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Effects of cardiac preload reduction and dobutamine on hepatosplanchnic blood flow regulation in porcine endotoxemia

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Jakob SM, Bracht H, Porta F, Balsiger BM, Brander L, Knuesel R, Feng H-Q, Kolarova A, Ma Y, Takala J. Effects of cardiac preload reduction and dobutamine on hepatosplanchnic blood flow regulation in porcine endotoxemia. *Am J Physiol Gastrointest Liver Physiol* 303: G247–G255, 2012. First published May 3, 2012; doi:10.1152/ajpgi.00433.2011.—Insufficient cardiac preload and impaired contractility are frequent in early sepsis. We explored the effects of acute cardiac preload reduction and dobutamine on hepatic arterial (Qha) and portal venous (Qpv) blood flows during endotoxin infusion. We hypothesized that the hepatic arterial buffer response (HABR) is absent during preload reduction and reduced by dobutamine. In anesthetized pigs, endotoxin or vehicle (n = 12, each) was randomly infused for 18 h. HABR was tested sequentially by constricting superior mesenteric artery (SMA) or inferior vena cava (IVC). Afterward, dobutamine at 2.5, 5.0, and 10.0 μg/kg per minute or another vehicle (n = 6, each) was randomly administered in endotoxemic and control animals, and SMA was constricted during each dose. Systemic (cardiac output, thermodilution) and carotid, splanchnic, and renal blood flows (ultrasound Doppler) and blood pressures were measured before and during administration of each dobutamine dose. HABR was expressed as hepatic arterial pressure/flow ratio. Compared with controls, 18 h of endotoxin infusion was associated with decreased mean arterial blood pressure [49 ± 11 mmHg vs. 58 ± 8 mmHg (mean ± SD); P = 0.034], decreased renal blood flow, metabolic acidosis, and impaired HABR during SMA constriction [0.32 (0.18–1.32) mmHg/ml vs. 0.22 (0.08–0.60) mmHg/ml; P = 0.043]. IVC constriction resulted in decreased Qpv in both groups; whereas Qha remained unchanged in controls, it decreased after 18 h of endotoxemia (P = 0.031; constriction-time-group interaction). One control and four endotoxemic animals died during the subsequent 6 h. The maximal increase of cardiac output during dobutamine infusion was 47% (22–134%) in controls vs. 53% (37–85%) in endotoxemic animals. The maximal Qpv increase was significant only in controls [24% (12–47%) of baseline (P = 0.043) vs. 17% (−7–32%) in endotoxemia (P = 0.109)]. Dobutamine influenced neither Qha nor HABR. Our data suggest that acute cardiac preload reduction is associated with preferential hepatic arterial perfusion initially but not after established endotoxemia. Dobutamine had no effect on the HABR.

hepatic arterial buffer response; sepsis; liver

INADEQUATE SPLANCHNIC PERFUSION is associated with multiple organ failure and death (11, 23), especially when hepatic dysfunction is present (28). Global hepatic blood flow is supposed to be relatively protected when gut blood flow decreases because hepatic arterial flow increases when portal venous flow decreases (the hepatic arterial buffer response, HABR) (25, 26). However, evidence suggests that the HABR is diminished or even abolished during endotoxemia (1) and when gut blood flow becomes very low (19, 31).

The HABR is usually assessed by decreasing superior mesenteric or portal venous blood flow and measurement of the associated changes in hepatic arterial blood flow and pressure. The clinical correlates to this maneuver, mesenteric embolism or acute portal vein thrombosis, are rare events. In sepsis, impaired mesenteric perfusion is more frequently the result of hypovolemia or insufficient cardiac output (CO). Whether the hepatic arterial buffer response is effective under these conditions is not known.

Dobutamine is commonly used to ameliorate CO. However, effects of dobutamine on hepatosplanchnic perfusion during sepsis are controversial. Some have argued that these effects depend largely on the effect of the drug on systemic hemodynamics (21, 32). Others have demonstrated improvement in regional (sublingual) perfusion in patients with septic shock, independent of dobutamine-induced changes in systemic blood flow (9). On the other hand, in endotoxemic rats, portal and sinusoidal perfusion did not change despite a dobutamine-associated increased in CO (35). Furthermore, data from patients with septic shock demonstrate that dobutamine fails to restore liver function, despite amelioration of splanchnic perfusion (20). The effects of dobutamine on HABR are not known.

This study has two aims: *1*) to assess changes in portal venous and hepatic arterial blood flows during acute cardiac preload reduction in a model of porcine endotoxemia and *2*) to evaluate the effects of dobutamine on regional hepatosplanchnic perfusion after prolonged endotoxemia. We hypothesized that acute preload reduction would lead to a parallel decrease in hepatic arterial and portal venous blood flows and that dobutamine-induced redistribution of hepatosplanchnic perfusion would diminish the increase in hepatic arterial blood flow during acute portal blood flow reduction.

MATERIALS AND METHODS

The study was performed in accordance with the National Institutes of Health guidelines for the care and use of experimental animals and with the approval of the Animal Care Committee of the Canton of Bern, Switzerland. Some of the data obtained during the 18 h of endotoxin infusion, prior to dobutamine exposure, have been published previously (6, 29, 30). CO values of some of the animals have been published in another study where thermodilution was compared with esophageal Doppler (38). Here we report otherwise unpublished data from acute reduction in portal venous flow by superior mesenteric artery (SMA) occlusion and acute reduction in cardiac preload by

