Hepatic oxygen metabolism in porcine endotoxemia: the effect of nitric oxide synthase inhibition

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Hepatic oxygen metabolism in porcine endotoxemia: the effect of nitric oxide synthase inhibition. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G1377–G1385, 1998.—The role of endotoxin (lipopolysaccharide, LPS) and nitric oxide in hepatic oxygen metabolism was investigated in 36 pigs receiving 1) LPS (1.7 μg·kg⁻¹·h⁻¹) for 7 h and N⁶-nitro-L-arginine methyl ester (L-NAME; 25 mg/kg) after 3 h, 2) LPS, NaCl and L-NAME, and 3) NaCl. Infusion of LPS reduced hepatic oxygen delivery (D O₂H) from 60 ± 4 to 30 ± 5 ml/min (P < 0.05) and increased the oxygen extraction ratio from 0.29 ± 0.07 to 0.68 ± 0.04 after 3 h (P < 0.05). Hepatic oxygen consumption (V O₂H) was maintained (18 ± 4 and 21 ± 4 ml/min, change not significant), but acidosis developed. Administration of L-NAME during endotoxemia caused further reduction of D O₂H from 30 ± 3 to 13 ± 2 ml/min (P < 0.05) and increased hepatic oxygen extraction ratio from 0.46 ± 0.04 to 0.80 ± 0.03 (P < 0.05). There was a decrease in V O₂H from 13 ± 2 to 9 ± 2 ml/min but that did not reach statistical significance, probably representing a type II error. Acidosis was aggravated. Administration of L-NAME in the absence of endotoxin also increased the hepatic oxygen extraction ratio, but no acidosis developed. In a different experiment, liver blood flow was mechanically reduced in the presence and absence of endotoxin, comparable to the flow reductions caused by L-NAME. The increase in hepatic oxygen extraction ratio (0.34) and maximum hepatic oxygen extraction ratio (0.90) was similar whether D O₂H was reduced by occlusion or by L-NAME. We concluded that L-NAME has detrimental circulatory effects in this model. However, neither endotoxin nor L-NAME seemed to prevent the ability of the still circulated parts of the liver to increase hepatic oxygen extraction ratio to almost maximum when oxygen delivery was reduced. The effect of L-NAME on oxygen transport thus seems to be caused by a reduction in D O₂H rather than by alterations in oxygen extraction capabilities.

N⁶-nitro-L-arginine methyl ester; liver circulation; liver oxygen consumption; septic shock

Inhibition of NO synthesis in sepsis and endotoxemia may therefore be of therapeutic benefit. However, the evidence of increased organ damage during endotoxemia and NOS inhibition makes this a controversial approach (2, 9, 10, 28).

NO produced by the endothelial constitutive NOS (eNOS) regulates vascular tone and blood pressure as well as organ blood flow distribution (18, 34). NO also modulates vascular permeability (14) and inhibits platelet aggregation and leukocyte adhesion to the endothelium (15).

A general problem in sepsis seems to be a failure in the ability of tissues and organs to increase oxygen extraction to compensate for reductions in oxygen delivery, which often appear in combination with increased oxygen demand (26). Treatment of septic shock should therefore not only be aimed at increasing arterial pressure, which NOS inhibition seems to do, but also should be aimed at improving tissue oxygenation and ultimately tissue viability.

Although there is substantial evidence that nonselective NOS inhibition (i.e., inhibition of both eNOS and iNOS) reduces regional blood flows and oxygen delivery (12, 25, 34), the role of NO in the regulation of oxygen metabolism is controversial (26, 27, 29, 33, 35).

The aim of the present study was to investigate the effect of N⁶-nitro-L-arginine methyl ester (L-NAME), a nonselective inhibitor of NO synthase activity, on hepatic oxygen metabolism in a porcine model of endotoxemia.

Changes in hepatic oxygen metabolism caused by L-NAME might occur secondary to vasoconstriction and reduced oxygen delivery or potentially by a specific NO-related effect on oxygen metabolism. To separate the two potential mechanisms of action, hepatic oxygen metabolism after NOS inhibition was compared with measurements obtained at comparable blood flow obtained by mechanical reduction of hepatic inflow.

