Hepatic portal venous delivery of a nitric oxide synthase inhibitor enhances net hepatic glucose uptake

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Am J Physiol Endocrinol Metab 294: E768–E777, 2008. First published January 22, 2008; doi:10.1152/ajpendo.00184.2007.—Hepatic portal venous infusion of nitric oxide synthase (NOS) inhibitors causes muscle insulin resistance, but the effects on hepatic glucose disposition are unknown. Conscious dogs underwent a hyperinsulinemic (4-fold basal) hyperglycemic (hepatic glucose load 2-fold basal) clamp, with assessment of liver metabolism by arteriovenous difference methods. After 90 min (P1), dogs were divided into two groups: control (receiving intraportal saline infusion; n = 8) and LN [receiving N^3-nitro-L-arginine methyl ester (L-NAME), a nonspecific NOS inhibitor; n = 11] intraportally at 0.3 mg·kg^{-1}·min^{-1} for 90 min (P2). During the final 60 min of study (P3), L-NAME was discontinued, and five LN dogs received the NO donor SIN-1 intraportally at 6 μg·kg^{-1}·min^{-1} while six received saline (LN/SIN-1 and LN/SAL, respectively). Net hepatic fractional glucose extraction (NHFE) in control dogs was 0.034 ± 0.016, 0.039 ± 0.015, and 0.056 ± 0.019 during P1, P2, and P3, respectively. NHFE in LN was 0.045 ± 0.009 and 0.111 ± 0.007 during P1 and P2, respectively (P < 0.05 vs. control during P2), and 0.087 ± 0.009 and 0.122 ± 0.016 (P < 0.05) during P3 in LN/SIN-1 and LN/SAL, respectively. During P2, arterial glucose was 204 ± 5 vs. 138 ± 11 mg/dl (P < 0.05) in LN vs. control to compensate for L-NAME’s effect on blood flow. Therefore, another group (LNlow; n = 4) was studied in the same manner as LN/SAL, except that arterial glucose was clamped at the same concentrations as in control. NHFE in LNlow was 0.052 ± 0.019 during P1, P2, and P3, respectively (P < 0.008, 0.093 ± 0.023, and 0.122 ± 0.021 during P1, P2, and P3, respectively (P < 0.05 vs. control during P2 and P3), with no significant difference in glucose infusion rates. Thus, NOS inhibition enhanced NHFE, an effect partially reversed by P2 and P3, with no significant difference in glucose infusion rates.

The reduction in insulin sensitivity due to hepatic NOS blockade resulted from inhibition of insulin action in skeletal muscle, with no effect on glucose uptake by the liver, gut, or adipose tissue (21). However, the aforementioned studies (16, 17, 32) were carried out under hyperinsulinemic euglycemic conditions, when the skeletal muscle is responsible for much of the glucose disposal and the role of the liver is minimized. Under hyperinsulinemic hyperglycemic conditions, the liver is responsible for a substantial portion of total glucose uptake (~25–33%, depending on the conditions of study (7)). This raises the question of what effect NO has on net hepatic glucose uptake (NHGU).

The regulation of NHGU is particularly relevant because hepatic extraction of glucose in the postprandial period is impaired in individuals with type 2 diabetes (3), contributing to postprandial hyperglycemia. Furthermore, postprandial hyperglycemia is strongly associated with morbidity and mortality in people with diabetes (5). There is evidence to link hepatic NO levels and the magnitude of NHGU. Infusion of SIN-1 into the hepatic portal circulation of conscious dogs significantly blunted NHGU in the presence of hyperinsulinemia, hyperglycemia, and intraportal glucose delivery (2), conditions that mimic the postprandial state. Therefore, the current study was undertaken to quantify NHGU under hyperglycemic and hyperinsulinemic conditions in the presence of a reduction of endogenous NO brought about by intraportal infusion of L-NAME.

RESEARCH DESIGN AND METHODS

Animals and Surgical Procedures

Studies were carried out on conscious 42-h-fasted adult mongrel dogs of either sex weighing 19–25 kg. Diet and housing were as described previously (28), and the protocol was approved by the Vanderbilt University Institutional Animal Care and Use Committee. Approximately 16 days before study, each dog underwent a laparotomy for placement of ultrasonic flow probes (Transonic Systems, Ithaca, NY) around the portal vein and the hepatic artery as well as insertion of silicone rubber catheters for sampling in a hepatic vein, the portal vein, and a femoral artery and for infusion into a splenic and a jejunal vein, as described in detail elsewhere (28). Criteria for health of the animals prior to study were as described previously (28). On the morning of the study, catheters and flow probe leads were exteriorized from their subcutaneous pockets. Intravenous access was established in three peripheral veins.