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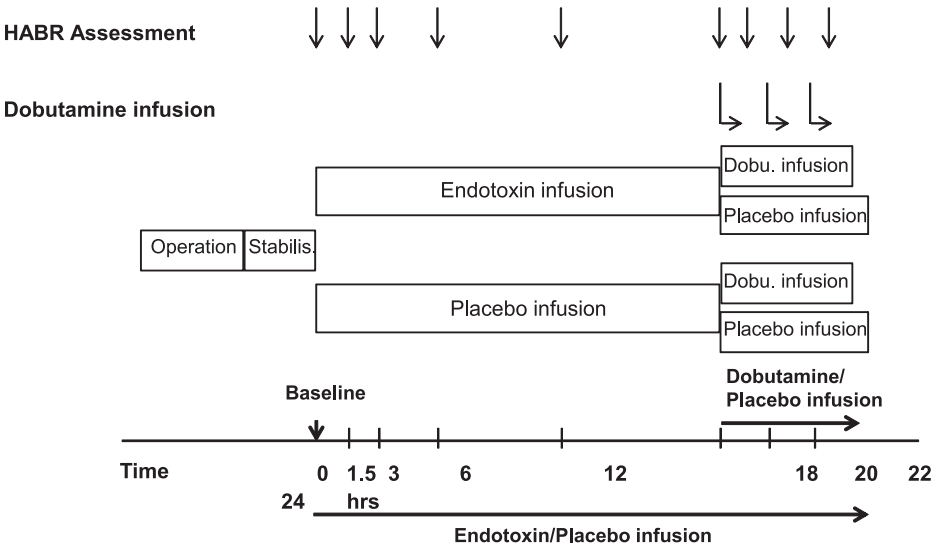

Fig. 1. Flow diagram outlining the experimental protocol. HABR, hepatic arterial buffer response; Dobu, dobutamine.

inferior vena cava occlusion during 18 h of endotoxemia and from the subsequent dobutamine-infusion period.

Anesthesia, monitoring, and animal preparation. For details we refer to a previous publication (30). Briefly, 24 anesthetized [thiopental (7 mg·kg^{-1}·h^{-1}) and fentanyl (30 μg·kg^{-1}·h^{-1} until the end of the operation, afterward 5 μg·kg^{-1}·h^{-1})] and mechanically ventilated pigs [volume-controlled ventilator (Servo ventilator 900 C; Siemens, Elema, Sweden) with 5-cmH_2O end-expiratory pressure and FI_{O_2} adjusted to keep Pa_{O_2} levels between 13.3 kPa (100 mmHg) and 20 kPa (150 mmHg)] were instrumented with carotid, pulmonary, and femoral artery catheters (CO/SVO_2 catheter; Edwards Lifesciences, Unterschleissheim, Germany) and a femoral vein catheter for fluid administration. An esophageal Doppler probe (CardioQ; Deltex Medical, Chichester, UK) was inserted after oro-tracheal intubation and connected to the monitor (CardioQ; Deltex Medical, Irving, TX). Afterward, the abdominal cavity was exposed by a midline abdominal incision; a drainage catheter was inserted into the urinary bladder; a hepatic artery catheter was inserted via the left gastric artery; superior mesenteric, hepatic, splenic, and kidney arteries and the celiac trunk and portal vein were exposed; ultrasound Doppler flow probes (Tran-

Table 1. *Systemic and regional hemodynamics at baseline and after 18 h of endotoxin and placebo infusion, respectively*

	Endotoxemia		Controls		*P* Value*	
	Baseline	18 h	Baseline	18 h	Time-group interaction group effect	Time effect
Systemic hemodynamics						
Cardiac output, l/min	3.77 ± 0.93	4.80 ± 0.83	4.44 ± 1.37	5.38 ± 0.99		<0.001
Mean carotid artery pressure, mmHg	69 ± 10	49 ± 11	73 ± 11	58 ± 8	0.004	<0.001
Heart rate, beats/min	101 ± 31	128 ± 22	109 ± 20	120 ± 22		0.001
Stroke volume, ml	37 ± 11	39 ± 8	41 ± 11	46 ± 11		0.003
Mean pulmonary artery pressure	18 ± 3	19 ± 3	19 ± 3	19 ± 3	0.034	0.003
Central vein pressure, mmHg	4 ± 1	6 ± 2	4 ± 1	5 ± 1	0.053	<0.001
Systemic vascular resistance, mmHg·ml^{-1}·min^{-1}	18,580 ± 5,120	9,600 ± 2,820	16,890 ± 5,700	10,080 ± 1,970	<0.001	0.039
Metabolic changes						
Arterial pH	7.43 ± 0.04	7.33 ± 0.08	7.45 ± 0.03	7.39 ± 0.05	0.045†	<0.001
Base excess	1.6 ± 1.7	−4.1 ± 3.9	2.3 ± 1.2	−1.9 ± 2.9	0.040†	<0.001
Arterial lactate, mmol/l	1.0 ± 0.3	0.8 ± 0.5	1.0 ± 0.2	0.7 ± 0.2		0.032
Regional flows, ml/min						
Carotid artery	143 ± 43	166 ± 74	144 ± 52	182 ± 34		0.038
Renal artery	209 ± 116	175 ± 74	190 ± 76	189 ± 66	0.041	0.026
Superior mesenteric artery	523 ± 219	740 ± 284	462 ± 155	687 ± 243		<0.001
Portal vein	751 ± 178	1095 ± 295	737 ± 254	978 ± 281		<0.001
Celiac trunk	298 ± 97	336 ± 74	361 ± 210	469 ± 201		0.004
Hepatic artery	133 ± 57	121 ± 49	176 ± 112	195 ± 91		
Splenic artery	47 ± 36	64 ± 62	36 ± 23	49 ± 28		0.014
Regional pressures, mmHg						
Portal vein	9 ± 3	11 ± 5	8 ± 2	9 ± 2		0.005
Hepatic vein	7 ± 2	8 ± 3	6 ± 2	6 ± 1		0.015
Hepatic artery	62 ± 15	39 ± 7	65 ± 9	48 ± 11		<0.001

Values are means ± SD. Systemic vascular resistance is calculated as mean arterial minus central venous pressure divided by cardiac output. *ANOVA repeated measures. For statistical analysis, all values (baseline, 90 min, 3, 6, 12, and 18 h) were included. †Group effect.

sonic Systems, Ithaca, NY) were placed around the vessels after in vitro calibration. Vascular occluders were placed around the superior mesenteric and the common celiac trunk artery and around the inferior vena cava proximal to the installed flow probes.

