptake and metabolism were largely unaffected. Interestingly, in the noninfected state, blockade of NO biosynthesis had little or no effect on liver blood flow or liver glucose metabolism. Thus, while the infection-induced increase in NO biosynthesis has marked effects on hepatic blood flow, the decrease in hepatic artery blood flow does not exacerbate the infection-induced decrease in liver glucose uptake.

The intraportal delivery of L-NNA was effective in decreasing hepatic NO biosynthesis while minimizing systemic effects. L-NNA was given into the portal vein as a constant infusion. This allowed us to give a lower dose of L-NNA. The total dose of L-NNA given over the 180-minute study (6.6 mg/kg) was 1 third of the dose (20 mg/kg) used to inhibit whole body NO synthesis in the dog when given as a bolus.[21] Consistent with the adequacy of the local blockade of the liver, L-NNA infusion completely suppressed nitrite and nitrate release by the liver. L-NNA was chosen because it is a potent inhibitor of both the inducible and noninducible forms of NO synthase, and it does not have any of the muscarinic effects of some other blockers.[22] Not surprisingly, despite the portal delivery of a low dose L-NNA, we did see some modest systemic effects. Arterial blood pressure increased (Δ10 mm Hg). The increase was, however, less than that seen with systemic blockade (Δ35 mm Hg).[21] Consistent with other studies, NO synthase inhibition did not decrease arterial nitrate and nitrite levels. This is likely due to the only partial inhibition of whole body nitrate synthesis and to the long half-life of nitrate (4 hours).[23]

The infection-induced increase in hepatic arterial blood flow is likely mediated by NO. As we have seen previously, infection increased hepatic arterial blood flow (≈ 2.6-fold).[2] The inducible form of NO synthase is increased in the liver in this model.[24] The administration of L-NNA into the portal vein reduced NO biosynthesis and consequently hepatic artery blood flow to that seen in the sham animal by increasing hepatic arterial resistance by 164% ± 15%. The dependency of hepatic artery blood flow on NO during inflammatory stress is consistent with previous studies.[6,25,26] Because hepatic artery blood flow decreased to, but not below, the rate seen in a sham animal, this suggests that in this model of infection, the associated increase in NO bio-