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Experimental Design

Each experiment consisted of a 90-min equilibration period (~120 to ~30 min), a 30-min basal period (~30 to 0 min), and a 240-min experimental period (0–240 min) that was divided into three subperiods (P1, 0–90 min; P2, 90–180 min; and P3, 180–240 min). At ~120 min, a continuous infusion of indocyanine green dye (0.08 mg/min; Sigma, St. Louis, MO) was begun in all dogs. At 0 min, a constant, peripheral infusion of somatostatin (0.8 μg·kg⁻¹·min⁻¹; Bachem, Torrance, CA) was begun to suppress endogenous insulin and glucagon secretion. Insulin was infused intraportally via the splenic and jejunal catheters at fourfold basal (1.2 μU·kg⁻¹·min⁻¹; Eli Lilly, Indianapolis, IN), and glucagon was replaced intraportally in basal amounts (0.57 μg·kg⁻¹·min⁻¹; GlucaGen; Bedford Laboratories, Bedford, OH). In addition, a primed, continuous, variable-rate 50% dextrose infusion was begun through a peripheral vein to create hyperglycemia. During P1, all dogs received intraportal saline infusion. In the control group (dogs receiving intraportal saline infusion; n = 8), the intraportal saline infusion continued throughout P2 and P3. In the LN group (dogs receiving l-NAME; n = 11), the dogs received intraportal L-NAME (Cayman Chemical, Ann Arbor, MI) at 0.3 mg·kg⁻¹·min⁻¹ during P2. This infusion rate, which is similar to that previously used in studies in humans (15), was derived from a canine dose-response study (data not shown). L-NAME infusion stopped at the end of P2, and the LN dogs were divided into two subgroups at 180 min. During P3, six dogs received intraportal saline after the cessation of l-NAME infusion (LN/SAL), and five received intraportal SIN-1 infusion (LN/SIN-1; Cayman Chemical) at 6 μg·kg⁻¹·min⁻¹. The large changes in hepatic blood flow brought about by l-NAME infusion raised questions about what effect this might have had on hepatic glucose balance. It was also unclear whether the hepatic glucose load or the concentration of glucose at the liver was most important in stimulating glucose uptake (35). Therefore, to determine whether the results could be due to the effects of the l-NAME infusion rate used or the elevation of the arterial glucose concentration, an additional group [dogs receiving a low rate of l-NAME infusion in P2 (LN/low); n = 4] was treated in the same manner as the LN group, except that the l-NAME infusion rate was 0.075 mg·kg⁻¹·min⁻¹ and the arterial glucose concentration was clamped at the same level as in the control group.

Femoral artery, portal vein, and hepatic vein blood samples were taken every 15–30 min throughout the study as described previously (28). Arterial samples were also taken every 5 min throughout the experimental period to monitor the plasma glucose level and allow appropriate adjustment of the glucose infusion rate. Blood pressure and heart rate were obtained with an arterial transducer (DigitMed; Micro-Med, Louisville, KY).

Processing and Analysis of Samples

Hematocrit, blood lactate, and glycerol and plasma glucose, insulin, glucagon, and cortisol were measured as described previously (26). In the LN/SAL, LN/low, and control groups, hepatic glycogen concentrations were determined by the method of Keppler and Decker (20).

Calculations and Data Analysis

Hepatic blood flow was measured using ultrasonic probes and indocyanine green extraction. The two methods yielded similar results, but the data reported here utilize the ultrasonic-determined flows, because their use did not require an assumption regarding the relative contribution of arterial and portal flow to total hepatic blood flow.

The rate of glucose delivery to the liver or hepatic glucose load, net hepatic substrate balance, net hepatic fractional substrate extraction, net hepatic carbon retention, and hepatic sinusoidal hormone concentrations were calculated as described previously (26). Nonhepatic glucose uptake equaled the glucose infusion rate minus NHGU; the rate was corrected for changes in the size of the glucose pool, using a pool fraction of 0.65 (11) and assuming that the volume of distribution for glucose equaled the volume of the extracellular fluid, or ~22% of the dog’s weight (37). Hepatic glucose clearance was calculated as NHGU divided by the weighted (for the proportion of hepatic blood flow contributed by the hepatic artery vs. the portal vein) inflowing arterial and portal venous glucose concentration. Net hepatic carbon retention, an index of hepatic glycogen storage, was calculated as the difference between NHGU and lactate output, assuming that net hepatic uptake of nonlactate gluconeogenic precursors was offset by the rate of hepatic glucose oxidation. We (26) have previously shown this calculated variable to be closely related to the actual rate of glycogen synthesis. For all glucose balance calculations, glucose concentrations were converted from plasma to blood values by using correction factors (ratio of the blood to the plasma concentration) previously established in our laboratory (19, 28). Net hepatic glycogen synthesized over the course of the study was calculated as the glycogen concentration in the terminal liver biopsies minus the concentrations in 42-h-fasted dogs that did not undergo study.