When all surgical procedures were completed, the abdominal wall was reapproximated, and towels were placed on the surface to minimize heat loss. During surgery and the subsequent study period, the animals received normal saline at a rate of 8 $ml \cdot kg^{-1} \cdot h^{-1}$. Additional volume boluses of 50 ml 4% gelatine (Physiogel; Braun, Emmenbrücke, Switzerland) were given if hypovolemia was suspected (mean arterial pressure <60 mmHg, heart rate >100 bpm, diuresis <0.5 $ml \cdot kg^{-1} \cdot h^{-1}$, arterial lactate concentration >2 mmol/l), even if target filling pressure had been reached. This was continued as long as stroke volume increased. Body temperature was kept constant by external cooling if necessary.

Experimental protocol. After preparation, 180 min were allowed for hemodynamic stabilization. Afterward, the animals were randomized into four different groups: control + placebo, control + dobutamine, endotoxin + placebo, and endotoxin + dobutamine (Fig. 1). Endotoxin or saline was infused into the right atrium [*Escherichia coli* lipopolysaccharide B0111:B4 (Difco Laboratories, Detroit, MI), 20 mg/l in 5% dextrose]. The initial infusion rate was 0.4 $\mu g \cdot kg^{-1} \cdot h^{-1}$ until the mean pulmonary artery pressure reached 40 mmHg. The infusion was then stopped and subsequently adjusted to maintain moderate pulmonary artery hypertension (mean pulmonary artery pressure 25–30 mmHg). The adjusted endotoxin infusion rate was maintained constant until the end of the experiment. Glucose 50% was administered when blood glucose concentration was <3.5 mmol/l. After 18 h of endotoxin or saline infusion, the animals in the dobutamine groups received dobutamine at 2.5, 5.0 and 10.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$ in a randomized order for 1 h and separated by 1 h each. The other animals in the endotoxin and control groups received a saline vehicle at the corresponding infusion speed. No other vasoactive medications were used. At the end of the experiment, the animals were killed with an overdose of intravenous potassium chloride.

Assessment of the HABR. The HABR was tested at baseline, after 1.5, 3, 6, 12, and 18 h, respectively, and then after each hour with dobutamine or placebo infusion. As a reference, SMA blood flow was first reduced to zero by using the vascular occluder. When hepatic artery flow ceased to increase, the occluder around the celiac trunk was used to establish baseline hepatic arterial pressure. In case of hemodynamic instability (low blood pressure, tachycardia, low mixed venous oxygen saturation), the HABR was not measured. The HABR was calculated as the ratio between hepatic arterial pressure and flow after SMA occlusion and as the relative change of this ratio before and after SMA occlusion (to account for baseline differences). In addition, absolute and relative changes in hepatic arterial blood flow, and the ratio between changes in hepatic arterial and portal venous blood flow, were also calculated. After SMA occlusion and reestablished stable hemodynamics, the inferior vena cava was occluded until arterial blood pressure dropped and portal venous blood flow had decreased by at least 10%. Longer lasting periods of inferior vena cava occlusion were avoided because they had been associated with prolonged hemodynamic instability in pilot endotoxemic animals.

Hemodynamic monitoring and recording. Heart rate and ECG were continuously monitored. All blood pressures were measured with quartz pressure transducers, displayed continuously on a multimodular monitor (Datex-Ohmeda Engström S/5 Compact Critical Care monitor; Datex-Ohmeda, Helsinki, Finland), and recorded. CO (l/min) was measured by the thermodilution technique (mean value of 3 measurements, CO module, Datex-Engström). Regional blood flows (ml/min) were displayed using flowmeters (T206 and T106; Transonic Systems) and recorded with a computer program for further analysis (Windaq 1.60; Dataq Instruments, Akron, OH).

Measurement of nitrate/nitrite and angiotensin II concentrations. Total plasma nitrate/nitrite concentrations were determined using a Colorimetric Assay Kit according to the manufacturer's instructions (Cayman Chemical, Ann Arbor, MI).

Plasma angiotensin II concentrations were determined using an ELISA assay kit (Enzo Life Sciences, Lausen, Switzerland). Samples were taken at baseline and after 18 h of endotoxin/placebo infusion.

