European Journal of Pharmaceutical Sciences 191 (2023) 106588

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Micro-lightguide spectrophotometry assessment of hepatic and intestinal microcirculation in endotoxemic rats during intravenous treatment with angiotensin II

Götz Schmidt, Laurenz Pitz, Melanie Markmann, Emmanuel Schneck, Michael Sander, Christian Koch *, Fabian Edinger

Department of Anesthesiology, Operative Intensive Care Medicine and Pain Therapy, Justus Liebig University of Giessen, Rudolf-Buchheim-Strasse 7, Giessen 35392, Germany

ARTICLE INFO

Keywords:
Sepsis
Vasopressor
Perfusion
Shock

ABSTRACT

Introduction: During septic shock, impairment of microcirculation leads to enhanced permeability of intestinal mucosa triggered by generalized vasodilation and capillary leak. Intravenous angiotensin II (AT-II) has been approved for the treatment of septic shock; however, no in-vivo data exist on the influence of AT-II on hepatic and intestinal microcirculation.

Material and methods: Sixty male Lewis rats were randomly assigned to six study groups (each $n = 10$): sham, lipopolysaccharide-induced septic shock, therapy with low- or high-dose AT-II (50 or 100 ng/kg/min, respectively), and septic shock treated with low- or high-dose AT-II. After median laparotomy, hepatic and intestinal microcirculation measures derived from micro-lightguide spectrophotometry were assessed for 3 h and included oxygen saturation (SO_2), relative blood flow (relBF) and relative hemoglobin level (relHb). Hemodynamic measurements were performed using a left ventricular conductance catheter, and blood samples were taken hourly to analyze blood gasses and systemic cytokines.

Results: AT-II increased mean arterial pressure in a dose-dependent manner in both septic and non-septic animals ($p < 0.001$). Lower hepatic and intestinal SO_2 (both $p < 0.001$) were measured in animals without endotoxemia who received high-dose AT-II treatment, however, significantly impaired cardiac output was also reported in this group ($p < 0.001$). In endotoxemic rats, hepatic relBF and relHb were comparable among the treatment groups; however, hepatic SO_2 was reduced during low- and high-dose AT-II treatment ($p < 0.001$). In contrast, intestinal SO_2 remained unchanged despite treatment with AT-II. Intestinal relBF ($p = 0.028$) and interleukin (IL)-10 plasma levels ($p < 0.001$) were significantly elevated during treatment with high-dose AT-II compared with low-dose AT-II.

Conclusions: A dose-dependent decrease of hepatic and intestinal microcirculation during therapy with AT-II in non-septic rats was observed, which might have been influenced by a corresponding reduction in cardiac output due to elevated afterload. While hepatic microcirculation was reduced during endotoxemia, no evidence for a reduction in intestinal microcirculation facilitated by AT-II was found. In contrast, both intestinal relBF and anti-inflammatory IL-10 levels were increased during high-dose AT-II treatment.

1. Introduction

Although major efforts have been made to improve the detection and therapy of sepsis and septic shock, mortality rates have remained high over the last decades (Fleischmann-Struzek et al., 2020; Fleischmann et al., 2016). Current guidelines of the Surviving Sepsis Campaign,

Abbreviation: AT-1R, angiotensin II receptor type 1; AT-2R, angiotensin II receptor type 2; AT-II, angiotensin II; AU, arbitrary units; BE, base excess; CO, cardiac output; EDV, end-diastolic volume; Hb, hemoglobin; HR, heart rate; IL, interleukin; LPS, lipopolysaccharide; MAP, mean arterial pressure; P_aO_2, partial pressure of oxygen; P_aCO_2, partial pressure of carbon dioxide; relBF, relative blood flow; relHb, relative hemoglobin; $S_{cv}O_2$, central venous oxygen saturation; SO_2, oxygen saturation; TNFα, tumor necrosis factor alpha.

* Corresponding author.
E-mail address: Christian.Koch@chiru.med.uni-giessen.de (C. Koch).

https://doi.org/10.1016/j.ejps.2023.106588
Received 14 March 2023; Received in revised form 27 August 2023; Accepted 19 September 2023
Available online 20 September 2023

define sepsis as a dysregulated host response to infection resulting in life-threatening organ dysfunction, and the necessity of early identification and immediate treatment initiation is underlined (Ruiqiang et al., 2021; Singer et al., 2016). During septic shock, which is defined as sepsis with persisting hypotension and a serum lactate above 2 mmol/L despite adequate volume resuscitation, vasopressors are used to achieve a mean arterial pressure (MAP) above 65 mmHg (Ruiqiang et al., 2021; Singer et al., 2016). The mechanisms leading to severe vasoplegia during septic shock are induced by cytokine release and consecutive endothelial damage (Burgdorff et al., 2018). These findings lead to severe dysregulation of the sympathetic nervous system, vasopressin system, and renin-angiotensin system, which are essential for maintaining sufficient vascular tone under physiological conditions (Lambden et al., 2018). To counteract these pathophysiological effects, the first-line agent used in septic shock is norepinephrine; other vasoconstricting agents, such as vasopressin, are added when inadequate MAP persists (Ruiqiang et al., 2021). However, the use of vasopressors can provoke additional dose-dependent complications, such as arrhythmias, myocardial injury, elevation of serum lactate, and peripheral ischemia (Rodriguez et al., 2020). Angiotensin II (AT-II) has been known to be a potent vasoconstrictor for years; however, its clinical use was restricted to preclinical and experimental settings. For clinical use, AT-II is now available as Giapreza® (2.5 mg/mL, La Jolla Pharmaceutical, San Diego, California, USA). The randomized controlled ATHOS-3 study investigated the value of AT-II in patients with refractory vasodilatory shock despite the administration of more than 0.2 μg/kg/min norepinephrine and demonstrated a rapid response in MAP, an improvement in cardiac Sequential Organ Failure Assessment score, and comparable mortality rates between the 163 patients randomized to the AT-II treatment group and the 158 patients in the placebo group (Khanna et al., 2017). In addition, the dosage of norepinephrine was reduced in the AT-II group (Chawla et al., 2014). Based on these results, the U.S. Food and Drug Administration and the European Medicines Agency approved AT-II for the treatment of septic or distributive shock in adults. Post-hoc analysis of the ATHOS-3 trial focused on renal outcomes and demonstrated that patients requiring renal replacement therapy (RRT) who received AT-II showed favorable outcomes compared with patients on RRT who received a placebo (Tumlin et al., 2018). The patients who received AT-II showed improved 28-day survival, more frequent termination of RRT, and increased response in MAP (Tumlin et al., 2018). These results confirmed preliminary experimental data from a septic shock rat model where AT-II alleviated abnormalities in serum creatinine compared with norepinephrine treatment (An et al., 2021). Hypertension, metabolic alkalosis, thrombosis, and delirium are the most frequent adverse effects of AT-II treatment (Chawla et al., 2014; Rodriguez et al., 2020). Much less is known about the possible effects of AT-II treatment on intestinal perfusion. Furthermore, no in-vivo data exist on the influence of AT-II on hepatic and intestinal microcirculation during septic shock. Due to generalized vasodilation and capillary leak during septic shock, intestinal microcirculation is impaired, leading to enhanced permeability of the intestinal mucosa, which facilitates the transfer of endotoxins and gut bacteria into the vascular system, resulting in further acceleration of systemic inflammation and subsequent organ failure (De Backer et al., 2002; Haussner et al., 2019). Although it has been hypothesized, a significant influence on the intestinal microcirculation following the administration of norepinephrine or vasopressin has not yet been demonstrated in animal studies (Asfar et al., 2003; Dancker et al., 2018; Qiu et al., 2014; Shi et al., 2020). Angiotensin II receptor type 1 (AT1-R) and angiotensin II receptor type 2 (AT2-R) receptors are found in the intestinal tract (Lumlertgul and Ostermann, 2020; Matrougui et al., 1999a). While AT1-R enhances vasoconstriction, thrombosis, aldosterone liberation, and activation of proinflammatory cells, AT2-R induces contrasting effects, resulting in vasodilation and anti-inflammatory effects (Lumlertgul and Ostermann, 2020). Although the effects of AT-II appear predominantly at AT1-R, the balance between AT1-R and AT2-R in the intestine during sepsis has not yet been fully investigated (Lumlertgul and Ostermann, 2020). Furthermore, the infusion of AT-II into non-septic rats was shown to induce stellate cell activation and upregulation of proinflammatory proteins in the liver, and AT-II was shown to decrease arterial and portal blood flow, resulting in enhanced glucose output and lactate release in isolated rat livers (Bataller et al., 2003; Reisenleiter et al., 1996). The in-vivo clinical effects of intravenous AT-II on intestinal and hepatic microcirculation during septic shock remain unclear; therefore, the aim of this study was to obtain evidence of the possible influence of AT-II on hepatic and intestinal microcirculation in a rat septic shock model.

2. Material and methods

2.1. Animals

All procedures involving animals were conducted in compliance with the European legislation for the protection of animals used for scientific purposes, the standards for animal experiments according to the German animal welfare law, and the ARRIVE guidelines. The experiments were approved by the local committee for animal care of the regional council (GI 20/28 No. G 74/2020 and GI 20/28 Nr. GI 17/2021, Regional Council Giessen, Germany). Male Lewis rats weighing 300 – 350 g were purchased from Janvier Labs (Le Genest-Saint-Isle, France). They were housed in conditions of 22 °C and 55% relative humidity with a day/night cycle of 14/10 h and free access to standard chow and water ad libitum.

2.2. Study design

Septic shock was induced by lipopolysaccharide (LPS) 1 mg/kg (LPS Ultrapure from E. coli 0111:B4 strain, InvivoGen, San Diego, California, USA), which was administered over 30 min. AT-II (Giapreza® 2.5 mg/ml) was obtained from La Jolla Pharmaceutical (San Diego, California, USA) and was handled according to the manufacturer's instructions. After dilution in a balanced full electrolyte solution for infusion therapy (Sterofundin ISO, B.Braun, Melsungen, Germany), AT-II was stored in single containers for each experiment at 4 °C. Sixty rats were randomly assigned to six study groups (each $n = 10$): sham, septic shock, low- or high-dose AT-II treatment (50 or 100 ng/kg/min, respectively), and septic shock treated with low- or high-dose AT-II. LPS and AT-II containers were under pseudonyms, and the allocation list was sealed until the end of the experiments; therefore, the investigators were blinded to the allocation during the procedures. Rats undergoing sham protocol, AT-II treatment, or LPS infusion instead received an equivalent amount of a balanced full electrolyte infusion (Sterofundin ISO, B.Braun, Melsungen, Germany). After the surgical procedure and consecutive administration of LPS or sham infusion, the rats were observed for 3 h. During the observation period, measurements were performed every 15 min, and blood samples were obtained each hour.