Statistical Analysis

All data are presented as means ± SE. Time course data were analyzed with repeated-measures ANOVA, and Tukey’s test was used for post hoc comparisons (SigmaStat, Point Richmond, CA). Statistical significance was accepted at P < 0.05. Where mean data for an experimental period are presented, they are the mean of the sampling points in the last 30 min of that period, when steady-state conditions prevailed.

RESULTS

High-Dose l-NAME With or Without SIN-1 Infusion

Hepatic blood flow and cardiovascular parameters. Hepatic blood flow did not differ between the control and LN groups during the basal period or P1 (Fig. 1). In both groups the portal vein blood flow decreased modestly with the onset of somatostatin infusion, whereas there was a small increase in hepatic artery flow, such that total hepatic flow decreased by ~15%. l-NAME infusion significantly reduced both hepatic artery and portal vein flow such that total hepatic blood flow was only 58% of that in the control group during the last 30 min of P2 (P < 0.05 between groups). In LN/SAL, portal vein flow increased significantly after cessation of l-NAME infusion, but hepatic artery flow did not change. Consequently, total hepatic flow rose but remained significantly suppressed. Infusion of SIN-1 during P3 had no effect on portal vein flow compared with the rate in LN/SAL, but it elevated hepatic artery flow (P < 0.05). The changes in hepatic blood flow were similar regardless of the method of measurement (indocyanine green-determined flow not shown).

Blood pressure and heart rate were stable during the basal period and P1 and did not differ between groups (Table 1). However, both systolic and diastolic blood pressure rose modestly in response to l-NAME, and the increase continued to be evident even after l-NAME infusion ceased. Infusion of SIN-1 post-l-NAME reduced systolic pressure to the level seen in the control group but failed to normalize diastolic pressure. Concomitant with the hypertensive changes, the heart rate was significantly reduced both during and after l-NAME infusion. SIN-1 partially reversed the bradycardia.

Hormone concentrations. The hepatic sinusoidal insulin concentrations rose ~35% (P < 0.05) in the LN group during P2 vs. P1; they increased further in LN/SAL during P3 but...
declined in LN/SIN-1 (Table 2). Arterial plasma glucagon concentrations remained basal throughout P1–P3 in all animals. Hepatic sinusoidal glucagon concentrations in the LN group were ~10–20% greater than those in the control group during P2, but this did not reach statistical significance; the concentrations in LN/SAL remained elevated ~14% (not significant) above control levels during P3. Arterial plasma cortisol concentrations did not change significantly from basal in either control or LN and differed between groups only during P1, when experimental conditions were identical in the groups. Arterial plasma norepinephrine did not differ significantly between the control and the LN groups during the basal period, P1, or P2 (Table 2). During P3 it was significantly greater than control values in the LN/SIN-1 group but not the LN/SAL group. Arterial plasma epinephrine concentrations were significantly greater in the LN group than in the control group during P3, and they remained higher in LN/SAL and LN/SIN-1 than in the control group during P3.

**Glucose metabolism.** Basal arterial blood glucose concentrations (~76–80 mg/dl) did not differ between the control and LN groups (Fig. 2). Similarly, the mean values during P1 were 139 ± 10 and 134 ± 4 mg/dl in the control and LN dogs, respectively (not significant). By design, the hepatic glucose load was increased ~60% over basal during the experimental period, with no differences between groups at any time (Fig. 2). Since hepatic blood flow did not change significantly during P1, P2, and P3 in the control group, the arterial blood glucose was also maintained at a constant level throughout the experimental period. However, the marked decline in hepatic blood flow during l-NAME infusion necessitated that sufficient glucose be infused to increase the arterial blood glucose concentrations ~50% (Fig. 2) to maintain a stable load of glucose to the liver. During P3, arterial blood glucose was gradually lowered in the LN subgroups to adjust for decrements in hepatic blood flow. During P1 and P3, the glucose infusion rates did not differ significantly between the control and LN dogs, but the glucose infusion rate during l-NAME infusion was ~45% greater than during the comparable time period in the control dogs.