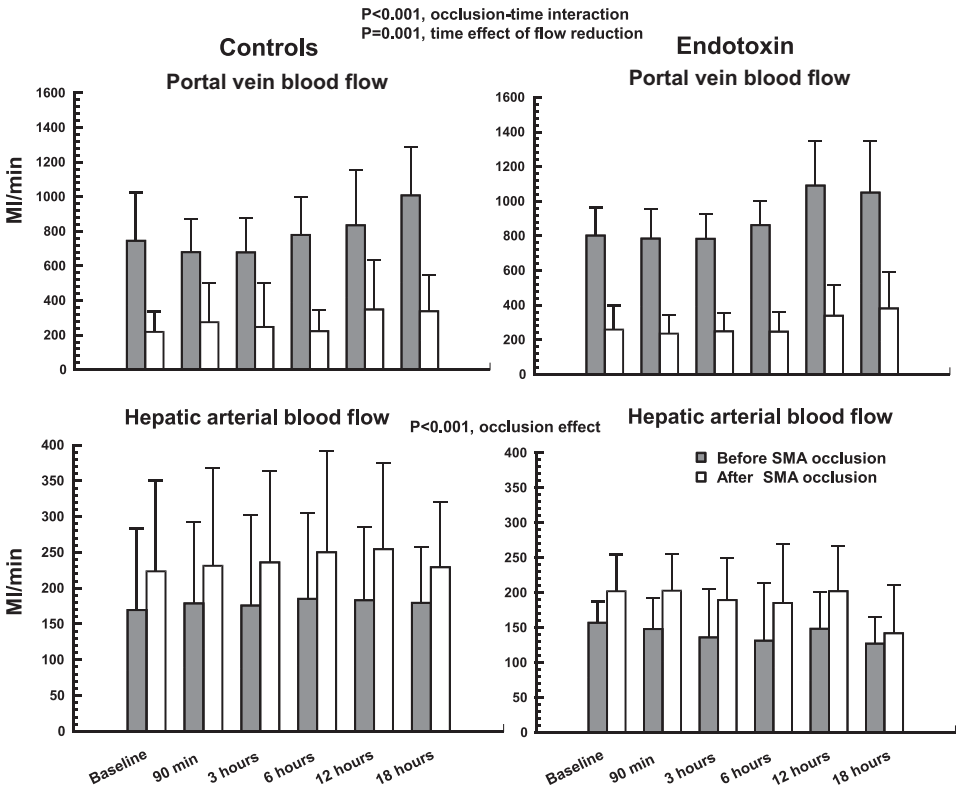

Fig. 2. The hepatic arterial buffer response. Changes in portal venous and hepatic arterial blood flows during occlusion of superior mesenteric artery (SMA) in controls and endotoxemic animals. Values are means ± SD.

Statistical analysis. For statistical analysis the PASW 18.0 software package was used. The Kolmogorov-Smirnov test was used to test for normal distribution. The effects of SMA and inferior vena cava occlusion before dobutamine infusion were analyzed using ANOVA for repeated measurements with two within-subject factors (occlusion and time) and one grouping factor (endotoxin or controls). In case of a significant occlusion-time-group interaction, blood flows before and after occlusion at 18 h were compared using paired *t*-tests. Baseline data for the subsequent period with dobutamine/placebo administration were compared using the unpaired *t*-test or the Mann-Whitney test. Changes over time were analyzed using parametric or nonparametric ANOVA (Friedman Test) for repeated measurements in each group separately. Due to significant mortality ($n = 5$) and the consequently small number of animals with all measurements in the septic groups, the effects of dobutamine and SMA constriction were only tested using comparisons between baseline and maximal values during dobutamine infusion, or last values (HABR), respectively (Wilcoxon Test). Plasma hormone concentrations were compared using the Mann-Whitney test. Data are presented as means ± SD or as median (range). A P value of <0.05 was considered significant.

RESULTS

There were no differences in the weights of the pigs between groups. During the first 18 h, animals received 261 ± 226 μg endotoxin. Central temperature slightly decreased from 39.8 ± 0.8°C at baseline to 39.4 ± 0.8°C after 18 h ($P = 0.022$), without differences between groups. Glucose support was numerically higher in endotoxemic pigs, but the difference was not statistically significant (213 ± 115 ml vs. 159 ± 69 ml glucose 50%; $P = 0.287$). Endotoxemic pigs received 8,490 ± 1,470 ml and control animals 5,740 ± 2,000 ml of fluids ($P = 0.001$).

Effects of 18 h of endotoxin infusion on systemic and regional hemodynamics, diuresis, arterial pH, and on nitrate/nitrite and angiotensin-II plasma concentrations. Eighteen hours of endotoxin infusion were associated with transient pulmonary artery hypertension (24 ± 9 mmHg vs. 19 ± 3 mmHg in controls at 90 min; time-group interaction $P = 0.034$), and shock with decreasing renal perfusion (Table 1). Diuresis tended to be lower in endotoxemic compared with control animals (660 ± 410 ml vs. 930 ± 320 ml; $P = 0.062$). Endotoxemic animals exhibited lower arterial pH and base excess, but lactate concentrations were not significantly different between groups (Table 1).

Nitrate/nitrite and angiotensin II concentrations were measured in six controls and in six endotoxic animals. Compared with controls, mean nitrate/nitrite plasma concentrations were similar at baseline (2.9 ± 5.4 μM vs. 3.0 ± 3.4 μM) and slightly but insignificantly higher after 18 h of endotoxemia (5.2 ± 4.8 μM vs. 2.0 ± 1.1 μM). Similarly, mean angiotensin II concentrations were equal at baseline (880 ± 322 pg/ml vs. 756 ± 391 pg/ml), and numerically but insignificantly higher at 18 h in endotoxic animals (1,231 ± 1,145 pg/ml vs. 857 ± 443 pg/ml).

Effects of 18 h of endotoxin infusion on hepato-splanchnic perfusion and the HABR. Portal venous blood flow increased in both groups, and hepatic arterial blood flow did not change in either group (Table 1). Although the buffer response following SMA constriction was not fully exhausted after 18 h of endotoxemia (Fig. 2), the hepatic arterial pressure/flow ratio was increased [0.32 (0.18–1.32) compared with 0.22 (0.08–0.60) in controls; $P = 0.043$; Fig. 3, *A* and *B*].