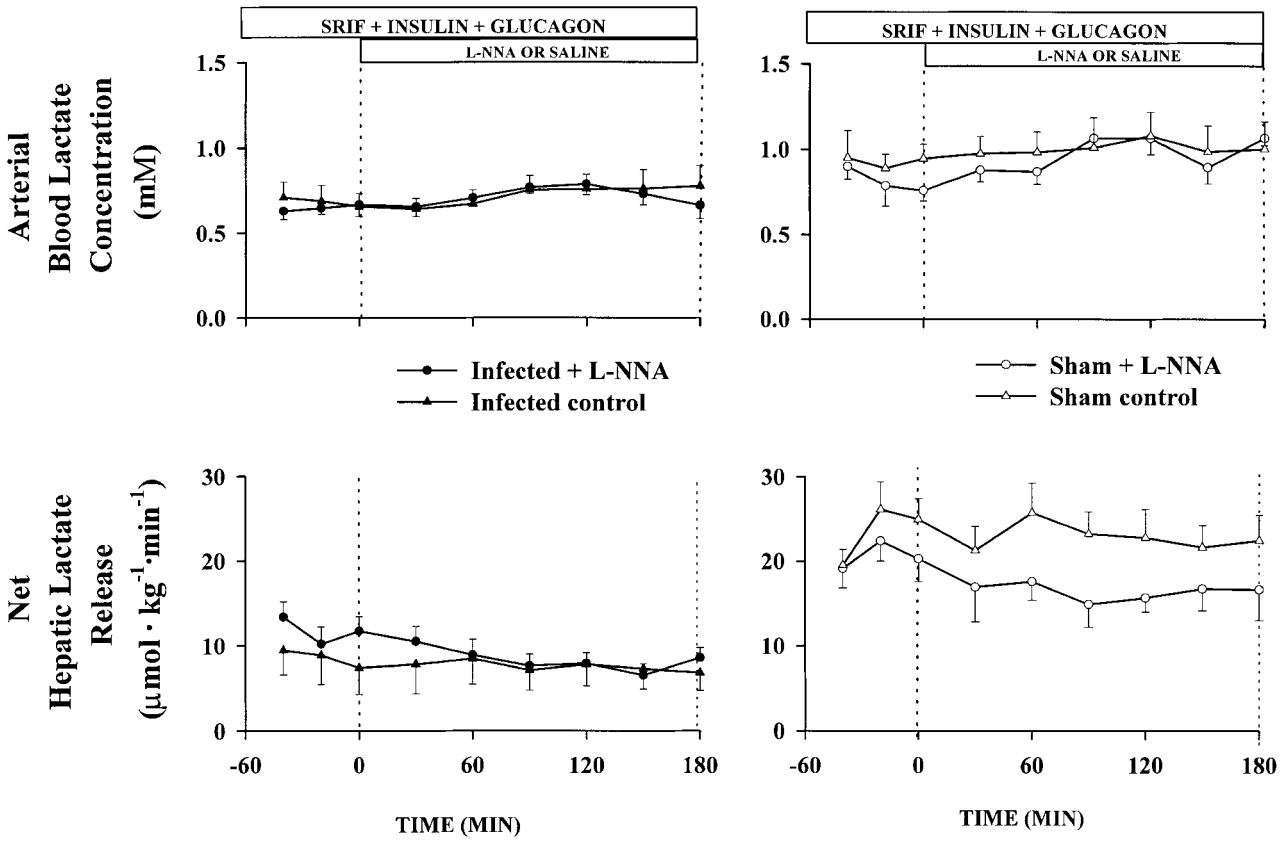

Fig 4. Arterial blood lactate concentration and net hepatic lactate release in sham and infected animals receiving saline (sham control; infected control) or L-NNA (sham + L-NNA; infected + L-NNA) into the portal vein during a pancreatic clamp (somatostatin [SRIF], insulin, and glucagon). Data are expressed as mean ± SEM.

synthesis is not masking the presence of a coexistent vasoconstrictor.

In contrast to its impact on the hepatic arterial blood flow, NO biosynthesis likely plays a relatively minor role in controlling portal vein blood flow during infection. Portal vein blood flow was not altered by L-NNA infusion in the infected animals. Portal vein vascular resistance did increase by 36% ± 4% with L-NNA infusion, however, an increase in arterial blood pressure limited the decrease in blood flow. The modest effect on portal vein vascular resistance is not consistent with previous reports. In prior studies, inhibition of NO biosynthesis decreased both hepatic artery and portal vein blood flow during

Table 3. Arterial Concentration, Net Hepatic Uptake and Net Fractional Hepatic Extraction in Chronically Catheterized Conscious Dogs Receiving Chronic TPN During Basal and Experimental Period in Sham and Sham + L-NNA Dogs

	Sham Control		Sham + L-NNA	
Parameter	Basal Period	Experimental Period	Basal Period	Experimental Period
Alanine				
Concentration (μmol/L)	501 ± 69	585 ± 99	455 ± 86	547 ± 103
Net hepatic uptake (μmol · kg^{-1} · min^{-1})	1.5 ± 0.3	1.4 ± 0.3	1.2 ± 0.3	0.8 ± 0.4
Net fractional hepatic extraction	0.11 ± 0.03	0.09 ± 0.02	0.10 ± 0.03	0.11 ± 0.05
Glycerol				
Concentration (μmol/L)	74 ± 6	88 ± 8	70 ± 6	85 ± 6
Net hepatic uptake (μmol · kg^{-1} · min^{-1})	1.2 ± 0.1	1.6 ± 0.1	1.1 ± 0.2	1.1 ± 0.1
Net fractional hepatic extraction	0.52 ± 0.03	0.60 ± 0.04	0.50 ± 0.05	0.59 ± 0.03
NEFA				
Concentration (μmol/L)	291 ± 32	275 ± 38	423 ± 45	372 ± 56
Net hepatic uptake (μmol · kg^{-1} · min^{-1})	0.6 ± 0.2	0.3 ± 0.2	1.9 ± 0.2	1.0 ± 0.3
Net fractional hepatic extraction	0.12 ± 0.04	0.03 ± 0.04	0.17 ± 0.03	0.14 ± 0.06

NOTE. Data are means ± SE of 6 dogs in each group.