MATERIALS AND METHODS

Animals

The experiments were performed on juvenile pigs of either sex, weighing 15–24 kg. Pigs were allowed free access to water but were not allowed food for 12 h before the experiments.

Anesthesia

The pigs were premedicated with ketamine chloride (25 mg/kg im) and atropine (0.3–0.5 mg im), and anesthesia was

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subsequently induced with pentobarbital sodium (5 mg/kg iv) and maintained with supplementary infusions of pentobarbital sodium (1–2 mg/kg) and morphine (10–20 mg·kg⁻¹·h⁻¹), when required. The animals were mechanically ventilated with room air on a Harvard volume-adjusted ventilator (tidal volume of 350–500 ml, rate of 12–18/min). Ventilator settings were adjusted during the stabilization period to achieve baseline blood gases (PO₂ > 73 mmHg, PCO₂ of 35–45 mmHg). Ventilation was not altered during the experiment, and no bicarbonate was administered.

Surgery

Surgery was performed under sterile conditions. The left internal jugular vein was cannulated for infusion of anesthetics and fluids. A tracheotomy was performed for mechanical ventilation. Through a midline incision, ultrasound transit time flow probes (Transonic Systems, Ithaca, NY) were placed around the hepatic artery and the portal vein for continuous flow measurements by a flowmeter (Transonic T201, Transonic Systems). Two mesenteric veins were cannulated for the infusion of either endotoxin or drugs. Catheters connected to pressure transducers (model AE840, SensoNOR, Horten, Norway) were passed from the right external jugular vein to a hepatic vein from the left femoral artery to the abdominal aorta and from a mesenteric vein into the portal vein. A Swan Ganz thermodilution catheter (model 93A-131H-7F, American Edwards Lab, Anasco, Puerto Rico) was passed from a femoral vein to a branch of the pulmonary artery and intermittently placed in a wedge position.

Recordings were performed on a multichannel recorder (Gould ES 2000 V20 recorder, Gould Recording Systems Division, Cleveland, OH). The Swan Ganz catheter was connected to a cardiac output computer (Edwards Lab, Santa Ana, CA). A Foley catheter was introduced into the bladder via a cystotomy, and urine output was measured. Rectal temperature was measured with a thermistor probe.

After surgery, the animals were allowed to stabilize for 1 h, and then baseline measurements and plasma samples were obtained.

Endotoxin was infused into the portal vein to mimic the possible event of endotoxemia caused by bacterial translocation from the gut. Because we wanted to study the effects of a treatment regimen on liver derangement caused by endotoxin, endotoxin was infused for 3 h before intervention.

Treatment Protocols

The animals were randomly assigned to one of the following protocols. Endotoxin (lipopolysaccharide, LPS), L-NAME, or their vehicles (NaCl) were infused into the portal vein. Group 1 pigs were given LPS and L-NAME (n = 9). Endotoxin (1.7 µg·kg⁻¹·h⁻¹; LPS B, Escherichia coli 026:B6, Difco Laboratories, Detroit, MI) was continuously infused over 7 h. L-NAME (Sigma Chemical, St. Louis, MO), 25 mg/kg dissolved in 2 ml NaCl, was given as a bolus injection after 3 h. Group 2 pigs were given LPS (1.7 µg·kg⁻¹·h⁻¹) and NaCl (n = 9). Two milliliters of isotonic saline were given as a bolus injection after 3 h. Group 3 pigs were given NaCl and L-NAME (n = 9). Isotonic saline was given in the same volume as the endotoxin solution, followed by L-NAME (25 mg/kg) as a bolus injection after 3 h. Group 4 pigs were the control group (n = 9). Isotonic saline infusion was given as in group 3, followed by 2 ml saline as a bolus injection after 3 h. The animals received a total fluid replacement of 20 ml·kg⁻¹·h⁻¹ (Ringer acetate and 5% glucose in saline). All animals were observed for 7 h after stabilization or until death. After the study period, the animals were killed with intravenous injection of pentobarbital sodium.