2.3. Anesthesia and surgery

Rats were transferred to a glass chamber where inhalation anesthesia was induced with 5% isoflurane (Baxter, Unterschleißheim, Germany) balanced with 95% oxygen. Then, rats were intubated orally with a 14 G catheter (64 mm, Surflash intravenous catheter, Terumo Europe, Shibuya, Japan) on a slanted board and mechanically ventilated in a weight-adjusted and volume-controlled manner with 50% oxygen. Isoflurane was adapted between 1.5% and 2.5%, according to the surgical procedure, heart rate (HR) and blood pressure. During the observation period, isoflurane was maintained constant at 1.3%. Tidal volume was adjusted to 6.2 ml × body weight $(kg)^{1.01}$, while respiratory rate was calculated as 53.3 × body weight $(kg)^{-0.26}$. Continuous electrocardiogram monitoring was established immediately after intubation using three subcutaneous needle electrodes. The lateral tail vein was punctured percutaneously with a 24 G catheter (0.7 mm, B.Braun,

Melsungen, Germany) and continuous infusion with Sterofundin ISO 5 mg/kg/h (B.Braun), midazolam 2 mg/kg/h, fentanyl 10 μg/kg/h, and pancuronium 0.3 mg/kg/h was started. To monitor arterial blood pressure, the tail artery was cannulated with a 24 G catheter (0.7 mm, B. Braun) after surgical dissection and connected to a pressure transducer. The right carotid artery was used to place a 2F pressure-volume catheter (SPR-838, Millar, Houston, Texas, USA) into the left ventricle to facilitate real-time hemodynamic measurements. The belly skin was incised using scissors, and the abdominal cavity was opened via median laparotomy using a cautery pen (AA01 Bovie high-temperature cautery, Bovie Medical Corporation, Clearwater, Florida, USA). The first measuring probe was placed on the right lobe of the liver beneath the abdominal skin. A small intestinal loop was then pulled out gently, and the mesenterial section without blood vessels was incised. A one-side opened silicone tube with a diameter of 8 mm containing a second measurement probe was carefully placed around the intestinal loop to ensure a loose fit before it was set back in its original position (Fig. 1**A**). After anticoagulation with intravenous administration of heparin (300 I. E./kg), the right internal jugular vein was cannulated with a 4F two-lumen central venous catheter (13 cm, Teleflex, Wayne, Pennsylvania, USA), which was placed into the right atrium via the superior vena cava. The catheter was used to ensure the reliable administration of LPS and AT-II, and the collection of central venous blood samples. All instruments used for surgery were properly sterilized in a steam sterilizer (DX-45, Systec GmbH & Co. KG, Linden, Germany).

2.4. Micro-lightguide spectrophotometry measurement of microcirculation

Microcirculation was assessed using micro-lightguide spectrophotometry (O2C, LEA Medizintechnik Giessen Gmbh, Heuchelheim, Germany, Fig. 1**B**) with one flat probe placed on the right lobe of liver (LFx-45) and one circular probe placed gently on a small intestine loop (LFx-152, 7 mm, both LEA Medizintechnik Giessen Gmbh). Each probe consisted of two different sources of light and its corresponding optical sensors. White light spectroscopy (450–1000 nm) was used to measure the percentage of tissue oxygenation saturation (SO_2), which is primarily composed of venous and secondarily arterial and capillary oxygen saturation. The magnitude of light absorption triggered by hemoglobin (Hb) was analyzed and expressed in relative Hb (relHb) arbitrary units (AU). The second light source emits laser light (820 nm, 30 mW) determining erythrocyte velocity, and relative blood flow (relBF, AU) was thus calculated using the Doppler effect.

2.5. Hemodynamic measurements

Blood pressure and HR were recorded continuously during the experiments to guide the depth of anesthesia during surgery. At the end of the surgery, the left ventricular pressure volume catheter was calibrated with parallel conductance with the central venous administration of 100 μL of 10% sodium chloride according to the manufacturer's instructions. The hemodynamic parameters assessed were HR, MAP, cardiac output (CO), and left ventricular end-diastolic volume (EDV). To rule out respiratory variations, all hemodynamic measurements were performed during a 5-second episode of apnea.

2.6. Blood analysis

Arterial blood analysis (100 μL) revealed pH, partial pressure of oxygen and carbon dioxide (p_aO_2 and p_aCO_2), Hb, base excess (BE), lactate, bicarbonate, and electrolytes, including sodium, potassium, calcium, and chloride. Central venous oxygen saturation ($S_{cv}O_2$) was obtained through central venous blood analysis (100 μL). These point-of-care measurements were performed after surgery (baseline) and every hour during the observation period (0 min, 60 min, 120 min, 180 min). The results were immediately available to the investigator (ABL800, Radiometer, Copenhagen, Denmark). At each timepoint during the observation period, 300 μL blood was obtained, centrifuged at 5000 rpm for 5 min, and plasma was stored at −80 °C until further measurements (see 2.8.). The extracted volume was replaced by an equivalent bolus of crystalloid infusion (Sterofundin ISO, B.Braun).

2.7. Euthanasia at the end of experiments

At the end of the observation period (180 min), isoflurane was increased to 5% and a bolus of midazolam and fentanyl was administered. Rats were euthanized by exsanguination; the whole blood volume was collected through the central venous catheter.

2.8. Enzyme-linked immunosorbent assay

To evaluate the systemic inflammatory response, plasma tumor necrosis factor alpha (TNFα), interleukin (IL)−6, and IL-10 were measured by enzyme-linked immunosorbent assay (ELISA). All measurements were conducted according to the manufacturer's instructions (ELISA Kit, R&D Systems, Wiesbaden, Germany), and the probes were unfrozen only once.

2.9. Statistical analysis

To achieve an average effect size of 0.55, a sample size of 10 animals was required, with an alpha and beta error of 0.05 and 0.2, respectively. Sample size calculation was performed using G*Power 3.1.9.2. Hemodynamic data were calculated using LabChart pro 7.3.8 (ADInstruments, Sydney, Australia), and microcirculation measurements were derived

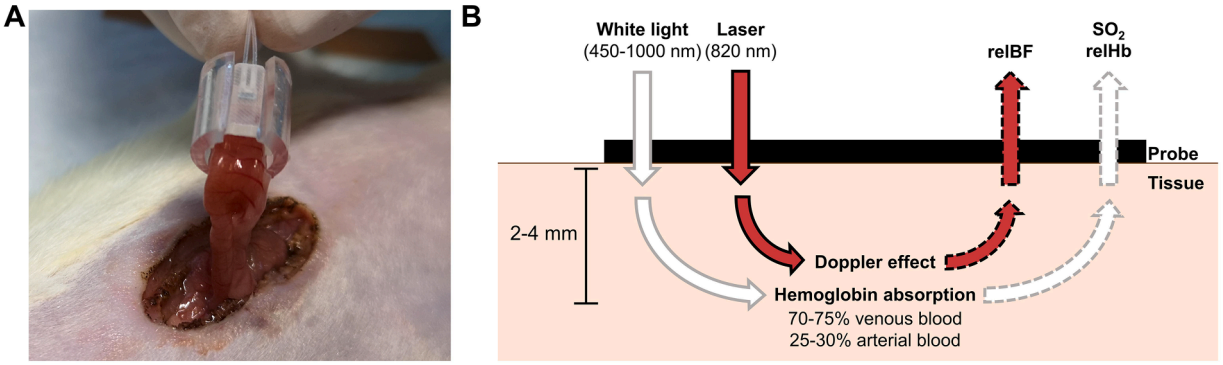

Fig. 1. Micro-lightguide spectrophotometry measurement of microcirculation.
Legend: A circular measurement probe was carefully placed around the intestinal loop before it was set back in its original position **B** Each probe measures tissue oxygenation saturation (SO_2) and relative hemoglobin (relHb) using white light spectroscopy. Relative blood flow (relBF) is calculated from laser light transmission using the Doppler effect.

from the O2C platform at each timepoint. To address translational aspects, MAP and blood analyses are reported as absolute values, as their dimensions are equal to human physiology in our model. Further hemodynamic parameters and microcirculation measurements were normalized to their baseline value and are presented as mean percentage and standard error of the mean (SEM). Blood analyses and cytokine measurements are described as median and interquartile range (IQR). Two-way ANOVA for repeated measurements models including the experimental groups were created, and inter-group differences over time were evaluated using post-hoc Bonferroni's test. Internal validation of our microcirculation measurement was ensured comparing the sham group to the group that received LPS only. The influence of AT-II on microcirculation was initially assessed in animals without septic shock (sham vs. AT-II 50 ng/kg/min vs. AT-II 100 ng/kg/min) followed by the analysis of the septic shock groups (LPS vs. LPS + AT-II 50 ng/kg/min vs. LPS + AT-II 100 ng/kg/min). Two-tailed values of $p < 0.05$ were

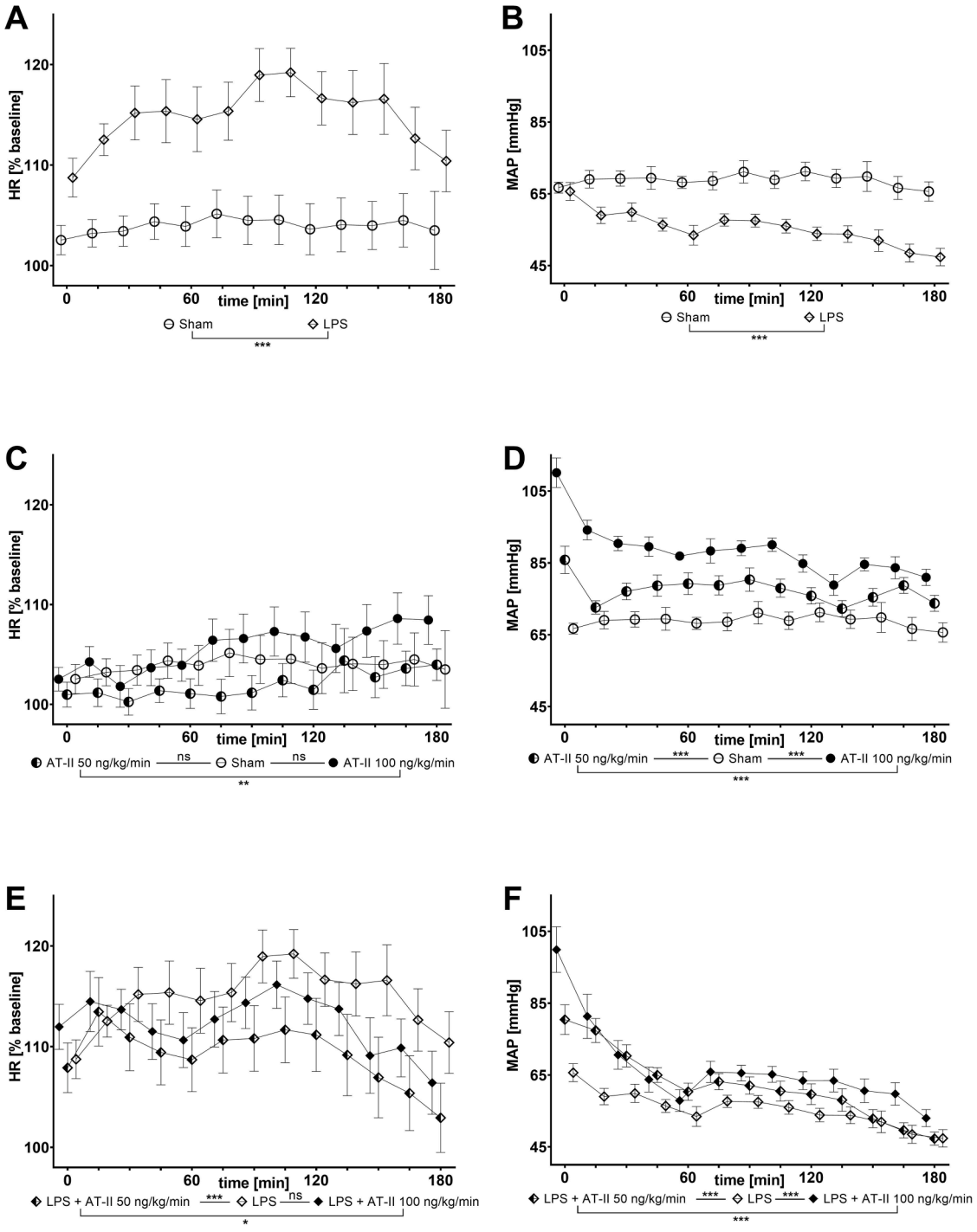

Fig. 2. Heart rate and mean arterial pressure.