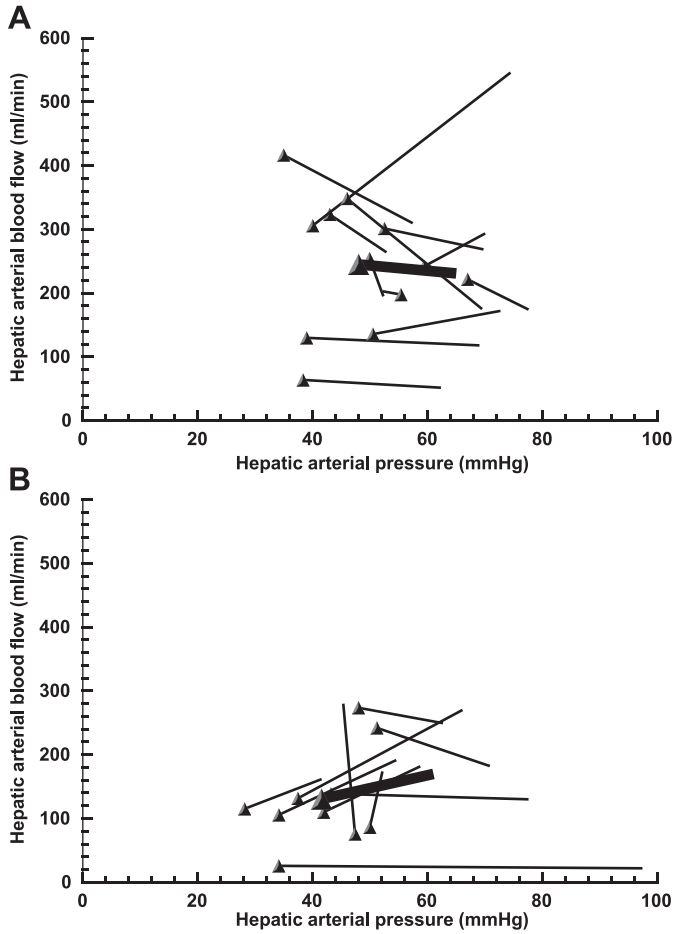

Fig. 3. *A* and *B*: hepatic arterial pressure and blood flow relationship at baseline and after 18 h of vehicle (data marker; Fig. 2*A*) or endotoxin infusion (Fig. 2*B*). Bold line and data marker: average value.

Effects of acute inferior vena cava constriction. Inferior vena cava constriction reduced femoral arterial blood pressure by an average of 9 ± 2 mmHg ($P = 0.074$). Portal venous flow decreased similarly in endotoxemia and control animals (on average 11 ± 2%; $P = 0.003$; Fig. 4). During inferior vena cava constriction and portal flow reduction, hepatic arterial flow remained unchanged in controls but decreased after 18 h of endotoxemia (occlusion-time-group interaction, $P = 0.031$; Fig. 4).

Effects of dobutamine infusion on systemic and regional hemodynamics. Five animals died between 18 and 24 h (endotoxin placebo group, $n = 1$, after 21 h; endotoxin dobutamine group, $n = 3$, after 18, 20, and 23 h; control dobutamine group, $n = 1$, after 20 h). Arterial blood pressure decreased between 18 and 24 h in both groups without dobutamine infusion, while cardiac output and regional blood flows remained unchanged (Table 2).

Dobutamine increased heart rate in both groups, increased cardiac output in controls, and tended to increase output in endotoxemia (Table 3). Maximal increases in carotid and superior mesenteric artery blood flow and in portal vein blood flow were significant only in controls (Table 3).

Effects of dobutamine infusion on the hepatic arterial buffer response. During the subsequent endotoxin infusion, the HABR did not deteriorate further. Dobutamine infusion did not alter HABR in any of the groups (Table 4, Fig. 5, *A* and *B*).

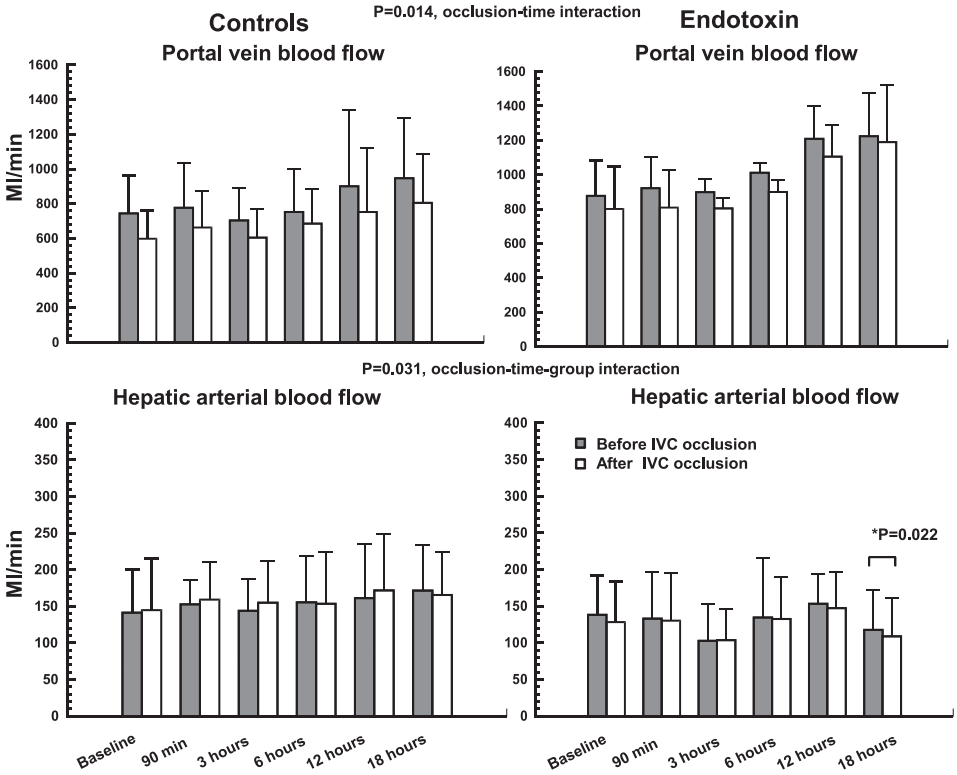

Fig. 4. Changes in portal venous and hepatic arterial blood flows during occlusion of inferior vena cava (IVC) in controls and endotoxemic animals. *Paired *t*-test. Values are means ± SD.

DISCUSSION

This study has two main findings: *1*) hepatic arterial blood flow was maintained during cardiac preload reduction early in endotoxemia but decreased after 18 h, and *2*) dobutamine did not alter the HABR.

The HABR is an acute mechanism that redistributes blood flows toward the hepatic artery and protects liver perfusion during portal venous flow reduction. During short-term endotoxemia, hepatic arterial blood flow decreases (37), and the HABR is abolished early and partially restored within the first hour (1). In our study, we found a decreased but still detectable HABR after 18 h of endotoxin infusion, which did not further deteriorate during continued endotoxin infusion. It has been shown that hepatic arterial resistance is increased for a given portal venous flow after several hours of endotoxin infusion (1). Lipopolysaccharide infusion decreases mRNA levels of constitutive nitric oxide synthase in liver tissue (37), and administration of the nitric oxide donor sodium nitroprusside during endotoxemia is able to both improve hepatic arterial flow and restore the autoregulatory response of the hepatic artery following reduction of hepatic blood flow (15, 37). Accordingly, nitric oxide seems to be an important regulator of hepatic arterial resistance (2).