In a separate set of experiments (n = 3), we measured the effect of repeated mechanical reductions of liver blood flow on hepatic oxygen extraction. Vascular occluders (In Vivo Metric, Healdsburg, CA) were placed around the hepatic artery (OC6, 6 mm) and the portal vein (OC12HD, 12 mm), and blood flow was reduced in a stepwise manner by 80–90% at baseline (n = 9 partial occlusions in the absence of endotoxin) and after 2–4 h of endotoxemia (n = 22 partial occlusions in the presence of endotoxin). The effect of mechanical reductions in hepatic oxygen delivery (DO₂H) was compared with the corresponding effects caused by L-NAME.

The experiments were carried out in accordance with the Norwegian National Guidelines for Animal Care and were approved by the local ethics committee.

Hemodynamic Measurements. The hemodynamic data have previously been published in part (25).

Portal venous blood flow (Qpv) and hepatic artery flow (Qha) were measured continuously, whereas cardiac output was measured and blood gases were analyzed at 180, 195, 240, 300, 360, and 420 min for statistical evaluation.

Calculation of DO₂H and Hepatic Oxygen Consumption

Blood samples were obtained from catheters in the aorta and the portal and hepatic veins and analyzed in a blood gas analyzer (AVL 945, AVL, Graz, Austria), which calculates PO₂, PCO₂, standard bicarbonate, and base excess. Oxygen saturation (SO₂) was measured in a cooximeter (model 282, Instrumentation Laboratories, Lexington, KY). Hemoglobin was measured in arterial blood samples by the hospital laboratory for clinical chemistry. Qha, Qpv, arterial oxygen saturation (SaO₂), portal venous oxygen saturation (SpvO₂), hepatic venous oxygen saturation (ShvO₂), and arterial hemoglobin (Hb) were used in the calculations for DO₂H (Eq. 1), hepatic oxygen consumption (VO₂H; Eq. 2), and hepatic oxygen extraction ratio (ERO₂H; Eq. 3), respectively

\[
DO₂H \text{ (ml/min)} = \frac{(S_aO₂ - \overline{Q}_ha + SpvO₂ \times \overline{Q}_pv) \times Hb \times 13.4 \times 10^{-5}}{1} \tag{1}
\]

\[
V Or H = \frac{(S_aO₂ - \overline{Q}_ha + SpvO₂ \times \overline{Q}_pv) \times Hb \times 13.4 \times 10^{-5}}{1} \tag{2}
\]

\[
ERO₂H = \frac{V Or H}{DO₂H} \tag{3}
\]

The calculations were repeated, implementing the individual hemoglobin in the portal and hepatic veins instead of the arterial hemoglobin that was used as an approximation. The difference in hemoglobin concentration between the three vessels was less than or equal to the interassay variation in each sample (5%). The dissolved oxygen content (PO₂ × 0.003) was incorporated as well. These additional calculations did not significantly change the results (most changes were <1%), confirming that in calculations of hepatic oxygen transport 1) the arterial hemoglobin can be used as a very good approximation of portal and hepatic venous hemoglobin and 2) the dissolved oxygen content (1–3% of the total oxygen content) can be disregarded.

Statistical Analysis

Results are expressed as means ± SE. Differences within and between groups were determined by ANOVA for repeated measurements, using the SPSS software (MANOVA), with the Greenhouse-Geisser correction for time dependency of
variables. When the recorded baseline values for any parameter differed between groups, the changes in that parameter were used in the MANOVA analysis. When the MANOVA procedure detected significant differences at up to 195 min with 34 of 36 animals still alive, it was followed by t-tests with appropriate corrections for multiple tests (Bonferroni). Evaluation of survival data was done by χ²-test. Probability values (after correction) of < 0.05 were considered statistically significant.

RESULTS

Survival

Five of nine animals died in group 1 (LPS + L-NAME) after 3.5, 4, 5, 6, and 6 h, respectively; however, they all lived long enough to be included in the evaluation of the L-NAME effects after 15 min. Two animals died in group 2 (LPS alone) after 3 and 6 h, and one animal died in group 3 (NaCl + L-NAME) after 5 h. There were no deaths in the control group (group 4). Group 1 survival was significantly reduced compared with the other groups (P < 0.05).