Legend: Lipopolysaccharide (LPS) infusion induced septic shock with **A** tachycardia and **B** hypotension. While C heart rate (HR) was lower during low-dose compared with high-dose angiotensin II (AT-II) treatment, AT-II caused a dose-dependent increase in **D** mean arterial pressure (MAP). During endotoxemia, a decrease in **E** HR was observed following low-dose AT-II treatment, while overall, AT-II was consistently able to restore **F** MAP to physiological levels. ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$; ns: not significant; ○ Sham; ◐ AT-II 50 ng/kg/min; ● AT-II 100 ng/kg/min; ⋄ LPS; ◈ LPS + AT-II 50 ng/kg/min; ◆ LPS + AT-II 100 ng/kg/min; ⊥ SEM.

considered statistically significant. While R statistics, version 4.0.4 (www.r-project.org) was used for statistical analysis, figures were created using GraphPad PRISM (Version 9.5.0, GraphPad Software, La Jolla, CA, USA).

3. Results

3.1. Hemodynamic measurements

Animals that received LPS showed significant elevation of HR and consecutive arterial hypotension with MAP < 65 mmHg (HR: sham 104.0 ± 2.3% vs. LPS 114.8 ± 2.8%, $p < 0.001$; Fig. 2**A**; MAP: sham

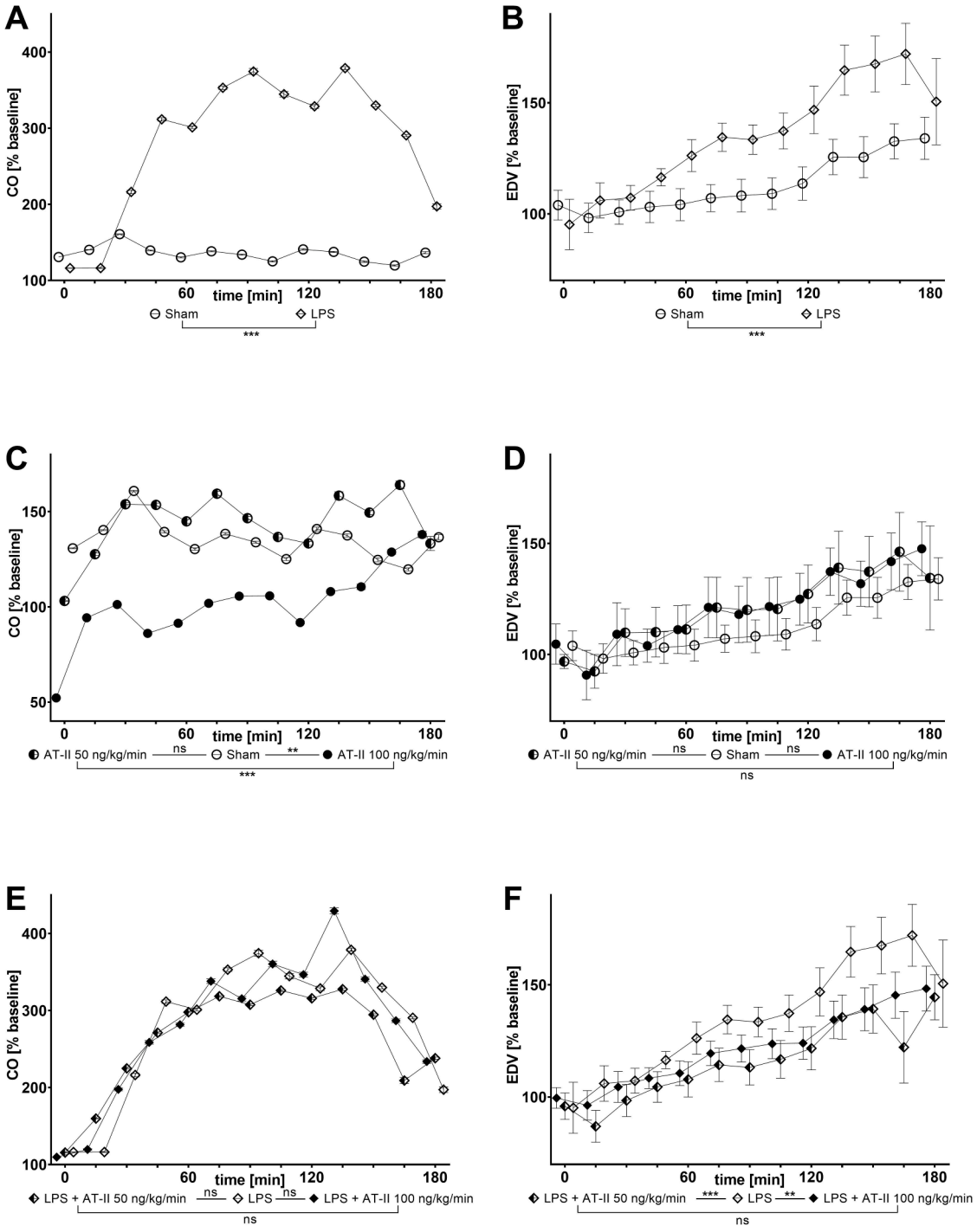

Fig. 3. Cardiac output and end-diastolic volume.

Legend: Compared with sham animals, endotoxemia induced hyperdynamic septic shock resulting in heavily **A** increased cardiac output (CO) with subsequent heart failure indicated by the intensifying rise in **B** end-diastolic volume (EDV). When angiotensin II (AT-II) was administered to sham animals, **C** CO was reduced in animals that received high-dose AT-II, while **D** EDV remained comparable. Animals that received AT-II after lipopolysaccharide (LPS) infusion had comparable **E** CO, and lower **F** EDV compared with endotoxemic animals that did not receive AT-II treatment. ***$p < 0.001$; **$p < 0.01$ ns: not significant; ○ Sham; ◐ AT-II 50 ng/kg/min; ● AT-II 100 ng/kg/min; ⬦ LPS; ⬖ LPS + AT-II 50 ng/kg/min; ◆ LPS + AT-II 100 ng/kg/min; ⊥ SEM.

68.8 ± 2.6 mmHg vs. LPS 55.5 ± 2.3 mmHg, $p < 0.001$; Fig. 2**B**). CO and EDV were significantly increased in the LPS group compared with the sham group (CO: sham 135.3 ± 0.7% vs. LPS 281.5 ± 2.2%, $p < 0.001$; EDV: sham 112.8 ± 7.4% vs. LPS 135.2 ± 9.6 mmHg, $p < 0.001$; Fig. 3**A** and **B**). Animals that received AT-II and were not treated with LPS exhibited a dose-dependent increase in MAP (low-dose 77.4 ± 2.6 mmHg vs. high-dose 88.6 ± 2.5 mmHg, each $p < 0.001$; Fig. 2**D**). In the low-dose AT-II group, HR, CO, and EDV did not differ significantly from the sham group (Fig. 2**C**, Fig. 3**C** and **D**). The high-dose group had comparable HR and EDV, but CO was significantly reduced compared with both the low-dose AT-II and sham group (CO: low-dose 143.4 ± 1.3%; high-dose 101.2 ± 0.8% both $p < 0.01$, Fig. 3**C**). Furthermore, HR was significantly higher in the high-dose AT-II group compared with the low-dose group (low-dose 102.0 ± 1.7 vs. high-dose 105.6 ± 2.1, $p < 0.01$). While HR following LPS infusion was comparable between the LPS group and the high-dose AT-II group, it was significantly lower in the low-dose AT-II group (low-dose 109.2 ± 3.4% vs. high-dose 112.3 ± 2.7%, $p = 0.038$; LPS $p < 0.001$; Fig. 2**E**). A dose-dependent increase in MAP was observed in endotoxemic animals that received AT-II (low-dose 62.1 ± 2.7 mmHg vs. high-dose 67.0 ± 3.4 mmHg, each $p < 0.001$; Fig. 2**F**). CO was comparable between the three groups that received LPS (Fig. 3**E**). However, EDV was significantly elevated in the group that received LPS without AT-II compared with the groups that received low-dose (115.5 ± 8.8%; $p < 0.001$) and high-dose AT-II (121.2 ± 7.1%; $p < 0.01$; Fig. 3**F**).

3.2. Hepatic microcirculation

Hepatic measurements initially dropped after 15 min in animals that received LPS, but no overall differences were measured in SO_2 and relBF (Fig. 4**A** and Fig. 4**B**). However, relHb was significantly elevated in the LPS group compared with the sham group (sham 93.7 ± 0.9% vs. LPS 95.5 ± 1.0%, $p < 0.001$; Fig. 4**C**). Low-dose AT-II infusion did not induce any changes in SO_2, relBF, or relHb compared with the sham group (Fig. 5**A-C**). However, following the administration of high-dose AT-II, SO_2 and relHb were significantly impaired (SO_2: sham 65.8 ± 7.5% vs. high-dose 48.5 ± 6.5%, $p < 0.001$, Fig. 5**A**; relHb: high-dose 90.6 ± 1,2%, $p < 0.001$, Fig. 5**C**), while relBF was preserved (Fig. 5**B**). Furthermore, SO_2 and relHb were significantly reduced in the high-dose AT-II group compared with the low-dose group (SO_2: low-dose 59.7 ± 5.6%, $p < 0.01$, Fig. 5**A**; relHb: low-dose 93.1 ± 1.0%, $p < 0.01$, Fig. 5**C**), while relBF was comparable between the two groups (Fig. 5**B**). Comparing the LPS and LPS with low-dose AT-II infusion groups, SO_2 was reduced in animals that received low-dose AT-II (LPS 67.1 ± 7.8% vs. low-dose 55.4 ± 8.7%, $p < 0.001$; Fig. 6**A**), while relBF and relHb were not significantly altered (Fig. 6**B** and **C**). Correspondingly, animals that received high-dose AT-II after LPS had lower SO_2, while relBF and relHb were comparable to the group that received LPS (SO_2: high-dose 49.3 ± 6.9%, $p < 0.001$, Fig. 6**A-C**). Comparing low-dose AT-II to high-dose AT-II after LPS infusion, no differences in SO_2, relHb, or relBF were detected.

3.3. Intestinal microcirculation

While intestinal SO_2 and relBF were significantly reduced in the LPS group compared with the sham animals, no differences were noted regarding relHb (SO_2: sham 95.5 ± 2.8% vs. LPS 85.0 ± 4.9%, $p < 0.001$, Fig. 4**D**; relBF: sham 96.7 ± 8.4% vs. LPS 82.6 ± 9.5%, $p < 0.01$; Fig. 4**E**). It must be noted that the difference in relBF equalized after 150 min (Fig. 4**D**). Additional low-dose infusion of AT-II did not provoke any changes in SO_2, relHb, or relBF compared to the sham group (Fig. 5**D-F**). However, when high-dose AT-II was administered, SO_2 was significantly impaired compared with the sham group, and no differences were observed in relBF or relHb (SO_2: high-dose 85.8 ± 3.1%; $p < 0.001$; Fig. 5**D**). When high-dose AT-II was compared with low-dose AT-II, SO_2 and relHb were significantly reduced in the high-dose AT-II group, while intestinal relBF was comparable (SO_2: low-dose 94.0 ± 2.3%, $p < 0.01$, Fig. 5**D**; relHb: low-dose 97.9 ± 5.0% vs. high-dose 89.6 ± 3.9%, $p < 0.01$, Fig. 4**F**). Animals that received low-dose or high-dose AT-II after LPS infusion showed comparable values of SO_2, relBF, and relHb to the