Table 4. Arterial Concentration, Net Hepatic Uptake and Net Fractional Hepatic Extraction in Chronically Catheterized Conscious Dogs Receiving Chronic TPN During Basal and Experimental Period in Infected and Infected + L-NNA Dogs

Parameter	Infected Control		Infected + L-NNA	
	Basal Period	Experimental Period	Basal Period	Experimental Period
Alanine				
Concentration (μmol/L)	220 ± 19*	195 ± 22*	197 ± 19*	197 ± 21*
Net hepatic uptake ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	3.1 ± 0.3	2.4 ± 0.3	2.1 ± 0.2	1.8 ± 0.2
Net fractional hepatic extraction	0.30 ± 0.02	0.28 ± 0.04	0.23 ± 0.03	0.33 ± 0.03
Glycerol				
Concentration (μmol/L)	65 ± 5	66 ± 6	63 ± 10	86 ± 14
Net hepatic uptake ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	1.6 ± 0.1	1.5 ± 0.1	1.5 ± 0.2	1.4 ± 0.3
Net fractional hepatic extraction	0.54 ± 0.03	0.55 ± 0.04	0.57 ± 0.05	0.65 ± 0.04
NEFA				
Concentration (μmol/L)	227 ± 18	217 ± 20	231 ± 20	241 ± 33
Net hepatic uptake ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	0.7 ± 0.2	0.6 ± 0.2	1.3 ± 0.3	1.2 ± 0.3
Net fractional hepatic extraction	0.10 ± 0.03	0.10 ± 0.04	0.15 ± 0.04	0.19 ± 0.03

NOTE. Data are means ± SE of 6 dogs in each group.
*Significantly different from sham group.

inflammatory stress,[7,26,27] despite increases in arterial blood pressure. One possible explanation is that by delivering L-NNA into the portal vein, we limited the extra-hepatic effects of L-NNA. However, in one study, we administered the same dose of L-NNA via a peripheral vein. While hepatic artery blood flow decreased (22 to 12 mL/kg/min), portal vein blood flow did not decrease. This suggests that the route of L-NNA infusion does not influence the hemodynamic response of the splanchnic bed. The majority of the studies examining the impact of inhibition of NO biosynthesis during infection and endotoxemia used models in which splanchnic blood flow was decreased,[26] most likely reflecting a greater stress.[6,7] In contrast, in our model, the animals were hemodynamically stable and had an elevated splanchnic blood flow. In a rodent model of cirrhosis, in which both hepatic artery and portal vein flow were elevated, blockade of NO biosynthesis decreased flow in both vascular beds.[27] However, in cirrhotic models, the source of NO may be from the endothelial rather than the inducible isoform of NO synthase.[28] Settings in which the inducible form of NO synthase is known to be increased, hepatic arterial blood flow is consistently decreased after suppression of NO biosynthesis, however, the effect on the portal vein varies. In part, this variability may be related to the severity of the stress.[25,26]

NO biosynthesis is likely low in the normal animal and plays a limited role in regulating splanchnic blood flow. We did, in fact, find that while hepatic artery and portal vein blood flow were unaltered by L-NNA infusion, resistance did increase by approximately 10% in both vascular beds. Blood flow was unaltered because arterial blood pressure increased, as well. The limited effects on splanchnic blood flow is not surprising because the availability of NO is likely very low in the basal state. It is possible that the infusion of somatostatin to suppress pancreatic insulin and glucagon secretion blunted the L-NNA–induced decrease in portal vein blood flow. This dose of somatostatin decreases portal vein blood flow by approximately 20%. The mechanism by which somatostatin induces vasoconstriction in the intestinal tract is unknown, but is not thought to be NO dependent.[29] However, the lowering of portal vein blood flow before administration of L-NNA may have limited the decrease we could detect.