Changes in Cardiac Output

LPS infusion caused within 3 h a reduction in cardiac output from 3.2 ± 0.2 to 2.4 ± 0.3 l/min (P < 0.05; Fig. 1). The injection of L-NAME caused a further reduction (by 41%) in cardiac output (LPS + L-NAME, group 1), and in the absence of endotoxin (NaCl + L-NAME, group 3) L-NAME also caused a marked reduction in cardiac output (by 30%; Fig. 1).

Changes in Liver Blood Flow

In the saline control group, there were no significant changes in liver blood flow.

Endotoxin effects. Continuous infusion of LPS for 3 h resulted in a reduced liver perfusion (Fig. 2). Qpv was reduced by 26% (from 450 ± 70 to 330 ± 40 ml/min, P < 0.05) and Qha by 41% (from 150 ± 15 to 80 ± 10 ml/min, P < 0.05). After 3 h, there were no further changes in liver blood flow. The hepatic arterial fractional flow (Qha/cardiac output) was reduced from 5.1 to 3.1% (P < 0.05; Fig. 3), indicating that endotoxin causes relatively larger reductions in Qha than in cardiac output.

L-NAME effects. During endotoxemia (LPS + L-NAME, group 1), the injection of L-NAME caused further reductions in liver perfusion by 50%, and maximal effects were observed after 15 min (Qpv from 320 ± 30 to 160 ± 20 ml/min and Qha from 100 ± 20 to 50 ± 10 ml/min, P < 0.05; Fig. 2). Compared with measurements before endotoxin infusion was started, liver blood flow was reduced by two-thirds. There was no further change in hepatic arterial fractional flow after L-NAME injection (Fig. 3).

Similarly, in the absence of endotoxin (NaCl + L-NAME, group 3), L-NAME reduced liver perfusion by 50% (P < 0.05, data not shown).

Fig. 1. Effect of endotoxin and Nω-nitro-L-arginine methyl ester (L-NAME) on cardiac output (CO). Results are means ± SE. ●, lipopolysaccharide (LPS) infusion followed by L-NAME after 180 min; ○, LPS infusion alone; ▲, saline + L-NAME; △, saline alone. *P < 0.05 compared with baseline; #P < 0.05 compared with 180 min (pre-L-NAME).

Fig. 2. Effect of endotoxin and L-NAME on liver blood flow. Results are means ± SE. Qpv, portal venous flow; Qha, hepatic artery flow. ●, LPS infusion (1.7 µg·kg⁻¹·h⁻¹ for 7 h) and L-NAME (25 mg/kg) intraportally after 180 min; ○, LPS infusion. *P < 0.05 compared with baseline (time 0); #P < 0.05 compared with 180 min (pre-L-NAME).
Changes in Blood Gases and Hepatic Oxygen Metabolism

In the saline control group (group 4), there were no significant changes in blood gases or oxygen metabolism.

Endotoxin effects. Continuous infusion of endotoxin caused within 3 h a reduction in DO$_2$H from 56 ± 4 to 30 ± 5 ml/min (P < 0.05, Fig. 4). The hepatic oxygen extraction ratio increased from 0.29 ± 0.07 to 0.68 ± 0.04 (P < 0.05), and VO$_2$H remained unchanged (from 18 ± 4 to 21 ± 4 ml/min, not significant; see Table 4). The increased extraction ratio was mirrored by a decrease in hepatic venous PO$_2$ from 31.9 ± 2.1 to 15.8 ± 2.7 mmHg (P < 0.05; Table 3).

During 3 h of endotoxemia, acidosis developed, as indicated by measurements of pH and base excess in aorta and the portal and hepatic veins (Tables 1–3, see Fig. 6). After 3 h, there were no further changes in blood gases.