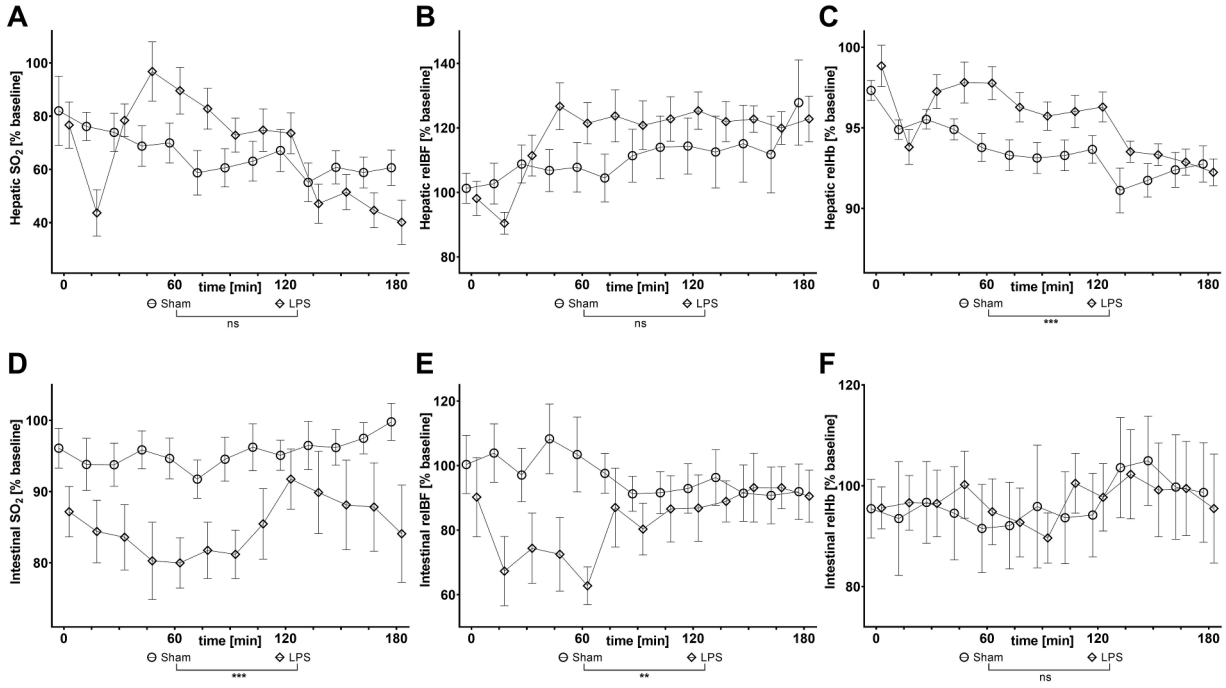

Fig. 4. Micro-lightguide spectrophotometry measurements during septic shock
Legend: Hepatic **A** oxygen saturation (SO_2) and **B** relative blood flow (relBF) did not change after lipopolysaccharide (LPS) infusion, but **C** relative hemoglobin (relHb) increased. Measurements of intestinal perfusion during endotoxemia showed **D** a reduction in intestinal SO_2 and **E** relBF, while **F** relHb levels remained unchanged. ***$p < 0.001$; **$p < 0.01$; ns: not significant; ○ Sham; ⋄ LPS; ⊥ SEM.

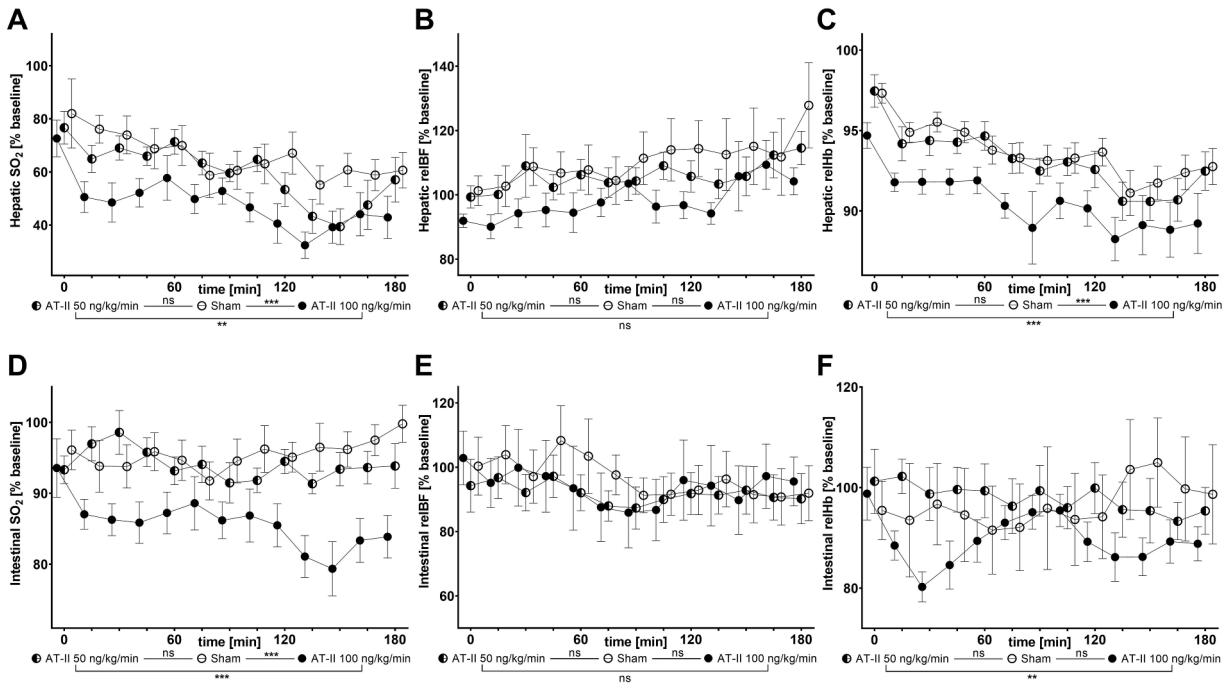

Fig. 5. Micro-lightguide spectrophotometry measurements during intravenous treatment with angiotensin II in rats without endotoxemia.
Legend: Low-dose angiotensin II (AT-II) treatment in animals without endotoxemia did not produce changes in any microcirculation measurement. However, hepatic **A** oxygen saturation (SO_2) and **C** relative hemoglobin (relHb) decreased significantly following high-dose AT-II administration, while **B** relative blood flow (relBF) was preserved. Animals that received high-dose AT-II treatment had lower **D** intestinal SO_2, while **E** relBF and **F** relHb were comparable.
$***p < 0.001$; $**p < 0.01$; ns: not significant; ○ Sham; ◐ AT-II 50 ng/kg/min; ● AT-II 100 ng/kg/min; ⊥ SEM.

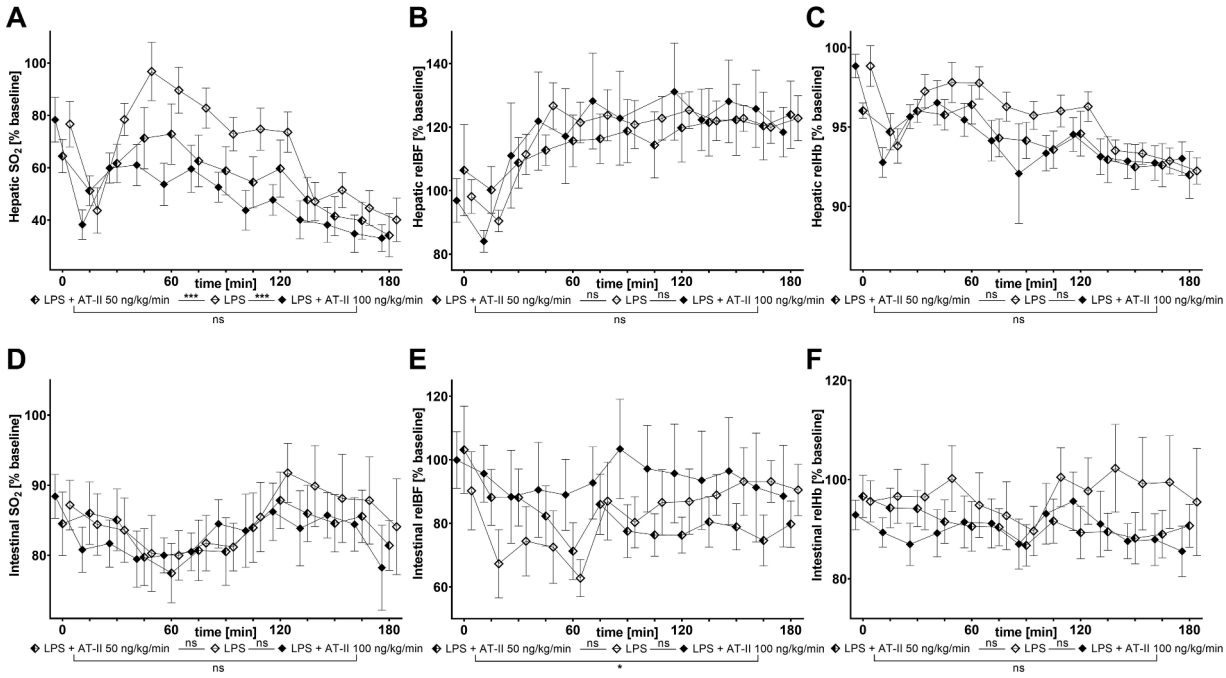

Fig. 6. Micro-lightguide spectrophotometry measurements during intravenous treatment with angiotensin II in rats with septic shock.
Legend: During endotoxemia, **A** hepatic oxygen saturation (SO_2) was reduced following angiotensin II (AT-II) treatment, while **B** relative blood flow (relBF) and **C** relative hemoglobin (relHb) were comparable among the treatment groups. **D** Intestinal SO_2 and **F** relHb remained unchanged despite the treatment with AT-II. In contrast, **E** relBF was increased following treatment with high-dose AT-II compared with low-dose AT-II.
$***p < 0.001$; ns: not significant; ⋄ LPS; ◈ LPS + AT-II 50 ng/kg/min; ◆ LPS + AT-II 100 ng/kg/min; ⊥ SEM.

animals that were not treated with AT-II after LPS infusion (Fig. 6**D-F**). However, comparing low-dose AT-II with high-dose AT-II after LPS infusion, relBF was significantly elevated in the high-dose AT-II group, but no differences in SO_2 and relHb were detected (relBF: low-dose 81,8 ± 8,5% vs. high-dose 94,0 ± 13,9%, $p = 0.03$, Fig. 6**E**).

3.4. Blood analyses

Point-of-care blood analyses evaluating arterial and central venous oxygen saturation, acid-base balance, and serum lactate are summarized in Table 1. Compared with the sham group, significantly higher levels of lactate ($p = 0.013$) and lower BE ($p < 0.01$), with a reduction in plasma bicarbonate levels ($p < 0.01$), resulting in a reduction in pH ($p < 0.01$), were measured in animals that received LPS. In the groups that did not receive LPS, significantly lower $S_{cv}O_2$ was observed in animals that received high-dose AT-II infusion compared with both the sham ($p < 0.001$) and low-dose AT-II ($p = 0.043$) groups. Metabolic alterations were only observed in the groups that received LPS. Although pH and serum lactate levels were comparable, lower BE and bicarbonate levels were measured in groups that received low-dose or high-dose AT-II infusion during endotoxemia (LPS vs. low-dose $p = 0.017$ for BE, $p = 0.029$ for bicarbonate; LPS vs. high-dose both $p < 0.01$). Further measurements obtained during the experiments, including Hb, hematocrit, blood gasses, and electrolytes are presented in the appendix (Table A.1).

3.5. Cytokine measurements

Plasma cytokine concentrations are shown in Table 2. While significantly elevated levels of TNFα, IL-6, and IL-10 were observed during endotoxemia (all $p < 0.001$), no differences were observed among the groups that received AT-II without septic shock. In contrast, despite comparable levels of IL-6 and TNFα, IL-10 was significantly increased in animals that received high-dose AT-II treatment during septic shock ($p < 0.001$).