The HABR has mostly been tested under circumstances with maintained CO. However, a few studies demonstrated that preferential hepatic arterial perfusion also occurs during hemorrhage (24, 31). Moreover, the HABR was still present in a study in which abdominal blood flow was selectively reduced (3). In our model of cardiac preload reduction resulting in a significant drop in portal vein blood flow, hepatic arterial perfusion was maintained in control animals and slightly but significantly reduced after 18 h of endotoxemia. Due to hemodynamic instability and impending death after 18 h of endotoxemia, inferior vena cava occlusion was not performed thereafter. Nevertheless, our data may suggest that hepatic blood flow regulation is exhausted when CO decreases in established septic shock. We assume that the different effects of superior mesenteric artery and inferior vena cava occlusion on the hepatic arterial buffer response (decreasing in the former, exhausted in the latter) are explained by the larger portal flow reduction during superior mesenteric artery occlusion. In addition, relative portal flow reduction during inferior vena cava occlusion even decreased during continuing endotoxemia.

Further studies should address specific effects of fluid administration on regional hepatic blood flow regulation in sepsis.

Dobutamine did not alter the HABR after prolonged endotoxemia. We postulated that a dobutamine-induced redistribution of hepatosplanchnic blood flow to the portal venous circulation could interfere with the increase in hepatic arterial perfusion during an acute reduction of portal blood flow. Under nonseptic conditions during reduced hepatosplanchnic blood flow, dobutamine has no effect on the HABR, whereas its effects on portal blood flow appear to be dose dependent, with increased portal blood flow only at a low dose (3). The present study demonstrated that a dobutamine-induced increase in portal venous blood flow per se had no effect on HABR in control animals. However, conflicting effects of dobutamine on hepatosplanchnic perfusion in human sepsis and experimental endotoxemia have been noted: in patients with severe sepsis or stabilized septic shock, dobutamine improved CO, total liver blood flow, and gastric mucosal perfusion (7, 20, 21, 32), whereas fractional hepatic perfusion was decreased in one study (21) but not in others (7, 32). In fluid-resuscitated

Table 2. *Time course of systemic and regional hemodynamics in endotoxemic and control animals receiving placebo*

	Endotoxemia				Controls			
	Before	After 1st	After 2nd	After 3rd	Before	After 1st	After 2nd	After 3rd
	Placebo Infusion				Placebo Infusion			
Systemic hemodynamics								
Cardiac output	4.18 (3.78–6.10)	3.80 (3.40–7.12)	3.84 (3.20–6.98)	4.10 (3.67–5.79)	4.69 (4.34–5.80)	5.38 (3.64–7.40)	4.57 (3.58–5.90)	4.47 (3.20–7.15)
Carotid artery pressure	50 (40–55)*	47 (36–54)	44 (34–54)	41 (28–50)	62 (46–68)*	59 (42–74)	53 (34–59)	46 (31–58)
Heart rate	118 (94–169)	127 (93–183)	127 (96–193)	111 (93–195)	127 (86–133)	129 (98–142)	108 (83–142)	106 (88–150)
Central vein pressure	5 (3–9)	5 (3–9)	6 (3–9)	6 (3–8)	5 (3–7)*	4 (3–5)	5 (4–7)	6 (5–7)
Regional hemodynamics								
Carotid artery blood flow	149 (132–313)	156 (138–294)	153 (134–174)	174 (139–199)	174 (130–236)	178 (125–212)	173 (132–270)	170 (125–293)
Renal artery blood flow	206 (70–240)	183 (67–200)	144 (64–195)	110 (48–203)	251 (154–287)	232 (148–310)	208 (120–292)	192 (96–234)
SMA blood flow	978 (577–1136)	895 (636–1037)	842 (586–1008)	902 (589–1099)	523 (450–1015)	550 (442–907)	472 (344–1118)	570 (318–738)
Portal vein blood flow	1087 (876–1620)	963 (854–1428)	941 (807–1015)	931 (842–1178)	832 (441–1127)	808 (579–1109)	841 (397–1096)	829 (540–925)
Celiac trunk blood flow	362 (345–422)	370 (305–383)	358 (286–427)	336 (197–470)	470 (346–851)	438 (327–855)	457 (281–784)	541 (395–787)
Hepatic artery blood flow	108 (18–161)	70 (24–105)	88 (25–140)	83 (15–140)	190 (120–361)	171 (91–338)	182 (67–313)	206 (155–289)
Splenic artery blood flow	47 (26–65)	46 (26–81)	44 (24–86)	53 (24–91)	40 (24–112)	34 (19–136)	43 (21–122)	53 (20–93)
Portal vein pressure	8 (6–14)	8 (6–11)	8 (6–11)	8 (7–13)	9 (7–11)*	9 (7–12)	10 (8–16)	10 (8–14)
Hepatic artery pressure	39 (27–46)	34 (26–45)	35 (22–44)	31 (18–51)	47 (37–72)*	48 (30–81)	46 (26–54)	36 (23–44)

Flows are l/min (cardiac output) and ml/min (regional blood flows). Pressures are mmHg. Values are median (range). SMA, superior mesenteric artery. *$P < 0.05$, Friedman Test including all measurements.