L-NAME effects. During endotoxemia, L-NAME injection caused further reductions in DO$_2$H from 30 ± 3 to 13 ± 2 ml/min after 15 min (P < 0.05; see Fig. 4). This was not only due to the reduced liver perfusion but also to the reduced portal venous oxygen saturation (Table 2). DO$_2$H was thus reduced by 80% compared with baseline. The hepatic oxygen extraction ratio was increased by 90% (from 0.45 ± 0.04 to 0.80 ± 0.03, P < 0.05; see Table 4), with a corresponding reduction in hepatic venous PO$_2$ from 22.0 ± 3 to 12.3 ± 2.1 mmHg, P < 0.05; Table 3). VO$_2$H tended to fall (from 13 ± 2 ml/min to 9 ± 2 ml/min, not significant; Table 4). At this point, the amount of oxygen delivered was 13 ± 2 ml/min, which is lower than the extracted amount of oxygen at baseline in this group (17 ± 3 ml/min; Fig. 5).

Similarly, in the absence of endotoxin (NaCl + L-NAME, group 3), L-NAME decreased DO$_2$H from 49 ± 7 to 32 ± 4 ml/min (P < 0.05; Fig. 4). Hepatic oxygen extraction ratio increased by 58 ± 27% (from 0.35 ± 0.03 to 0.56 ± 0.06, P < 0.05, range 0.2–0.9; Table 4), and VO$_2$H remained unchanged. Hepatic venous PO$_2$ was reduced from 30.4 ± 2.3 to 23.7 ± 1.5 mmHg (P < 0.05; Table 3).

During endotoxemia, L-NAME further increased acidosis. The reductions in plasma base excess (−10 mmol/l in all vessels) reached their maxima after 1 h (Tables 1–3 and Fig. 6). In the absence of endotoxin, L-NAME caused only minor changes in blood gases except for a decrease in PO$_2$ (Tables 1–3).

Thus the oxygen delivery after injection of L-NAME alone (saline group) was numerically equal to the oxygen delivery after 3 h of endotoxin infusion (Fig. 4), but acidosis was observed exclusively in the endotoxin group.

The effect of mechanical reductions in liver blood flow on hepatic oxygen extraction. After 2–4 h of endotoxemia, stepwise reductions in liver blood flow (n = 22 partial occlusions in 3 pigs) to 10–30% of baseline (average total liver blood flow reduction from 320 to 80 ml/min) caused a 90% increase in oxygen extraction ratio from 0.46 ± 0.03 to 0.80 ± 0.02 (Fig. 7). Individual extraction ratios after partial occlusion during endotoxemia ranged from 0.67 to 0.92. Compared with the...
increase in extraction ratio after L-NAME injection during endotoxemia (from 0.46 ± 0.04 to 0.80 ± 0.03), there was no difference at comparable total liver blood flow (80 and 100 ml/min, respectively; Fig. 7). Individual extraction ratios after L-NAME ranged from 0.72 to 0.97.

Similarly, partial occlusion of liver blood flow in the absence of endotoxin (n = 9 episodes of partial occlusion in 3 pigs; reduction of average total liver blood flow from 460 to 150 ml/min) caused an increase in hepatic oxygen extraction ratio (from 0.30 ± 0.04 to 0.74 ± 0.04; Fig. 7). Individual extraction ratios ranged from 0.58 to 0.88. The increase in extraction ratio after L-NAME injection in the absence of endotoxin (from 0.32 to 0.56, n = 9) was lower than during occlusion, but this corresponded to the reduction in total liver blood flow (from 480 to 310 ml/min), which was also lower than after occlusion (310 vs. 150 ml/min). Individual extraction ratios ranged from 0.38 to 0.90.

Thus comparable reductions in DO₂H, caused by L-NAME or vascular occlusion, resulted in similar increases in hepatic oxygen extraction ratios with the maximum extraction ratio at 0.9. The increases in oxygen extraction ratios were similar whether endotoxin was present or not.