4. Discussion

Our rat model represented hyperdynamic septic shock with tachycardia, hypotension, elevated plasma lactate, and subsequently increased CO with systolic dysfunction indicated by a stronger increase in EDV over time. Micro-lightguide spectrophotometry revealed sepsis-induced impairment of intestinal microcirculation evidenced by reduced intestinal SO_2 and relBF, while hepatic SO_2 and relBF remained unchanged. Compared with sham animals, hepatic relative Hb was slightly increased in septic animals potentially indicating intravascular fluid concentration through capillary leakage. When AT-II was administered, MAP was restored in a dose-dependent manner; however, the measured microcirculation was not improved by AT-II to the same extent. These results are supported by the concept that end-organ damage is associated with both macrohemodynamic disturbances and changes in the end-organs' microcirculation. Therefore, micro-lightguide spectrophotometry appears to be appropriate in our hemodynamic model for the evaluation of hepatic and intestinal microcirculation following AT-II infusion under septic or non-septic conditions.

Dose-dependent effects of AT-II were seen on hepatic microcirculation. While low-dose AT-II did not induce any changes in non-septic animals, SO_2 and relHb were significantly reduced during treatment with high-dose AT-II. In animals with septic shock, reduction in hepatic SO_2 was observed during both low-dose and high-dose AT-II treatment, although relBF and relHb remained unchanged. Nascimento et al. and Kimura et al. analyzed the effect of AT-II on isolated rat livers and reported a hypertensive response in the portal vein and hepatic artery during treatment with AT-II (Kimura et al., 2017; Nascimento et al., 2005). Furthermore, reduced arterial and portal venous blood flows were reported, and these changes altered hepatic metabolic function, resulting in increased oxygen consumption and glucose release (Reisenleiter et al., 1996). Moreover, these experiments revealed a global metabolic disturbance indicated by increased release of lactate during in-situ liver perfusion in rats (Reisenleiter et al., 1996). In comparison to our study, we administered lower AT-II doses, and increased lactate release was not observed in animals receiving AT-II. However, metabolic alterations evidenced by lower BE and plasma bicarbonate levels were

Table 1
Arterial and central venous oxygen saturation, acid-base balance, and serum lactate.

Parameter	Group	Baseline	0 min	60 min	120 min	180 min
S_aO_2	Sham	100 [98 - 100]	99 [99 - 100]	99 [98 - 100]	99 [98 - 100]	99 [98 - 100]
[%]	AT-II 50 ng/kg/min	100 [100 - 100]	100 [100 - 100]	100 [99 - 100]	100 [100 - 100]	100 [99 - 100]
	AT-II 100 ng/kg/min	100 [99 - 100]	99 [98 - 100]	100 [99 - 100]	99 [98 - 100]	99 [98 - 99]
	LPS	100 [99 - 100]	100 [100 - 100]	99 [96 - 100]	97 [95 - 98]	95 [92 - 97]
	LPS + AT-II 50 ng/kg/min	100 [100 - 100]	100 [99 - 100]	97 [96 - 99]	96 [95 - 98]	93 [91 - 95]
	LPS + AT-II 100 ng/kg/min	100 [98 - 100]	100 [99 - 100]	97 [95 - 98]	97 [94 - 99]	96 [95 - 96]
$S_{cv}O_2$	Sham	70 [69 - 73]	69 [63 - 71]	65 [63 - 69]	69 [65 - 73]	65 [54 - 68]
[%]	AT-II 50 ng/kg/min	73 [68 - 78]	64 [61 - 70]	66 [63 - 67]	57 [56 - 59]	58 [53 - 62]
	AT-II 100 ng/kg/min$^{++,=}$	67 [65 - 72]	63 [60 - 66]	61 [54 - 63]	53 [48 - 55]	50 [47 - 57]
	LPS	70 [65 - 75]	65 [62 - 72]	68 [59 - 71]	57 [53 - 62]	54 [53 - 66]
	LPS + AT-II 50 ng/kg/min	70 [67 - 71]	67 [64 - 69]	65 [60 - 69]	58 [55 - 60]	47 [44 - 52]
	LPS + AT-II 100 ng/kg/min	72 [71 - 74]	64 [62 - 69]	65 [57 - 72]	57 [53 - 64]	53 [47 - 57]
pH	Sham **	7.40 [7.35 - 7.43]	7.39 [7.37 - 7.44]	7.37 [7.33 - 7.40]	7.36 [7.34 - 7.39]	7.36 [7.35 - 7.37]
	AT-II 50 ng/kg/min	7.42 [7.39 - 7.42]	7.38 [7.35 - 7.40]	7.36 [7.34 - 7.38]	7.36 [7.33 - 7.36]	7.33 [7.30 - 7.34]
	AT-II 100 ng/kg/min	7.40 [7.39 - 7.43]	7.38 [7.36 - 7.42]	7.37 [7.35 - 7.39]	7.35 [7.34 - 7.38]	7.34 [7.34 - 7.37]
	LPS	7.42 [7.40 - 7.45]	7.40 [7.38 - 7.43]	7.30 [7.28 - 7.33]	7.29 [7.27 - 7.34]	7.29 [7.27 - 7.34]
	LPS + AT-II 50 ng/kg/min	7.40 [7.37 - 7.41]	7.37 [7.34 - 7.41]	7.28 [7.26 - 7.31]	7.28 [7.25 - 7.32]	7.28 [7.27 - 7.30]
	LPS + AT-II 100 ng/kg/min	7.39 [7.38 - 7.40]	7.37 [7.34 - 7.38]	7.28 [7.25 - 7.29]	7.25 [7.24 - 7.28]	7.28 [7.27 - 7.31]
HCO3 -	Sham **	24.1 [23.5 - 24.5]	25.1 [24.7 - 26.1]	24.8 [24.4 - 25.0]	24.2 [23.4 - 24.6]	23.4 [21.1 - 24.1]
[mmol/l]	AT-II 50 ng/kg/min	24.9 [23.3 - 25.5]	23.8 [23.4 - 25.0]	23.1 [22.9 - 23.6]	23.1 [22.7 - 23.9]	22.2 [20.8 - 22.6]
	AT-II 100 ng/kg/min	24.4 [23.0 - 25.4]	23.5 [22.1 - 26.1]	23.6 [22.0 - 24.6]	22.7 [22.1 - 23.8]	22.8 [22.6 - 23.8]
	LPS	24.9 [24.7 - 25.9]	25.4 [23.6 - 25.4]	21.3 [21.0 - 22.4]	22.2 [21.5 - 22.8]	21.8 [21.1 - 22.6]
	LPS + AT-II 50 ng/kg/min$^{\$}$	24.1 [22.7 - 24.6]	23.5 [23.2 - 24.0]	21.0 [20.3 - 21.3]	21.1 [20.4 - 21.8]	21.5 [20.8 - 22.5]
	LPS + AT-II 100 ng/kg/min§§	24.1 [23.8 - 24.8]	23.1 [22.9 - 23.3]	21.1 [20.1 - 21.4]	20.7 [18.9 - 21.7]	20.7 [20.2 - 22.0]
Base excess	Sham **	−0.3 [−1.1 - 1.4]	1.0 [0.6 - 1.9]	0.3 [0.0 - 0.7]	−0.3 [−1.2 - 0.2]	−1.2 [−3.2 - (−0.4)]

(continued on next page)

Table 1 (*continued*)

Parameter	Group	Baseline	0 min	60 min	120 min	180 min
	AT-II 50 ng/kg/min[#]	−0.2 [−1.5 - 1.2]	−0.9 [−2.0 - 0.5]	−1.5 [−1.7 - (−1.0)]	−1.2 [−2.0 - (−0.6)]	−2.8 [−3.3 - (−2.3)]
	AT-II 100 ng/kg/min	0.6 [−1.4 - 2.1]	−0.7 [−2.8 - 1.7]	−0.9 [−3.0 - 0.2]	−2.0 [−2.7 - (- 0.4)]	- 1.5 [−2.1 - (−0.7)]
	LPS	0.5 [0.3 - 1.6]	0.9 [−0.8 - 1.1]	−3.1 [−4.0 - (−2.4)]	−2.3 [−3.1 - (−1.8)]	−2.6 [−3.8 - (−2.0)]
	LPS + AT-II 50 ng/kg/min[$]	−0.4 [−2.0 - 0.1]	−1.1 [−1.5 - (−0.3)]	−4.1 [−5.0 - (−3.9)]	−4.0 [−4.8 - (−3.2)]	−3.3 [−4.4 - (−2.2)]
	LPS + AT-II 100 ng/kg/min[ßß]	−0.6 [−0.8 - 0.4]	- 1.6 [−1.8 - (−1.4)]	−4.0 [−5.3 - (−3.5)]	- 4.2 [−5.7 - (−3.1)]	- 4.2 [−5.0 - (−2.7)]
Lactate	Sham *	1.8 [1.8 - 2.2]	1.4 [1.0 - 1.5]	1.2 [1.0 - 1.4]	1.2 [1.1 - 1.3]	1.1 [1.0 - 1.4]
[mmol/l]	AT-II 50 ng/kg/min	1.8 [1.5 - 2.1]	1.6 [1.5 - 1.7]	1.5 [1.1 - 1.7]	1.1 [1.0 - 1.3]	1.0 [0.8 - 1.2]
	AT-II 100 ng/kg/min	1.7 [1.5 - 1.9]	1.4 [1.0 - 1.7]	1.4 [1.2 - 1.6]	1.4 [1.2 - 1.5]	1.0 [0.9 - 1.2]
	LPS	1.6 [1.4 - 1.8]	1.2 [1.0 - 1.3]	2.1 [1.9 - 2.7]	1.5 [1.2 - 1.8]	1.8 [1.5 - 1.9]
	LPS + AT-II 50 ng/kg/min	1.7 [1.5 - 2.0]	1.6 [1.5 - 1.8]	2.2 [2.1 - 2.5]	1.6 [1.4 - 1.9]	1.5 [1.4 - 2.2]
	LPS + AT-II 100 ng/kg/min	1.7 [1.6 - 1.8]	2.0 [1.5 - 2.2]	2.2 [2.1 - 2.6]	1.5 [1.3 - 2.2]	1.7 [1.3 - 1.9]

Legend: Sham vs. LPS: *$p < 0.05$; **$p<0.01$; Sham vs. AT-II 50 ng/kg/min: [#]$p<0.05$; Sham vs. AT-II 100 ng/kg/min: [+++]$p<0.001$; AT-II 50 ng/kg/min vs. AT-II 100 ng/kg/min: [=]$p<0.05$; LPS vs. LPS + AT-II 50 ng/kg/min: [$]$p<0.05$; LPS vs. LPS + AT-II 100 ng/kg/min: [ßß]p<0.01;.

seen, even though these effects were not strong enough to provoke an overall reduction in pH. Interestingly, these effects were observed in endotoxemic animals to a larger extent; therefore, it can be hypothesized that the preserved metabolic function of the liver may be further impaired by additional treatment with AT-II during septic shock. Increased oxygen consumption was also observed in our study, evidenced by a decreased hepatic SO_2. The previously reported effects on non-septic rat livers were also observed during our endotoxemia experiments. In contrast, relBF remained comparable in our study, underlining that our measurements primarily reflected end-organ microcirculation, which cannot be directly transferred to macroscopic portal vein or hepatic artery measurements. Interestingly, restoring MAP and subsequent hepatic perfusion in mice with septic shock was reported to impair the metabolic capacity of the liver (Albuszies et al., 2005). Another series of experiments reported that prolonged AT-II infusion provokes liver damage indicated by elevated liver enzymes, increased oxidative stress, upregulated inflammatory proteins, and proinflammatory cytokine release (Bataller et al., 2003). Bucher et al. reported AT1-R downregulation in the adrenal gland, kidney, liver, lung, and heart during endotoxemia that was attributed to the release of proinflammatory cytokines, like TNFα and IL-1β (Bucher et al., 2001). These findings may contribute to the fact that the increase in MAP was lower in endotoxemic rats in our study; however, we did not observe significantly increased proinflammatory cytokine levels following AT-II treatment in our experiments. It may be possible that these observations could not be made due to the short observation period; however, we observed increased levels of anti-inflammatory IL-10 during high-dose AT-II treatment. Activating the anti-inflammatory axis may be beneficial in counter-regulating the dysregulated proinflammatory host response to infection. Downregulation of AT1-R in these end-organs could be beneficial when exogenous AT-II is administered because

Table 2
Plasma cytokine concentrations.