During the basal period, both the control and LN groups exhibited net hepatic glucose output at 1.5 mg·kg⁻¹·min⁻¹ (Fig. 3). In the presence of hyperinsulinemia and hyperglycemia, both groups shifted to NHGU, with the rates during the last 30 min of P1 averaging 1.3 ± 0.6 and 1.6 ± 0.3 mg·kg⁻¹·min⁻¹ in control and LN, respectively (not significant). Infusion of l-NAME markedly enhanced NHGU, with rates during P2 averaging 1.5 ± 0.5 vs. 3.5 ± 0.2 mg·kg⁻¹·min⁻¹ in control and LN, respectively (P < 0.001). NHGU remained significantly enhanced even after l-NAME infusion ceased (i.e., LN/SAL), but infusion of SIN-1 partially

This text is a description of a scientific experiment and its results, focusing on the effects of L-NAME and SIN-1 on hepatic blood flow and glucose metabolism. The data shows that the arterial plasma epinephrine concentrations were significantly greater in the LN group than in the control group during P3, and they remained higher in LN/SAL and LN/SIN-1 than in the control group during P3. The glucose infusion rate during l-NAME infusion was ~45% greater than during the comparable time period in the control dogs. During the basal period, both the control and LN groups exhibited net hepatic glucose output at 1.5 mg·kg⁻¹·min⁻¹ (Fig. 3). In the presence of hyperinsulinemia and hyperglycemia, both groups shifted to NHGU, with the rates during the last 30 min of P1 averaging 1.3 ± 0.6 and 1.6 ± 0.3 mg·kg⁻¹·min⁻¹ in control and LN, respectively (not significant). Infusion of l-NAME markedly enhanced NHGU, with rates during P2 averaging 1.5 ± 0.5 vs. 3.5 ± 0.2 mg·kg⁻¹·min⁻¹ in control and LN, respectively (P < 0.001). NHGU remained significantly enhanced even after l-NAME infusion ceased (i.e., LN/SAL), but infusion of SIN-1 partially.
reversed the l-NAME-induced enhancement of NHGU (rates in control, LN/SAL, and LN/SIN-1: 2.2 ± 0.7, 4.7 ± 0.8, and 2.8 ± 0.5 mg·kg⁻¹·min⁻¹, respectively; *P < 0.05 for LN/SAL vs. control and LN/SIN-1). During P2, hepatic glucose clearance averaged 1.00 ± 0.32 vs. 1.79 ± 0.11 ml·kg⁻¹·min⁻¹ in the control vs. LN groups, respectively (*P < 0.01), and during P3 it was 1.48 ± 0.39 vs. 2.33 ± 0.26 ml·kg⁻¹·min⁻¹ in control vs. LN/SAL, respectively (*P < 0.05). The infusion of SIN-1 tended to reverse the l-NAME-induced stimulation of hepatic glucose clearance (1.83 ± 0.19 ml·kg⁻¹·min⁻¹ in LN/SIN-1, *P = 0.07 vs. LN/SAL and *P = 0.10 vs. control during P3). The rates of NHGU and hepatic glucose clearance obtained with the indocyanine green-derived blood flows did not differ significantly from those with the ultrasonic blood flow data. Net hepatic fractional extraction of glucose mirrored the results for NHGU, with l-NAME infusion increasing the value >2.5-fold. The enhancement continued after cessation of l-NAME and was partially reversed by infusion of SIN-1 (Fig. 3). Nonhepatic glucose uptake in LN was significantly greater than in control during the first 30 min of P2, but it declined so that it was no different from control during the last half of P2.

The mass of hepatic glycogen synthesized over the course of the study tended to be greater in LN/SAL than in control (1.74 ± 0.40 vs. 1.29 ± 0.37 g/100 g liver, *P = 0.20).

Lactate, glycerol, and nonesterified fatty acid metabolism: net hepatic carbon retention. Arterial blood lactate concentrations increased with the onset of hyperglycemic hyperinsulinemia in both groups, and the concentrations did not differ between groups at any time (Table 3). Net hepatic lactate balance shifted from uptake to output during P1 in both groups. Net hepatic carbon retention, an index of glycogen storage, was significantly enhanced by l-NAME infusion (Table 3).