### DISCUSSION

Endotoxin infusion caused significant reductions in liver blood flow and oxygen delivery followed by a marked increase in the hepatic oxygen extraction ratio. There was no significant change in VO₂H, but acidosis developed, indicating inadequate organ perfusion. Administration of L-NAME during endotoxemia caused immediate and profound further reductions in liver perfusion and in DO₂H, accompanied by aggravated acidosis. The oxygen extraction ratio increased to 0.80, and VO₂H tended to decrease. This indicates that the

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Values are means ± SE. *P < 0.05 compared with baseline; †P < 0.05 compared with 180 min (pre-L-NAME).
hepatic oxygen extraction capability was remarkably well preserved even during late (>3 h) endotoxemia, which is somewhat contradictory to the impairments in oxygen extraction during sepsis and endotoxemia observed in other studies (23). Increased VO2H in early sepsis has, however, previously been reported in the pig (1). Also, similar experiments in our porcine model show that, during 6 h of endotoxemia, the VO2H is unchanged (8).

In a canine model, Nelson and co-workers (20) demonstrated that endotoxin caused a reduction in intestinal oxygen extraction ability: critical DO2 (the DO2 below which VO2 becomes supply dependent) was increased by 88%, and the corresponding critical extraction ratio was reduced by 30% (from 0.69 to 0.47), whereas maximum intestinal extraction ratio was reduced from 0.83 to 0.71. In the present study, we have not calculated the critical DO2H, but the pooled data show that the maximum hepatic oxygen extraction ratio during endotoxemia was not reduced. On the contrary, the maximum hepatic oxygen extraction ratio during reductions in DO2H (vascular occlusion) tended to be even higher in the presence than in the absence of endotoxin (hepatic oxygen extraction ratio of 0.92 and 0.84, respectively). Thus oxygen transport during endotoxemia seems to be better preserved in the porcine liver than, at least, in the canine intestine.

Although there was no significant decrease in total VO2H during endotoxemia, the low hepatic venous PO2 and acidosis indicated that in some regions DO2 probably was too low to meet tissue metabolic needs. After L-NAME was given in the saline + L-NAME group, DO2H was abruptly reduced from 50 ml/min to the same level as in the endotoxin alone group (30 ml/min), but there were no signs of oxygen supply dependency or ischemia, i.e., VO2H, pH, and base excess were unchanged.

Therefore, the observed acidosis during endotoxemia cannot be explained exclusively by a global reduction in DO2H, but more likely it reflects heterogeneous perfusion causing focal ischemia.

This interpretation is consistent with previous studies that have demonstrated marked heterogeneity in liver perfusion during endotoxemia, ranging from areas with shutdown of sinusoids (16) to areas with apparent hyperperfusion (4). There are several possible explanations for this flow pattern, including vasospasm, swelling of sinusoidal lining cells, sinusoidal contraction of Ito cells, and platelet plugs/thrombosis. The poorly

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Values are means ± SE. *P < 0.05 compared with baseline; †P < 0.05 compared with 180 min (pre-L-NAME).
circulated areas of the liver will produce H⁺, which causes acidosis in the main hepatic vein.

The marked decrease in liver blood flow caused by L-NAME may have been accompanied by increasing heterogeneity of flow (21) and thus caused increasing acidosis and organ damage. The ischemic areas producing H⁺ during anaerobe glycolysis will contribute less to oxygen consumption than well-perfused areas, and the overall hepatic Vportal will logically decrease.

The reduction in liver Vportal after L-NAME was not statistically significant in the present study. This may be due to a type II error because there were large variations in oxygen transport between pigs. A power analysis of the test shows that, to detect a true difference in means of 8 ml/min (the observed difference in Vportal at baseline and 15 min after L-NAME during endotoxemia) with 80% probability, a sample size of 23 is required, assuming a standard deviation of differences of 9, using a paired t-test with a 0.05 two-sided significance level.

During the last 3 h of the study, several of the pigs in the LPS + L-NAME group (group 1) died, making the group too small (n = 7, 6, and 4, respectively) to draw definite statistical conclusions. As the sickest animals (having probably the lowest oxygen uptake) died, the values reported may not be representative of values for the survivors.