Parameter	Group	0 min	60 min	120 min	180 min
IL-6	Sham***	0.0 [0.0 - 0.0]	0.0 [0.0 - 58.4]	103.6 [0.0 - 219.0]	204.8 [43.8 - 252.1]
[pg/ml]	AT-II 50 ng/kg/min	0.0 [0.0 - 0.0]	999.0 [295.1 - 3214.9]	657.2 [405.1 - 1772.8]	756.5 [436.8 - 1713.2]
	AT-II 100 ng/kg/min	0.0 [0.0 - 0.0]	9.3 [0.0 - 728.8]	197.2 [142.4 - 586.7]	406.4 [254.8 - 506.5]
	LPS	0.0 [0.0 - 12.8]	883.8 [615.5 - 28,792.8]	3223.4 [2381.8 - 141,220.1]	3427.9 [2349.6 - 186,555.3]
	LPS + AT-II 50 ng/kg/min	0.0 [0.0 - 92.1]	13,253.0 [737.6 - 36,319.2]	43,733.6 [2915.2 - 129,192.3]	84,353.1 [3339.4 - 151,713.5]
	LPS + AT-II 100 ng/kg/min	0.0 [0.0 - 0.0]	29,020.8 [6588.8 - 34,844.1]	115,129.8 [103,861.0 - 147,183.5]	124,197.1 [30,599.5 - 154,716.0]
TNFα	Sham***	11.6 [5.2 - 24.1]	13.1 [12.3 - 33.5]	12.7 [11.6 - 21.6]	12.7 [11.3 - 17.9]
[pg/ml]	AT-II 50 ng/kg/min	70.0 [33.4 - 149.5]	461.9 [38.5 - 2269.6]	91.4 [29.6 - 147.1]	58.2 [21.2 - 97.1]
	AT-II 100 ng/kg/min	10.8 [10.7 - 42.5]	15.5 [11.9 - 301.7]	15.2 [12.0 - 63.6]	35.2 [11.5 - 44.3]
	LPS	212.1 [195.8 - 276.2]	35,335.4 [31,164.0 - 39,990.1]	6962.0 [5534.8 - 7547.3]	789.6 [576.1 - 996.5]
	LPS + AT-II 50 ng/kg/min	260.4 [177.6 - 485.5]	21,576.4 [11,603.5 - 27,713.2]	4589.9 [1937.3 - 12,745.1]	967.7 [668.7 - 1129.5]
	LPS + AT-II 100 ng/kg/min	255.2 [189.5 - 348.6]	27,501.2 [20,735.9 - 34,777.2]	5558.9 [4231.5 - 6912.2]	634.0 [603.3 - 832.2]
IL-10	Sham***	21.7 [8.0 - 78.6]	42.6 [26.7 - 63.6]	47.5 [31.6 - 63.5]	44.1 [30.7 - 72.1]
[pg/ml]	AT-II 50 ng/kg/min	323.9 [237.9 - 423.2]	272.9 [123.0 - 466.7]	93.6 [77.5 - 105.5]	184.6 [122.8 - 258.9]
	AT-II 100 ng/kg/min	29.7 [20.1 - 134.4]	80.9 [23.1 - 314.5]	74.0 [39.8 - 91.6]	76.9 [19.2 - 204.5]
	LPS	406.1 [288.7 - 554.3]	1442.0 [1216.3 - 1681.5]	734.7 [584.3 - 993.3]	402.2 [360.6 - 518.8]
	LPS + AT-II 50 ng/kg/min	461.4 [337.1 - 581.5]	1736.8 [1551.5 - 1963.9]	984.3 [762.5 - 1406.0]	495.1 [433.2 - 553.9]
	LPS + AT-II 100 ng/kg/min[ßßß]	526.8 [335.2 - 825.3]	2072.0 [1659.9 - 2756.5]	1117.3 [999.4 - 1285.7]	494.6 [432.4 - 658.6]

Legend: Sham vs. LPS: ***$p<0.001$; LPS vs. LPS + AT-II 100 ng/kg/min: [ßßß]p<0.001;.

potential adverse effects could be reduced due to the elevated endogenous AT-II resistance. Low-dose AT-II even reduced hepatic SO_2 during endotoxemia, while hepatic SO_2 was comparable in non-septic animals. Unfortunately, clinical data addressing these concerns are still lacking. The ATHOS trial did not report any difference in the occurrence of metabolic disorders following treatment with AT-II; however, the validity of this observation could be impaired by the small sample size of only 10 patients per treatment group [9]. Neither ATHOS nor ATHOS-III reported any laboratory measurements, such as liver enzymes, precise course of serum lactate or cytokine levels [8,9]. It is questionable

whether slightly accelerated lactate release from the liver may be clinically relevant when sufficient circulation is restored by AT-II, leading to better organ perfusion, which itself should decrease serum lactate levels during septic shock. However, concerns about impairments in liver function accelerated by AT-II treatment could not be negated in the context of preliminary data and our study. Therefore, these findings should be taken into careful consideration in septic shock patients with preexisting severe liver disease or septic hepatic insufficiency when considering treatment with AT-II.

Endogenous receptor downregulation processes may have been induced by AT-II infusion in our experiments because the initial rise in MAP diminished during the observation period, an effect that was seen in all animals treated with AT-II, whether endotoxemia was present or not. However, MAP remained significantly elevated during the entire observation period. No reports on the necessity of higher AT-II dosage over time were made in the ATHOS trials. In contrast, compared with our data, MAP increased rapidly in most patients in the ATHOS-III trial after AT-II treatment initiation with 20 ng/kg/min, and most of the patients required immediate dose reduction within the first 30 min after treatment initiation (Khanna et al., 2017).

Intestinal microcirculation was not altered by low-dose AT-II infusion in non-septic and septic animals in our study. However, in animals without septic shock, high-dose AT-II treatment led to an impairment of intestinal SO_2 and relHb. These results could be attributed to infusion of AT-II or to the altered hemodynamic conditions in our experiments. During high-dose AT-II therapy, we observed significantly reduced CO compared with low-dose AT-II and the sham group. CO may have been reduced due to unphysiologically high afterload from peripheral vasoconstriction induced by AT-II. Therefore, it was not surprising that $S_{cv}O_2$ was also impaired in this treatment group. In contrast, it is possible that AT-II contributed more to these changes than the reduction in CO. Although the difference was significant compared with the other treatment groups, CO was only reduced slightly below its baseline value. A reduction in $S_{cv}O_2$ could also be explained by significantly reduced intestinal microcirculation leading to overall increased oxygen consumption. Therefore, a significant reduction in intestinal and hepatic microcirculation during high-dose AT-II treatment in non-septic rats cannot be ruled out in our study, although the results must be interpreted with caution.

CO was comparable among the endotoxemic treatment groups. Interestingly, EDV was lower in the groups treated with low-dose and high-dose AT-II, indicating preserved systolic function compared with the LPS treatment group. Similar observations were made for HR; however, these effects were only significant in the low-dose AT-II group. Therefore, treatment with AT-II might have mediated the beneficial effects in our experimental groups. The intestine is one of the greatest end-organs impaired by septic shock; therefore, beneficial systemic effects could be explained by the improvement of its circulation. RelBF was significantly increased in septic animals that received high-dose AT-II compared with those that received low-dose AT-II, even though intestinal SO_2 was comparable among all LPS groups. It is difficult to discuss these findings in light of other data because – to our knowledge – our experiments were the first to assess intestinal microcirculation under the influence of exogenous intravenous AT-II. The administration of the selective AT1-R antagonist losartan in endotoxemic pigs with hypodynamic shock was shown to improve oxygen delivery in the gut while mucosal pH and mucosal-arterial PO_2-gap remained unchanged (Oldner et al., 1999). Similar effects were observed in dogs with hemorrhagic shock treated with topic losartan, where losartan did not improve gut perfusion assessed by micro-lightguide spectrophotometry (Truse et al., 2019). Unfortunately, the hyperdynamic septic shock induced in our model cannot be compared to other hypodynamic or hemorrhagic forms of shock. However, these results suggest that administering AT-II might not impair intestinal microcirculation; AT-II may even increase intestinal microcirculation, evidenced by slightly enhanced intestinal relBF in our experiments. Although these conclusions should be made with caution and should be consolidated in further trials, experimental data on treatment with norepinephrine or vasopressin have also highlighted unaltered intestinal microcirculation parameters during septic shock (Nakajima et al., 2006). AT-II has a wide range of clinical effects depending on the receptor availability at the effector organ, and both AT1-R and AT2-R can be found at the mesenteric vascular bed (Matrougui et al., 1999b). Interestingly, although AT-II has a higher affinity for AT1-R, both receptors are sensitive to AT-II and produce complementary effects.

Similar observations were made for anti-inflammatory IL-10, which was enhanced in our high-dose AT-II treatment group during septic shock, although the proinflammatory response was originally mediated by AT1-R. Increased IL-10 levels could also be induced by AT2-R stimulation because an increase in IL-10 was observed in macrophages and renal proximal tubule epithelial cells after AT2-R stimulation (Dhande et al., 2015, 2013). Interestingly, TNFα and IL-6 levels were reduced in these experiments, which highlights the anti-inflammatory effects of AT2-R. However, pro-inflammatory cytokine levels remained unchanged in our experiments despite treatment with AT-II, suggesting that anti-inflammatory effects induced by AT-II were weaker than those produced by isolated in-vitro AT2-R stimulation. Whether these cytokine changes occur in humans remains unclear, but our data can be interpreted as hypothesis-generating. Furthermore, these observations could serve as an explanation for the beneficial effects of AT-II seen in an ATHOS-III secondary analysis that reported increased 28-day survival and a high rate of renal replacement therapy discontinuation (Tumlin et al., 2018).

Several limitations of our experiments must be acknowledged. First, drug dosage is not interchangeable between humans and rats as their pharmacodynamics and pharmacokinetics differ. A higher dosage of AT-II was necessary to achieve a sufficient increase in MAP in rats, and the mean AT-II dosage needed to produce relevant MAP improvements was considerably lower in humans, as reported in the ATHOS-III trial. While vasopressor dosage is titrated to achieve a certain MAP in septic patients, AT-II infusion rate was fixed to produce comparable treatment groups in our model. Furthermore, decreasing MAP was observed after the initial AT-II peak, which could be attributed to counter-regulatory effects or the worsening of cardiocirculatory function due to the septic shock. However, this phenomenon was observed in all animals that received AT-II, even in animals that did not receive LPS infusion. Despite a higher basal metabolic rate in rats, the observation time was only 3 h, and the longer-term effects of AT-II thus could not be studied in our experiments; these effects might become relevant in septic shock patients in intensive care units. Second, the animals received AT-II alone, whereas in humans, AT-II is administered to treat persistent hypotension despite initial treatment with vasopressors like norepinephrine or vasopressin. It must be noted that our experiments aimed to evaluate the effects of AT-II; therefore, any other potential distorting substances were avoided. Further microcirculatory alterations that may occur when AT-II is combined with other vasopressors cannot be excluded. Third, LPS-induced experimental septic shock does not reflect cytokine kinetics as they are seen in septic patients or animals after cecal ligature and puncture. LPS was used in our experiments to achieve a hyperdynamic model of septic shock due to animal welfare regulations. Finally, despite sample size calculation, the size of our study groups consisting of 10 animals was quite low; however, animal welfare regulations required the use of the smallest possible number of animals, and significant differences were measured despite the small sample size.