Table 3. *Effects of dobutamine on systemic and regional hemodynamics in endotoxemic and control animals*

	Endotoxemia				Controls			
	Before	2.5 mg/h	5 mg/h	10 mg/h	Before	2.5 mg/h	5 mg/h	10 mg/h
	Dobutamine Infusion				Dobutamine Infusion			
Systemic hemodynamics								
Cardiac output	4.9 (4.5–5.6)	5.5 (4.8–7.7)	7.4 (6.4–9.1)	6.3 (6.1–8.2)	5.3 (4.7–7.8)*	6.2 (5.3–7.0)	7.0 (4.6–9.6)	8.5 (5.7–12.2)
Carotid artery pressure	47 (33–70)*	42 (20–56)	45 (30–59)	45 (26–50)	55 (48–66)*	53 (46–67)	50 (45–62)	45 (33–59)
Heart rate	132 (101–153)*	137 (100–162)	145 (128–204)	172 (146–189)	127 (90–153)*	142 (123–164)	157 (136–176)	171 (162–173)
Central vein pressure	4 (3–7)	4 (4–6)	5 (3–6)	4 (3–6)	4 (3–7)	4 (4–6)	5 (3–6)	4 (3–6)
Regional hemodynamics								
Carotid artery blood flow	156 (47–270)	202 (153–276)	242 (167–242)	201 (162–217)	178 (150–216)*	197 (169–196)	229 (192–300)	262 (180–352)
Renal artery blood flow	197 (13–239)	200 (107–243)	184 (163–241)	169 (133–177)	164 (65–206)	151 (62–176)	144 (59–169)	125 (53–143)
SMA blood flow	590 (290–708)	592 (371–681)	635 (350–701)	488 (316–702)	805 (268–1054)*	867 (243–1020)	930 (235–1293)	767 (364–936)
Portal vein blood flow	911 (678–1385)	1213 (851–1539)	1451 (1083–1507)	1331 (990–1404)	1128 (962–1335)*	1232 (1001–1538)	1397 (990–1968)	1235 (1008–1633)
Celiac trunk blood flow	303 (176–404)	302 (249–462)	322 (223–470)	252 (210–416)	355 (173–594)	342 (207–449)	343 (264–399)	249 (221–395)
Hepatic artery blood flow	159 (67–184)	146 (95–191)	144 (102–173)	132 (74–150)	195 (36–299)	153 (83–357)	216 (106–326)	114 (103–227)
Splenic artery blood flow	51 (12–219)	61 (18–270)	95 (15–284)	73 (11–246)	31 (28–75)	48 (33–93)	48 (31–112)	72 (30–155)
Portal vein pressure	9 (7–21)	8 (7–16)	8 (7–11)	8 (6–10)	8 (5–13)	8 (8–9)	9 (7–10)	9 (6–9)
Hepatic artery pressure	41 (26–49)	36 (30–39)	40 (32–41)	35 (30–35)	46 (32–58)*	39 (34–48)	35 (30–43)	36 (20–43)

Flows are l/min (cardiac output) and ml/min (regional blood flows). Pressures are mmHg. Values are median (range). *$P < 0.05$, Wilcoxon test [before dobutamine vs. values at highest cardiac output (systemic hemodynamics) and at highest portal vein flow (regional hemodynamics), respectively, induced by dobutamine].

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Table 4. *Hepatic arterial buffer response: changes during SMA occlusion after 18 h of endotoxemia/placebo*

	Animals Receiving Subsequent Placebo				Animals Receiving Subsequent Dobutamine			
	18 h	End of Experiment	18 h	End of Experiment	18 h		18 h	
		Controls		Endotoxemia	Controls	Dobutamine Max	Endotoxemia	Dobutamine Max
HAP /HAF after SMA occlusion, mmHg/ml	0.22 (0.08–0.30)	0.16 (0.09–0.56)	0.32 (0.28–1.32)	0.32 (0.24–0.61)	0.23 (0.13–0.60)	0.17 (0.13–0.38)	0.41 (0.18–0.63)	0.27 (0.16–0.83)

HAP, mean hepatic arterial blood pressure; HAF, hepatic artery blood flow; SMA, superior mesenteric artery. Values are median (range).

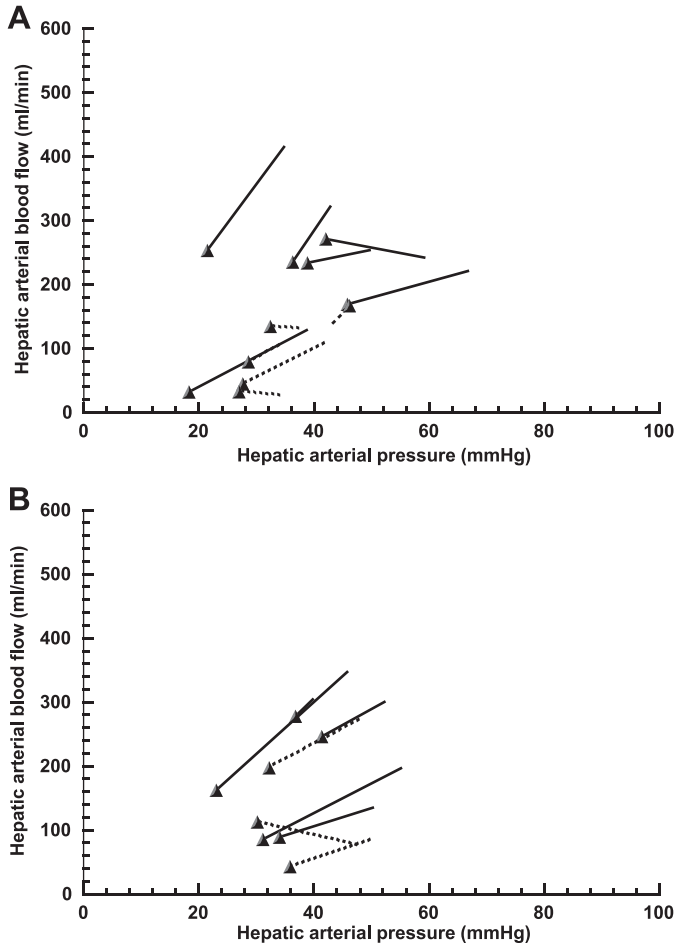

Fig. 5. *A* and *B*: hepatic arterial pressure and blood flow relationship at 18 h and at the end of the experiment in placebo groups (data marker, *A*) or at highest dobutamine doses (Fig. *B*). Continuous line is controls; dotted line is endotoxemic animals.

endotoxemic rats, pigs, and dogs, dobutamine administration maintained or increased hepatic blood flow and oxygen delivery compared with endotoxin alone (5, 8, 35). Conversely, in sheep subjected to endotoxin, intestinal oxygen delivery and mucosal perfusion decreased during dobutamine administration despite maintained systemic oxygen supply (12). Furthermore, in fecal porcine peritonitis, catecholamines were able to improve superior mesenteric artery blood flow but did not change local microcirculatory perfusion (16). In endotoxemic animals in our study, we found no significant increase in regional hepatosplanchnic perfusion with dobutamine, whereas carotid blood flow was augmented.