Arterial blood glycerol concentrations declined from basal during hyperinsulinemic hyperglycemia in all animals and did not differ between groups at any time. Similarly, net hepatic carbon retentions were similar in all groups.
during P3. In P1, the hepatic glucose load was equivalent in the two groups (34 ± 3 and 33 ± 2 mg·kg⁻¹·min⁻¹ in the control and LNlow groups, respectively). Because of the fall in hepatic blood flow, the hepatic glucose load during and after L-NAME infusion was lower (P < 0.05) in LNlow than in control (25 ± 1 vs. 36 ± 4 mg·kg⁻¹·min⁻¹ in P2 and 24 ± 2 vs. 38 ± 4 mg·kg⁻¹·min⁻¹ in P3). NHGU tended to be increased in the LNlow vs. control group during P2 and P3 (61 and 35%, respectively, P = 0.15 and 0.23; Fig. 5) despite the smaller glucose load. Net hepatic fractional extraction of glucose was enhanced (P < 0.05) during and after L-NAME infusion (0.093 ± 0.023 and 0.122 ± 0.021 in LNlow vs. 0.039 ± 0.015 and 0.056 ± 0.019 in control during P2 and P3, respectively).

Nonhepatic glucose uptake was significantly reduced during P3 but not P2 in LNlow vs. control (Fig. 5). Arterial lactate concentrations, net hepatic lactate balance, and net hepatic carbon retention in the LNlow group did not differ from those in the control group (Table 5). The mass of hepatic glycogen synthesized in the LNlow group tended to be greater than in the control group (1.78 ± 0.43 vs. 1.29 ± 0.37 g/100 g liver, P = 0.16). Arterial blood glycerol and net hepatic glycerol uptake declined in a similar manner to the control group; there was a significant increase in both parameters in LNlow at the final time point.

**DISCUSSION**

The current report is the first to examine the effects of NOS inhibition on glucose metabolism in vivo under controlled hyperinsulinemic hyperglycemic conditions, when substantial NHGU occurs. Intraportal administration of L-NAME at 0.3 mg·kg⁻¹·min⁻¹ significantly increased the uptake and fractional extraction of glucose by the liver. In this investigation we did not use intraportal glucose delivery, which augments NHGU, due to our concern that it could result in our erroneously concluding that suppression of hepatic NOS has no effect on NHGU. Such an error would occur if NOS inhibition is the method by which portal glucose delivery (the “portal signal”) stimulates NHGU or if NOS inhibition and the portal signal are unrelated but not additive mechanisms for enhancing NHGU. The effects of L-NAME on NHGU and fractional glucose extraction did not wane during P3, after L-NAME infusion ceased, consistent with the finding (10) that the vasoactive effects of L-NAME in rats persist for as long as 6 h after an injection of the drug.

The blood glucose concentrations during L-NAME infusion at 0.3 mg·kg⁻¹·min⁻¹ were clamped at higher levels than in the control group to maintain a stable hepatic glucose load [a key determinant of NHGU (7)], raising the question of whether the increased hyperglycemia might have been responsible for the enhancement of NHGU. However, even when NHGU was adjusted for the glucose concentrations (i.e., hepatic glucose clearance), a significant augmentation of hepatic glucose disposal was evident in the group receiving the NOS inhibitor. Nevertheless, because of concern that the additional hyperglycemia might have affected the results, an additional group of dogs (LNlow) was studied in which no attempt was made to clamp the hepatic glucose load at the same level as in the control group. Instead, the arterial glucose concentration was clamped at control levels; as L-NAME brought about a fall in hepatic blood flow, the hepatic glucose load declined to basal.

uptakes of glycerol fell during P1 in all dogs and did not differ among treatments through P2 (Table 3). Infusion of SIN-1 tended to increase arterial blood glycerol (P = 0.14) and significantly stimulated net hepatic glycerol uptake.

**Low-Dose L-NAME Infusion**

In LNlow, total hepatic blood flow decreased ~25% during P2 compared with P1 (P < 0.05; Table 4). Neither the systolic nor the diastolic blood pressure increased significantly between P2 and P1, but the heart rate fell ~20% (P < 0.05; Table 4).

The arterial insulin concentrations did not differ from those in the control group during any period, but the hepatic sinusoidal concentrations were significantly higher in LNlow than in control throughout P1, P2, and P3 (Table 4). The arterial and hepatic sinusoidal glucagon concentrations in LNlow were not significantly different from those in control throughout P1, P2, and P3.