However, the marked decrease in liver blood flow, the very low hepatic venous oxygen saturation, and the severe acidosis are consistent with severe hepatic ischemia and tissue injury. This will probably cause irreversible organ damage. Therefore, in a larger study, we predict there will be a significant decrease in Vportal in endotoxemia.

One of the questions in this study was whether endotoxin or L-NAME affected the ability of the liver to increase its oxygen extraction ratio when oxygen delivery was reduced. Therefore, separate experiments with vascular occluders were performed to evaluate the effect of profound reductions in oxygen delivery. Even after 4 h of endotoxemia, extraction ratios up to 0.9...
were measured, comparable with the extraction ratios after L-NAME injection (see Fig. 7). The very high oxygen extraction ratios suggest that those parts of the liver that still circulate function well, and these regions appear to have a potential for increasing the oxygen extraction. We suggest that the main reason for the acidosis during endotoxemia is the contribution of $H^+$ from anaerobic metabolism in poorly circulating areas, possibly combined with specific cellular effects of endotoxin.

Another possible mechanism for the acidosis in endotoxemia might be increased NO production, which inhibits mitochondrial respiration. This would be in accordance with studies in cell cultures in which increases in NO, either exogenously applied or endogenously induced, cause an inhibition of mitochondrial respiration due to enzyme inhibition (28, 30). In a model of porcine endotoxemia comparable to ours, enhancement of NO production was indeed demonstrated (13). With the assumption that L-NAME, in the dose used in the present study, causes complete NOS inhibition, the increase in the hepatic oxygen extraction ratio might be explained by reactivation of mitochondrial enzymes. However, the similar increases in the oxygen extraction ratio after reductions in oxygen delivery caused by L-NAME and liver blood flow occlusion makes this explanation less plausible. Thus the effect of L-NAME on hepatic oxygen metabolism in the present study can most logically be explained by the reduction in oxygen supply.

Endotoxin is known to inhibit eNOS production (19, 22), which is of fundamental importance in regulation of microvascular functions. The finding that further inhibition of endothelial NOS by L-NAME had detrimental effects on liver perfusion supports the notion that some basal NO production is necessary to secure adequate organ perfusion and oxygen delivery. Thus the unfavorable inhibition of the constitutive NOS caused by endotoxin as well as by L-NAME would probably by far outweigh any favorable effects of reduced NO production from iNOS caused by L-NAME.

The present study does not exclude specific effects of endotoxin on cellular metabolism causing increased energy requirements or uncoupling of oxidative phosphorylation compromising ATP production (6). Under these conditions, “normal” levels of VO$_2$ would be inadequate and acidosis would subsequently develop. Endotoxin may cause defects in cellular metabolic pathways in such a manner that anaerobic glucose utilization is preferred, thus leading to tissue acidosis independent of either tissue P$_O_2$ or VO$_2$ (7).

In a clinical setting without invasive measurements of organ blood flow and oxygen transport, treatment with NOS inhibitors in septic shock could seem attractive due to the increase in arterial pressure observed in animals (32) and humans (24). However, any agent reducing organ blood flow and oxygen delivery, as demonstrated in this study, is potentially hazardous.

In conclusion, we found that, whereas endotoxin caused a 50% reduction in DO$_2$H, oxygen extraction ratios increased, and there was no significant decrease in oxygen consumption. Still, the acidosis indicates insufficient tissue oxygen delivery relative to oxygen demand.

The NOS inhibitor L-NAME caused further reductions in DO$_2$H, and, whereas the oxygen extraction ratio increased (by 87%), VO$_2$ tended to fall. The accompanying aggravated hepatic venous acidosis indicates that parts of the liver were ischemic. However, neither endotoxin nor L-NAME seemed to reduce the capacity of the liver to increase oxygen extraction when oxygen delivery was reduced. Maximal oxygen extraction ratios during endotoxemia were similar after L-NAME and after comparable reductions in DO$_2$H caused by vascular occluders. Thus the L-NAME-induced changes in hepatic oxygen metabolism in endotoxemia appear to be mediated predominantly by vasoconstriction, reduced liver inflow, and the subsequent reduction in oxygen delivery rather than by alterations in oxygen extraction capabilities.

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