To be able to explore the effects of dobutamine alone on regional blood flow, we did not use norepinephrine to maintain blood pressure. The use of norepinephrine, which has β-mimetic properties, may both increase and redirect regional blood flow (39). It is also possible that the low regional perfusion pressures in endotoxemic animals prevented a significant increase in regional flow. In fact, portal vein blood flow tended to increase in control animals receiving dobutamine. Accordingly, it is possible that regional splanchnic blood flows would have increased with dobutamine in septic animals as well if higher arterial blood pressures had been achieved.

This study has strengths and limitations. So far, no other study has sequentially assessed the HABR after prolonged endotoxemia in intact pigs. Furthermore, effects of acute preload reduction and dobutamine on the HABR in endotoxemia have not been described before. In the landmark papers on HABR by the groups of Lautt (25) and Robotham (1), tributaries of the portal vein and all branches of the common hepatic artery and celiac trunk were ligated, and the spleen was removed. Although a nearly perfect pressure-flow relationship can be established by using this model, such an approach does not reflect the situation in intact animals or humans, where anastomoses between vascular regions and anatomical variants are common (33). We have chosen to use a more physiological and maybe clinically more relevant model with intact anatomy. However, our approach, which includes major surgery to place flow probes and vascular occluders, increases tissue trauma and includes repeated splanchnic hypoperfusion-reperfusion events by the HABR assessments also in control animals.

In our model, not all of the hemodynamic changes expected with septic shock, or their metabolic consequences, were present: CO increased moderately, stroke volume and filling pressure were maintained, and arterial plasma lactate did not increase. This was at least in part the result of the design, which featured a rather low endotoxin infusion rate and fluid filling according to clinical signs of hypovolemia. In pilot animals, higher endotoxin doses or less filling was associated with early death. Increased plasma norepinephrine and angiotensin II concentrations in endotoxemic animals may have helped to stabilize hemodynamics, too. It has been shown that blood norepinephrine levels are increased in sepsis (36). However, although norepinephrine concentrations are inversely correlated with systemic blood pressure, they do not correlate with systemic vascular resistance or CO, suggesting decreased or lack of responsiveness of resistance vessels or the heart to catecholamines (36). It seems therefore impossible to estimate plasma norepinephrine concentrations from systemic hemodynamics in sepsis or during endotoxemia.

In patients with sepsis, especially those with septic shock, total serum nitrite and nitrate concentrations are also increased, indicating increased endogenous nitric oxide production (14, 41). The reduced sensitivity to the vasoconstrictive effects of norepinephrine can be reversed by selective inhibition of nitric oxide synthase (17, 18, 40). At least in the rat hepatosplanchnic and skeletal muscle vasculature, all of the resting nitric oxide-mediated vasodilation in sepsis is secondary to endogenous adenosine action (34). Although numerically higher, the nitrate/nitrite concentrations in our samples from endotoxemic animals were not significantly increased above those from controls.

In patients with resuscitated sepsis, angiotensin II concentration and plasma renin activity are also increased, and the degree of elevation correlates with microvascular dysfunction and the extent of early organ failure (10). In rabbit cardiac myocytes, angiotensin II and endotoxin have synergistic effects on the activation of nitric oxide and cyclic GMP pathways to induce dose-dependent impairment of contractile functions (42). In mice and rats, endotoxin also alters the vascular reactivity to angiotensin II (13, 22), and the blood pressure response to exogenous angiotensin II is significantly diminished (4). It has been shown that angiotensin II type 1 receptor expression is markedly downregulated in septic rats, an effect that could be reproduced in vitro by administration of nitric oxide as well as by a combination of proinflammatory cytokines to rat renal mesangial cells (4). In our study, angiotensin II concentrations were slightly but not significantly higher in endotoxemic compared with control animals.

In summary, both effects of our design and neurohumoral activation with overexpression of various substances may have contributed to the phenotype of our sepsis model. Obviously, our findings cannot be extrapolated readily to situations of more severe septic shock.

The mortality rate of our animals (21%) is comparable to mortality rates in patients with sepsis without shock or organ failure (27). This obviously reduced the number of measurements, so that our findings are of preliminary character. Furthermore, the administration of dobutamine alone in hypotensive states can be questioned; we do not know whether our results can be reproduced in situations with higher blood pressures.

In conclusion, our data suggest that acute cardiac preload reduction is associated with preferential hepatic arterial perfusion during early but not after prolonged endotoxemia. Similarly, HABR provoked by acute SMA blood flow reduction is also impaired after prolonged endotoxemia. In contrast, dobutamine had no effect on HABR.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: S.M.J. and J.T. conception and design of research; S.M.J., H.B., F.P., and A.K. analyzed data; S.M.J. interpreted results of experiments; S.M.J., B.M.B., L.B., R.K., H.-Q.F., A.K., Y.M., and J.T. edited and revised manuscript; S.M.J., H.B., F.P., B.M.B., L.B., R.K., H.-Q.F., A.K., Y.M., and J.T. approved final version of manuscript; H.B., F.P., L.B., R.K., H.-Q.F., and Y.M. performed experiments; H.B. and F.P. prepared figures; H.B. and F.P. drafted manuscript.

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