The arterial blood glucose concentration was clamped at 135 ± 2, 134 ± 2, and 134 ± 3 mg/dl in LNlow during P1, P2, and P3, respectively, not different from the concentrations in the control group (Fig. 4). The glucose infusion rate did not differ significantly between groups, although it tended to be lower (~35%, P = 0.36) in LNlow than in the control group.
The fact that NHGU was not higher during L-NAME infusion fractional extraction of glucose was significantly stimulated. Despite of the fall in hepatic glucose load, NHGU tended to be is basal or near basal. Galassetti et al. (13) carried out studies contribution to glucose disposal when the hepatic glucose load is not surprising, given that the liver does not make a major Table 4. Blood flow, cardiovascular parameters, and plasma hormone concentrations in LNlow

<table>
<thead>
<tr>
<th>Group</th>
<th>Basal Period</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>491±112</td>
<td>560±130</td>
<td>829±186</td>
<td>805±132</td>
</tr>
<tr>
<td>LN</td>
<td>388±65</td>
<td>552±62</td>
<td>796±83</td>
<td>726±68</td>
</tr>
<tr>
<td>LN/SAL</td>
<td>420±110</td>
<td>512±107</td>
<td>793±109</td>
<td>750±108</td>
</tr>
<tr>
<td>LN/SIN-1</td>
<td>450±120</td>
<td>550±110</td>
<td>810±110</td>
<td>780±110</td>
</tr>
</tbody>
</table>

Net hepatic lactate balance, μmol·kg⁻¹·min⁻¹

Control -6.8±1.2 | 0.4±2.5  | 3.9±2.4  | 1.7±2.1  | 0.5±2.1  | 1.2±3.0  | -0.5±2.5  | 1.2±2.4  | 0.0±2.6  |
LN -6.1±0.9      | 2.3±1.6  | 5.1±2.3  | 3.9±0.8  | 4.7±1.0  | 5.5±0.9  | 3.6±0.5  | 1.2±4.0  | 3.3±0.8  |
LN/SAL           | 1.6±0.3   | 0.6±0.2   | 0.5±0.2   | 0.5±0.1   | 0.5±0.1   | 0.3±0.1   | 0.3±0.1   | 0.5±0.2   |
LN/SIN-1         | 1.7±0.3   | 0.9±0.2   | 0.6±0.1   | 0.5±0.1   | 0.2±0.1   | 0.3±0.1   | 0.3±0.1   | 0.6±0.2   |

Net hepatic carbon retention, mg glucose equivalents·kg⁻¹·min⁻¹

Control 0.1±0.3 | 0.6±0.3   | 1.1±0.4   | 1.8±0.5   | 1.3±0.5   | 1.6±0.4   | 2.0±0.5   | 2.3±0.5   |
LN 0.4±0.2      | 0.9±0.3   | 1.5±0.3   | 2.7±0.2   | 3.4±0.3   | 3.2±0.3   | 3.1±0.4   | 3.8±0.4   | 4.5±0.7   |
LN/SAL          | (3.2±0.3) | (3.2±0.3) | (3.2±0.3) | (3.4±0.3) | (3.2±0.3) | (3.1±0.4) | (3.8±0.4) | (4.5±0.7) |
LN/SIN-1        | (3.2±0.3) | (3.2±0.3) | (3.2±0.3) | (3.4±0.3) | (3.2±0.3) | (3.1±0.4) | (3.8±0.4) | (4.5±0.7) |

Data are means ± SE. See legend to Table 1 for details of groups and study conditions. Values in parentheses are those in the subgroups at the last time point in P2. At that time, all animals in the subgroups were treated identically. *P < 0.05 vs. control.

Despite of the fall in hepatic glucose load, NHGU tended to be enhanced during and after L-NAME infusion, and net hepatic fractional extraction of glucose was significantly stimulated. The fact that NHGU was not higher during L-NAME infusion is not surprising, given that the liver does not make a major contribution to glucose disposal when the hepatic glucose load is basal or near basal. Galassetti et al. (13) carried out studies in conscious dogs in which glucagon was delivered intraportal-ly at a basal rate, insulin was infused intraportally at either three- or 15-fold basal rates (resulting in arterial plasma insulin concentrations of ~15 and 120 μU/mL, respectively), and the hepatic glucose load was maintained at basal levels by peripheral venous glucose infusion. NHGU during the final 2 h of these 4-h clamp studies averaged only ~0.1 and 0.5

Table 4. Blood flow, cardiovascular parameters, and plasma hormone concentrations in LNlow