Conclusions

In conclusion, our findings indicate that AT-II induces alterations in hepatic microcirculation with and without septic shock that might not only reflect ischemia but also altered hepatic metabolic conditions, as described in non-septic animal models. Furthermore, our study revealed a dose-dependent decrease in intestinal microcirculation following AT-II

infusion in non-septic rats; however, this effect might be influenced by the corresponding reduction in CO that was attributed to the high afterload generated by high-dose AT-II infusion. We did not find evidence for a reduction in intestinal microcirculation facilitated by AT-II during septic shock. Anti-inflammatory IL-10 was even increased in our septic shock group treated with high-dose AT-II, indicating possible beneficial effects of AT-II through restoring or even preserving intestinal microcirculation. Although difficult to conduct, further experiments should assess the influence of AT-II treatment on cardiac and cerebral microcirculation. The hypotheses derived from the animal experiments should be evaluated in clinical studies during septic shock, focusing on the side effects of AT-II treatment that can be attributed to intestinal or hepatic perfusion.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability

Data will be made available on request.

Acknowledgments

The authors thank Ilona Magel for her excellent technical assistance as well as the whole team of the experimental animal husbandry for taking care of the animals.

Appendices

Table A.1
Blood gas analysis and hemoglobin, electrolyte, and glucose measurements.

Parameter	Group	Baseline	0 min	60 min	120 min	180 min
Hb	Sham	14.1 [13.8 - 14.5]	14.1 [14.0 - 14.4]	13.3 [13.0 - 13.6]	13.0 [11.8 - 13.1]	11.6 [11.2 - 12.5]
[g/dl]	AT-II 50 ng/kg/min	14.2 [13.5 - 14.5]	14.2 [13.3 - 14.6]	13.8 [12.9 - 14.1]	12.8 [12.3 - 13.2]	12.1 [11.7 - 12.2]
	AT-II 100 ng/kg/min	14.3 [14.1 - 14.5]	14.1 [13.5 - 14.7]	13.8 [12.4 - 14.1]	13.3 [10.9 - 13.5]	12.4 [12.3 - 12.8]
	LPS	14.2 [13.7 - 14.6]	14.2 [14.0 - 15.1]	13.9 [13.3 - 14.0]	12.5 [11.9 - 12.7]	10.9 [10.7 - 11.4]
	LPS + AT-II 50 ng/kg/min	14.2 [13.5 - 14.5]	14.6 [14.2 - 14.9]	13.6 [13.2 - 14.0]	12.6 [12.0 - 12.8]	11.2 [10.6 - 11.9]
	LPS + AT-II 100 ng/kg/min	14.7 [14.3 - 14.8]	14.5 [14.1 - 15.0]	13.2 [12.8 - 13.9]	12.0 [11.6 - 13.1]	10.9 [10.2 - 11.6]
Hematocrit	Sham	43.2 [42.5 - 44.5]	43.3 [43.0 - 44.1]	40.8 [39.9 - 41.8]	39.8 [36.3 - 40.4]	35.6 [34.6 - 38.5]
[%]	AT-II 50 ng/kg/min	43.9 [41.4 - 44.6]	43.7 [40.9 - 44.6]	42.3 [39.8 - 43.2]	39.5 [38.0 - 40.4]	37.1 [36.0 - 37.8]
	AT-II 100 ng/kg/min	43.8 [43.3 - 44.6]	43.2 [41.4 - 45.0]	42.3 [38.2 - 43.3]	40.9 [33.8 - 41.5]	38.3 [37.9 - 39.2]
	LPS	43.5 [42.3 - 44.7]	43.8 [42.7 - 46.1]	42.5 [40.7 - 43.0]	38.5 [36.5 - 38.9]	33.8 [33.1 - 35.2]
	LPS + AT-II 50 ng/kg/min	43.5 [41.3 - 44.6]	44.7 [43.6 - 45.7]	41.7 [40.6 - 42.8]	38.7 [37.0 - 39.3]	34.5 [32.5 - 36.6]
	LPS + AT-II 100 ng/kg/min	45.0 [43.9 - 45.5]	44.5 [43.2 - 46.0]	40.5 [39.3 - 42.5]	37.0 [35.7 - 40.2]	33.5 [31.5 - 35.9]
p_aO_2	Sham	142.0 [107.0 - 152.5]	120.5 [106.2 - 127.8]	112.5 [109.5 - 128.8]	114.0 [104.0 - 127.0]	112.0 [108.0 - 136.5]
[mmHg]	AT-II 50 ng/kg/min	140.5 [119.8 - 175.2]	141.5 [131.8 - 160.8]	141.0 [124.5 - 152.8]	132.0 [121.8 - 135.8]	127.0 [117.0 - 128.5]
	AT-II 100 ng/kg/min	145.5 [131.0 - 151.5]	125.5 [114.0 - 147.2]	124.0 [117.2 - 139.8]	125.0 [116.0 - 152.0]	117.0 [107.8 - 129.2]
	LPS	130.0 [122.2 - 147.2]	134.5 [122.0 - 146.8]	127.5 [106.5 - 132.8]	110.0 [98.4 - 113.5]	97.8 [83.3 - 100.5]
	LPS + AT-II 50 ng/kg/min	150.5 [128.0 - 165.2]	132.5 [120.8 - 157.0]	112.5 [106.5 - 124.5]	104.0 [96.7 - 116.8]	117.0 [107.8 - 129.2]
	LPS + AT-II 100 ng/kg/min	129.0 [107.0 - 155.2]	123.5 [111.5 - 137.5]	109.0 [103.0 - 119.0]	108.5 [103.0 - 113.2]	87.2 [83.5 - 95.0]
p_aCO_2	Sham	40.2 [36.5 - 44.7]	42.2 [37.6 - 44.7]	44.8 [40.5 - 46.6]	43.7 [42.2 - 47.2]	43.3 [42.6 - 46.3]
[mmHg]	AT-II 50 ng/kg/min	39.1 [38.1 - 40.2]	41.2 [39.8 - 41.9]	43.2 [42.3 - 44.4]	42.9 [39.4 - 45.0]	43.7 [42.2 - 46.3]
	AT-II 100 ng/kg/min	40.4 [35.9 - 42.4]	41.0 [36.1 - 42.5]	41.0 [38.8 - 44.4]	42.1 [40.4 - 44.3]	44.0 [41.3 - 46.4]
	LPS	38.2 [35.6 - 40.3]	39.7 [38.6 - 43.7]	47.3 [45.0 - 50.0]	51.1 [47.4 - 53.2]	50.4 [44.1 - 52.9]
	LPS + AT-II 50 ng/kg/min	37.0 [35.4 - 41.9]	42.2 [37.7 - 45.6]	49.9 [45.0 - 53.8]	50.0 [46.0 - 56.2]	51.2 [48.1 - 52.6]
	LPS + AT-II 100 ng/kg/min	40.7 [37.9 - 42.3]	41.3 [39.5 - 43.8]	50.5 [46.7 - 52.0]	52.0 [49.0 - 58.3]	49.1 [46.5 - 54.6]
Sodium	Sham	141.5 [140.2 - 142.8]	142.0 [141.0 - 142.0]	142.0 [141.0 - 142.0]	143.0 [142.0 - 143.0]	143.0 [141.5 - 143.5]
[mmol/l]	AT-II 50 ng/kg/min	140.5 [140.0 - 142.5]	142.0 [141.0 - 143.8]	142.0 [141.0 - 143.8]	143.0 [141.2 - 144.0]	142.0 [140.5 - 142.5]
	AT-II 100 ng/kg/min	141.0 [140.0 - 143.5]	141.5 [141.0 - 143.8]	141.5 [140.2 - 144.2]	141.5 [141.0 - 144.2]	142.0 [141.8 - 143.0]
	LPS	140.0 [139.0 - 142.0]	142.0 [141.0 - 143.5]	142.0 [141.0 - 143.8]	142.0 [142.0 - 144.0]	142.0 [142.0 - 145.0]
	LPS + AT-II 50 ng/kg/min	142.0 [141.2 - 143.8]	142.5 [140.5 - 144.0]	143.5 [142.0 - 145.0]	144.0 [142.2 - 146.0]	144.0 [143.0 - 145.0]
	LPS + AT-II 100 ng/kg/min	142.0 [141.2 - 142.8]	143.0 [142.0 - 144.0]	143.0 [142.0 - 145.0]	143.0 [142.2 - 144.0]	143.0 [142.0 - 144.0]
Potassium	Sham***	4.3 [4.2 - 4.8]	4.7 [4.4 - 4.9]	4.6 [4.3 - 4.8]	4.8 [4.6 - 5.0]	4.7 [4.6 - 4.8]
[mmol/l]	AT-II 50 ng/kg/min	4.6 [4.5 - 4.8]	4.6 [4.5 - 4.7]	4.7 [4.5 - 4.8]	5.0 [4.7 - 5.0]	4.9 [4.8 - 4.9]
	AT-II 100 ng/kg/min	4.4 [4.2 - 4.6]	4.6 [4.5 - 4.7]	4.7 [4.6 - 4.8]	4.9 [4.6 - 5.1]	5.1 [5.0 - 5.3]
	LPS	4.5 [4.1 - 4.6]	4.5 [4.2 - 4.6]	3.8 [3.7 - 3.9]	4.4 [4.2 - 4.5]	4.7 [4.6 - 4.8]
	LPS + AT-II 50 ng/kg/min	4.5 [4.3 - 4.5]	4.6 [4.4 - 4.7]	4.0 [3.9 - 4.0]	4.5 [4.4 - 4.6]	4.8 [4.6 - 4.8]
	LPS + AT-II 100 ng/kg/min	4.3 [4.2 - 4.7]	4.3 [4.3 - 4.5]	3.9 [3.8 - 4.0]	4.3 [4.2 - 4.4]	4.6 [4.5 - 4.7]
Calcium	Sham***	1.5 [1.4 - 1.5]	1.5 [1.5 - 1.5]	1.5 [1.5 - 1.5]	1.5 [1.5 - 1.5]	1.5 [1.5 - 1.5]
[mmol/l]	AT-II 50 ng/kg/min	1.5 [1.5 - 1.5]	1.5 [1.5 - 1.6]	1.5 [1.5 - 1.6]	1.5 [1.5 - 1.5]	1.5 [1.4 - 1.5]
	AT-II 100 ng/kg/min	1.5 [1.5 - 1.5]	1.5 [1.5 - 1.6]	1.5 [1.4 - 1.5]	1.5 [1.5 - 1.5]	1.5 [1.4 - 1.5]
	LPS	1.4 [1.4 - 1.5]	1.5 [1.5 - 1.5]	1.4 [1.4 - 1.4]	1.4 [1.4 - 1.4]	1.4 [1.3 - 1.4]
	LPS + AT-II 50 ng/kg/min$^{\$\$}$	1.5 [1.5 - 1.6]	1.5 [1.5 - 1.6]	1.4 [1.4 - 1.5]	1.4 [1.4 - 1.5]	1.4 [1.4 - 1.4]
	LPS + AT-II 100 ng/kg/min	1.5 [1.5 - 1.5]	1.6 [1.5 - 1.6]	1.4 [1.4 - 1.5]	1.4 [1.3 - 1.5]	1.4 [1.3 - 1.4]
Chloride	Sham **	108.5 [107.0 - 110.0]	109.0 [107.5 - 110.8]	110.5 [108.5 - 111.0]	112.0 [110.0 - 113.0]	114.0 [110.5 - 114.0]
[mmol/l]	AT-II 50 ng/kg/min	109.0 [108.0 - 110.5]	110.0 [108.2 - 111.0]	111.5 [110.0 - 112.0]	112.5 [112.0 - 114.0]	113.0 [113.0 - 114.5]
	AT-II 100 ng/kg/min	109.0 [108.0 - 109.0]	110.5 [108.2 - 111.8]	110.5 [109.2 - 113.8]	111.5 [111.0 - 113.0]	113.0 [111.0 - 114.5]
	LPS	110.0 [109.2 - 110.0]	110.0 [109.0 - 111.0]	111.5 [110.0 - 112.8]	113.0 [112.0 - 113.0]	115.0 [113.0 - 115.0]
	LPS + AT-II 50 ng/kg/min	109.0 [107.2 - 110.5]	110.5 [109.2 - 111.0]	111.0 [110.0 - 112.8]	112.0 [111.0 - 113.0]	112.0 [112.0 - 113.8]
	LPS + AT-II 100 ng/kg/min	109.0 [108.2 - 110.0]	110.0 [109.0 - 111.0]	111.5 [109.2 - 112.0]	112.5 [112.0 - 113.0]	112.0 [112.0 - 116.0]
Glucose	Sham	168.0 [164.2 - 183.8]	144.5 [134.8 - 147.8]	137.0 [129.2 - 150.5]	127.0 [126.0 - 135.0]	138.0 [131.0 - 147.5]
[mg/dl]	AT-II 50 ng/kg/min$^{\#\#}$	178.0 [149.0 - 190.2]	133.5 [113.2 - 141.0]	126.5 [110.2 - 132.8]	108.0 [101.2 - 114.0]	115.0 [110.5 - 122.5]
	AT-II 100 ng/kg/min	172.5 [166.2 - 192.0]	139.0 [127.8 - 144.5]	131.0 [113.2 - 137.5]	115.5 [106.0 - 125.8]	112.5 [107.2 - 132.5]
	LPS	188.0 [174.8 - 198.2]	143.5 [139.0 - 157.8]	199.0 [191.8 - 207.8]	152.0 [138.0 - 159.0]	112.0 [104.0 - 121.0]
	LPS + AT-II 50 ng/kg/min	175.0 [159.0 - 187.2]	137.5 [135.2 - 148.8]	185.5 [164.5 - 214.2]	150.5 [135.5 - 159.2]	119.0 [103.0 - 126.8]
	LPS + AT-II 100 ng/kg/min	179.0 [175.8 - 183.8]	144.5 [138.2 - 153.5]	204.5 [195.5 - 217.2]	147.5 [133.5 - 161.8]	114.0 [86.0 - 127.0]