The lack of a change in liver glucose metabolism despite a decrease in liver blood flow is surprising. This contrasts with studies in muscle in which modulation of muscle blood flow regulates insulin action.[30] Acetylcholine, a known stimulator of NO biosynthesis, increased liver glucose uptake[10] and hepatic arterial blood flow. The increase in blood flow was similar to the magnitude seen in infection. Direct effects on the liver parenchymal cell, however, may mediate the effects of acetylcholine on liver glucose uptake. Liver glucose uptake did not decrease in the present study, because net fractional hepatic glucose extraction increased. An analogous effect was seen with oxygen extraction; hepatic oxygen extraction increased as liver blood flow decreased.[7] Explanations for the apparent increase in the ability of the liver to remove glucose include preferential removal of glucose from the portal vein, blood flow heterogeneity within the liver lobule, and enhanced mean transit time. If the majority of the glucose were removed from the portal vein as opposed to the hepatic artery, then any decrease in hepatic arterial blood flow would not alter liver glucose uptake. A lower hepatic artery blood flow increased the average mean sinusoidal transit time, thus allowing more time for glucose uptake. In muscle vascular beds, capillary recruitment plays a more important role in determining glucose uptake, and whether this is also seen in the liver is unknown. It is clear that decreases in liver blood flow in the setting of substantial liver glucose uptake have no effect on liver metabolism.

Hind limb glucose uptake and blood flow also were not altered by L-NNA infusion. It is recognized that muscle glucose uptake can be dependent upon muscle blood flow.[30] The mechanism is complex and likely involves capillary recruitment and redistribution of flow between nutritive and nonnutritive vessels.[31] Recent evidence indicates that blockade of NO synthesis in the liver induces peripheral insulin resistance.[32] While the dose of L-NNA was inadequate to cause complete blockade of NO synthase in the muscle, we found no evidence that NO is modulating muscle glucose uptake in our infection model.

It is unlikely that direct effects of NO on the hepatic parenchymal cell may have obscured the vascular effects of NO blockade on hepatic glucose uptake. High concentrations of NO

can inhibit hepatic glucose output, gluconeogenesis,[33] and protein synthesis and can aggravate hepatic injury.[34,35] However, others have reported that NO has no effect on glucose metabolism.[36] One of the difficulties of interpreting previous in vivo studies is that subtle changes in insulin and glucagon could either hide or amplify L-NNA–induced changes in liver glucose metabolism. By controlling the insulin and glucagon levels, we could determine that there is no direct effect of NO on hepatic glucose metabolism in vivo in a setting of a nonlethal inflammatory stress. If NO exerted an inhibitory effect on glucose production, we would have expected glucose production to increase and consequently net hepatic glucose uptake to decrease during L-NNA infusion. However, we may have been unable to detect an increase in glucose production, because glucose production was suppressed by the TPN. Other metabolic data also indicate that L-NNA had no metabolic effects on the liver. NO is an inhibitor of glucagon action in vitro.[37] Because glucagon is elevated in infection and it is a potent stimulator of hepatic amino acid transport,[38] we expected an increase in net hepatic fractional alanine extraction during L-NNA infusion. We were unable to detect an increase. However, it is possible that in more severe forms of inflammatory stress, in which NO biosynthesis is markedly elevated, a metabolic effect of NO could be detected.

In summary, during infection acute intraportal infusion of a NO synthase inhibitor decreases hepatic arterial blood flow and total hepatic blood flow. However, despite the marked hemodynamic effects, inhibition of NO biosynthesis does not alter the net hepatic glucose metabolism in the TPN-adapted state.

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