<table>
<thead>
<tr>
<th>Group</th>
<th>Basal Period</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
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<td>Hepatic blood flow, ml·kg⁻¹·min⁻¹</td>
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<tr>
<td>Hepatic artery</td>
<td>4.3±0.1</td>
<td>5.4±0.8</td>
<td>3.4±0.6*</td>
<td>3.5±0.7*</td>
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<tr>
<td>Portal vein</td>
<td>24.3±3.0</td>
<td>19.4±2.0</td>
<td>15.1±1.4*</td>
<td>14.4±2.2*</td>
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<td>Blood pressure, mmHg</td>
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<tr>
<td>Systolic</td>
<td>156±8</td>
<td>163±6</td>
<td>173±6</td>
<td>179±6</td>
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<td>Diastolic</td>
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<td>83±2</td>
<td>90±4</td>
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<td>Heart rate, beats/min</td>
<td>75±5</td>
<td>74±8</td>
<td>59±6*</td>
<td>59±8*</td>
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<td>Plasma insulin, μU/ml</td>
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<td>Artery</td>
<td>8±2</td>
<td>22±1</td>
<td>21±1</td>
<td>23±1</td>
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<td>Hepatic sinusoidal</td>
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<td>Plasma glucagon, μg/ml</td>
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<tr>
<td>Artery</td>
<td>32±6</td>
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<td>Hepatic sinusoidal</td>
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</tr>
<tr>
<td>Artery</td>
<td>2.6±0.6</td>
<td>3.6±0.9</td>
<td>2.4±0.4</td>
<td>2.2±0.3</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 4. LNlow, dogs receiving a low rate of L-NAME infusion in P2. See legend to Table 1 for study conditions. LNlow followed the same procedures as LN/SAL, except that the L-NAME infusion rate was only 1/4 as great and the arterial glucose concentration was matched to that in control. *P < 0.05 vs. control (shown in Table 2).
In contrast to our current findings with NOS inhibition during acute hyperglycemia and hyperinsulinemia, in normal dogs that had received total parenteral nutrition (TPN) for 5 days, initiation of an intraportal infusion of the NOS inhibitor Nω-nitro-l-arginine (l-NNA, the active metabolite of l-NAME) had no impact on NHGU (6). The delivery rate of the NOS inhibitor in the current study was ~6.6- and 1.6-fold greater in the LN and LNlow groups, respectively, than that in the TPN-infused dogs, and this might explain the difference in the results. Additionally, the TPN-adapted dogs exhibited high rates of NHGU before the l-NNA (with the liver extracting ~45% of a glucose load under clamp conditions) was begun despite the presence of euglycemia (115–120 mg/dl) and only mild hyperinsulinemia (~16 µU/ml) (23). Thus, the hepatic fractional extraction of glucose in the TPN-adapted dogs was approximately four times greater than that observed in the control group in the current study, and it could be that NOS inhibition was ineffective in increasing hepatic glucose uptake.

**Fig. 4.** Arterial blood glucose, glucose infusion rate, and hepatic glucose load in the control and LNlow (dogs receiving a low rate of l-NAME infusion in P2) groups. The control group is the same one shown in the previous figures. *P < 0.05 vs. control.

mg·kg⁻¹·min⁻¹ with three- and 15-fold basal insulin, respectively. Thus, even with marked hyperinsulinemia, NHGU was very low. On the other hand, net hepatic fractional extraction of glucose was significantly stimulated. Fractional extraction takes into account the magnitude of the hepatic glucose load, making a difference between groups more readily apparent.

Calculation of NHGU is dependent on the accuracy of hepatic blood flow measurements. With a vasoconstrictive agent such as l-NAME, there is a concern that blood flow might not be measured accurately by the ultrasonic method, which is dependent on the diameter of the vessel. However, Transonic flow probes have previously been reported (42) to function appropriately during vasoconstrictive changes. In addition, the rates of NHGU obtained with the ultrasonic technique did not differ significantly from those obtained with blood flow derived from the dye method, a technique that is not dependent on vessel diameter.