Legend: Sham vs. LPS: ***$p<0.001$, **$p<0.01$; LPS vs. LPS + AT-II 50 ng/kg/min: $^{\$\$}p<0.01$; Sham vs. AT-II 50 ng/kg/min: $^{\#\#}p<0.01$;.
(Table A1)

References

Albuszies, G., Radermacher, P., Vogt, J., Wachter, U., Weber, S., Schoaff, M., et al., 2005. Effect of increased cardiac output on hepatic and intestinal microcirculatory blood flow, oxygenation, and metabolism in hyperdynamic murine septic shock. Crit. Care Med. 33, 2332–2338. https://doi.org/10.1097/01.CCM.0000182817.20977.E9.

An, H., Hu, Z., Chen, Y., Cheng, L., Shi, J., Han, L., 2021. Angiotensin II-mediated improvement of renal mitochondrial function via the AMPK/PGC-1α/NRF-2 pathway is superior to norepinephrine in a rat model of septic shock associated with acute renal injury. Ann. Transl. Med. 9, 481. https://doi.org/10.21037/atm-21-621. –481.

Asfar, P., Pierrot, M., Veal, N., Moal, F., Oberti, F., Croquet, V., et al., 2003. Low-dose terlipressin improves systemic and splanchnic hemodynamics in fluid-challenged endotoxic rats. Crit. Care Med. 31, 215–220. https://doi.org/10.1097/00003246-200301000-00033.

Bataller, R., Gäbele, E., Schoonhoven, R., Morris, T., Lehnert, M., Yang, L., et al., 2003. Prolonged infusion of angiotensin II into normal rats induces stellate cell activation and proinflammatory events in liver. Am. J. Physiol. - Gastrointest. Liver Physiol. 285, 642–651. https://doi.org/10.1152/ajpgi.00037.2003.

Bucher, M., Ittner, K.P., Hobbhahn, J., Taeger, K., Kurtz, A., 2001. Downregulation of angiotensin II type 1 receptors during sepsis. Hypertension 38, 177–182. https://doi.org/10.1161/01.HYP.38.2.177.

Burgdorff, A.M., Bucher, M., Schumann, J., 2018. Vasoplegia in patients with sepsis and septic shock: pathways and mechanisms. J. Int. Med. Res. 46, 1303–1310. https://doi.org/10.1177/0300060517743836.

Chawla, L.S., Busse, L., Brasha-Mitchell, E., Davison, D., Honiq, J., Alotaibi, Z., et al., 2014. Intravenous angiotensin II for the treatment of high-output shock (ATHOS trial): a pilot study. Crit. Care 18, 1–9. https://doi.org/10.1186/s13054-014-0534-9.

Dancker, C., Hopster, K., Rohn, K., Kästner, S.B., 2018. Effects of dobutamine, dopamine, phenylephrine and noradrenaline on systemic haemodynamics and intestinal perfusion in isoflurane anaesthetised horses. Equine Vet. J. 50, 104–110. https://doi.org/10.1111/evj.12721.

De Backer, D., Creteur, J., Preiser, J.C., Dubois, M.J., Vincent, J.L., 2002. Microvascular blood flow is altered in patients with sepsis. Am. J. Respir. Crit. Care Med. 166, 98–104. https://doi.org/10.1164/rccm.200109-016OC.

Dhande, I., Ali, Q., Hussain, T., 2013. Proximal tubule angiotensin AT2 receptors mediate an anti-inflammatory response via interleukin-10: role in renoprotection in obese rats. Hypertension 61, 1218–1226. https://doi.org/10.1161/HYPERTENSIONAHA.111.00422.

Dhande, I., Ma, W., Hussain, T., 2015. Angiotensin AT2 receptor stimulation is anti-inflammatory in lipopolysaccharide-activated THP-1 macrophages via increased interleukin-10 production. Hypertens. Res. 38, 21–29. https://doi.org/10.1038/hr.2014.132.

Fleischmann, C., Scherag, A., Adhikari, N.K.J., Hartog, C.S., Tsaganos, T., Schlattmann, P., et al., 2016. Assessment of global incidence and mortality of hospital-treated sepsis current estimates and limitations. Am. J. Respir. Crit. Care Med. 193, 259–272. https://doi.org/10.1164/rccm.201504-0781OC.

Fleischmann-Struzek, C., Mellhammar, L., Rose, N., Cassini, A., Rudd, K.E., Schlattmann, P., et al., 2020. Incidence and mortality of hospital- and ICU-treated sepsis: results from an updated and expanded systematic review and meta-analysis. Intensive Care Med. 46, 1552–1562. https://doi.org/10.1007/s00134-020-06151-x.

Haussner, F., Chakraborty, S., Halbgebauer, R., Huber-Lang, M., 2019. Challenge to the intestinal mucosa during sepsis. Front. Immunol. 10 https://doi.org/10.3389/fimmu.2019.00891.

Khanna, A., English, S.W., Wang, X.S., Ham, K., Tumlin, J., Szerlip, H., et al., 2017. Angiotensin II for the treatment of vasodilatory shock. N. Engl. J. Med. 377, 419–430. https://doi.org/10.1056/nejmoa1704154.

Kimura, D.C., Nagaoka, M.R., Borges, D.R., Kouyoumdjian, M., 2017. Angiotensin II or epinephrine hemodynamic and metabolic responses in the liver of L-NAME induced hypertension and spontaneous hypertensive rats. World J. Hepatol. 9, 781–790. https://doi.org/10.4254/wjh.v9.i17.781.

Lambden, S., Creagh-Brown, B.C., Hunt, J., Summers, C., Forni, L.G., 2018. Definitions and pathophysiology of vasoplegic shock. Crit. Care 22. https://doi.org/10.1186/s13054-018-2102-1.

Lumlertgul, N., Ostermann, M., 2020. Roles of angiotensin II as vasopressor in vasodilatory shock. Fut. Cardiol. 16, 569–583. https://doi.org/10.2217/fca-2020-0019.

Matrougui, K., Loufrani, L., Heymes, C., Lévy, B.I., Henrion, D., 1999a. Activation of AT2 receptors by endogenous angiotensin II is involved in flow-induced dilation in rat resistance arteries. Hypertension 34, 659–665. https://doi.org/10.1161/01.HYP.34.4.659.

Matrougui, K., Loufrani, L., Heymes, C., Lévy, B.I., Henrion, D., 1999b. Activation of AT2 receptors by endogenous angiotensin ii is involved in flow-induced dilation in rat resistance arteries. Hypertension 34, 659–665. https://doi.org/10.1161/01.HYP.34.4.659.

Nakajima, Y., Baudry, N., Duranteau, J., Vicaut, E., 2006. Effects of vasopressin, norepinephrine, and L-arginine on intestinal microcirculation in endotoxemia. Crit. Care Med. 34, 1752–1757. https://doi.org/10.1097/01.CCM.0000218812.73741.6C.

Nascimento, É.A., Gioli-Pereira, L., Carvalho, L.T., Santos, E.L., Pesquero, J.B., Kouyoumdjian, M., et al., 2005. Hemodynamic and metabolic effects of angiotensin II on the liver. Peptides 26, 315–322. https://doi.org/10.1016/j.peptides.2004.09.017.

Oldner, A., Wanecek, M., Weitzberg, E., Rundgren, M., Alving, K., Ullman, J., et al., 1999. Angiotensin II receptor antagonism increases gut oxygen delivery but fails to improve intestinal mucosal acidosis in porcine endotoxin shock. Shock 11, 127–135. https://doi.org/10.1097/00024382-199902000-00010.

Qiu, X., Huang, Y., Xu, J., Qiu, H., Yang, Y., 2014. Effects of terlipressin on microcirculation of small bowel mesentery in rats with endotoxic shock. J. Surg. Res. 188, 503–509. https://doi.org/10.1016/j.jss.2014.01.053.

Reisenleiter, F., Katz, N., Gardemann, A., 1996. Control of hepatic carbohydrate metabolism and haemodynamics in perfused rat liver by arterial and portal angiotensin II. Eur. J. Gastroenterol. Hepatol. 8, 279–286. https://doi.org/10.1097/00042737-199603000-00017.

Rodriguez, R., Cucci, M., Kane, S., Fernandez, E., Benken, S., 2020. Novel vasopressors in the treatment of vasodilatory shock: a systematic review of angiotensin ii, selepressin, and terlipressin. J. Intensive Care Med. 35, 327–337. https://doi.org/10.1177/0885066618818460.

Ruiqiang Z., Yifen Z., Ziqi R., Wei H., Xiaoyun F. Surviving Sepsis Campaign: international guidelines for management of sepsis and septic shock 2021, interpretation and expectation. vol. 33. 2021. https://doi.org/10.3760/cma.j.cn121430-20211009-01442.

Shi, R., Hamzaoui, O., De Vita, N., Monnet, X., Teboul, J.-L., 2020. Vasopressors in septic shock: which, when, and how much? Ann. Transl. Med. 8, 794. https://doi.org/10.21037/atm.2020.04.24. –794.

Singer, M., Deutschman, C.S., Seymour, C., Shankar-Hari, M., Annane, D., Bauer, M., et al., 2016. The third international consensus definitions for sepsis and septic shock (sepsis-3). JAMA - J. Am. Med. Assoc. 315, 801–810. https://doi.org/10.1001/jama.2016.0287.

Truse, R., Voß, F., Herminghaus, A., Schulz, J., Weber, A.P.M., Mettler-Altmann, T., et al., 2019. Local gastric RAAS inhibition improves gastric microvascular perfusion in dogs. J. Endocrinol. 241, 235–247. https://doi.org/10.1530/JOE-19-0030.

Tumlin, J.A., Murugan, R., Deane, A.M., Ostermann, M., Busse, L.W., Ham, K.R., et al., 2018. Outcomes in patients with vasodilatory shock and renal replacement therapy treated with intravenous angiotensin II. Crit. Care Med. 46, 949–957. https://doi.org/10.1097/CCM.0000000000003092.