**Fig. 5.** Net hepatic glucose uptake and fractional extraction and nonhepatic glucose uptake in the control and LNlow groups. The control group is the same one shown in Figs. 1–3. *P < 0.05 vs. control.
change in hepatic sinusoidal hormone concentrations resulting in enhancement of NHGU, other explanations must be sought. The global knockout of endothelial NOS is a result of impaired hepatic microcirculation (4), and it has been suggested (34) that enhanced NHGU 70% under hyperglycemic hyperinsulinemic clamp concomitant with portal glucose infusion (1) and in vivo (24). Conversely, intraportal injection or ligation of the hepatic artery reduces liver glucose uptake in vitro (1) and in vivo (24). Complete occlusion or ligation of the hepatic artery reduces liver glucose uptake in vitro (1) and in vivo (24). Conversely, intraportal delivery of acetylcysteine, which increased hepatic artery blood flow more than twofold but did not alter portal vein blood flow, enhanced NHGU 70% under hyperglycemic hyperinsulinemic conditions (35). NO is regarded as a key regulator of the hepatic microcirculation (4), and it has been suggested (34) that the hepatic insulin resistance previously reported in mice with global knockout of endothelial NOS is a result of impaired hepatic perfusion.

The change in hepatic blood flow does not explain the enhancement of NHGU, other explanations must be sought. The change in hepatic sinusoidal hormone concentrations resulting from the reduction of hepatic blood flow during l-NAME infusion could have impacted both positively (insulin) and negatively (glucagon) on NHGU, but the magnitude of the impacts is difficult to quantify. In our previous work (27), a doubling of the sinusoidal insulin concentration was sufficient to increase NHGU and fractional extraction of glucose (91 and 102 mg·kg⁻¹·min⁻¹ and two- to threefold, respectively. Thus the 35% increase in sinusoidal insulin that we observed in LN/SAL is unlikely to have accounted for a large proportion of the enhancement in liver glucose uptake. Moreover, the increase in sinusoidal insulin in LNlow was much more modest (10 and 12% in P2 and P3, respectively, compared with P1) and would have little impact on NHGU and fractional extraction. Also, our previous data (27) were obtained in the presence of basal glucagon concentrations. In the current studies, glucagon tended to increase concomitantly with insulin, and this would have offset insulin’s stimulatory effect on liver glucose uptake (12, 18, 33). Additionally, epinephrine concentrations were elevated in the LN group vs. control and this would have stimulated hepatic glucose output (8), thereby reducing the net glucose uptake and fractional extraction of glucose by the liver. Another possible explanation for our data is that NOS inhibition limits the activity of AMP-activated protein kinase (AMPK). One pathway for activation of AMPK is triggered by peroxynitrite, which can be generated by the spontaneous reaction of NO with superoxide anion (43). Consistent with this possibility, intraportal infusion of the AMPK activator 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside induced acute hepatic insulin resistance in the conscious dog (29).

Nonhepatic glucose disposal was difficult to evaluate in the high-dose LN groups because the dogs were exposed to very different peripheral glucose loads during P1–P3. Nonhepatic glucose uptake in the LNlow group reached a relative plateau during P2 and P3, but it steadily increased in the control group over the course of the experiment. During P3, the divergence in response between groups reached statistical significance. The reduction in nonhepatic glucose uptake in association with an enhancement of liver glucose disposal is in agreement with our previous findings (14, 25, 28) of a reciprocal relationship between the liver and nonhepatic tissues in disposition of glucose under a variety of experimental conditions. The glucose infusion rate, in absolute terms, was ~3.5 mg·kg⁻¹·min⁻¹ less in LNlow than in the control group during P3, but because of the variability of the measurement, it did not reach statistical significance. Therefore, it is not clear whether the l-NAME infusion reduced whole body glucose disposal or merely redirected glucose toward the liver and away from the peripheral tissues. In lean, standard-diet-fed mice with or without l-NAME added to their drinking water for 12 wk, there were no differences in glucose tolerance, insulin tolerance, or insulin signaling in
skeletal muscle between groups (40). This suggests that β-NAME treatment in our studies simply directed glucose toward the liver.

Blood pressure and heart rate during the basal period and P1 were within the ranges previously reported by our group (9) and others (39) in dogs similar in size to those in the current investigation. The hypertensive and bradycardic effects of β-NAME were consistent with those observed in humans, dogs, and rats (30, 31, 41) and appeared to be dose dependent. Despite the observed cardiovascular changes, the arterial concentrations of norepinephrine in the combined LN group did not differ significantly from those in the control group during P2, suggesting little change in sympathetic output. Only SIN-1 infusion was associated with a consistent and significant rise in catecholamine concentrations.

In conclusion, acute intraportal infusion of β-NAME stimulated NHGU under hyperinsulinemic and hyperglycemic conditions. The increase in NHGU was accompanied by an increase in hepatic carbon retention (glycogen storage) and may reflect an enhancement of hepatic insulin signaling in the presence of β-NAME (38).

REFERENCES

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GRANTS

NOS INHIBITION AND LIVER GLUCOSE DISPOSAL


