Immediate norepinephrine in endotoxic shock: effects on regional and microcirculatory flow

Supplemental Data

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Suppl. MATERIALS AND METHODS

Animal preparation and anesthesia. The present study was approved by the institutional Animal Research Committee (CIECUAE 0021/2019). Fifteen female Landrace pigs (32–38 kg) were kept fasting for a 12-h period, with free access to water. After a preconditioning period of at least 24 hours, they were sedated with intramuscular injections of ketamine (5–10 mg/kg) and xylazine (0.1 mg/kg). Afterward, a venous access (Insyte Autoguard, Infusion therapy system; Sandy, Utah, USA) was inserted in the ear to ensure administration of sedative agents. Initial intravenous sedation was provided with a combination of propofol (2 – 4 $mg \cdot kg^{-1}$), and fentanyl (2 – 5 $\mu gr \cdot kg^{-1}$), and an endotracheal tube was placed while remaining in ventral position. Then, animals were positioned in supine and connected to mechanical ventilation (Dräger Fabius plus XL anaesthesia machine; Lübeck, Germany) in assist control mode, setting a tidal volume of 12 $ml \cdot Kg^{-1}$ and adjusting minute ventilation to maintain arterial PCO_2 at 36 – 42 mmHg. A side-stream capnometer (Dräger, Scio four plus gas measurements module; Lübeck, Germany) was connected to the expiratory branch from the ventilator circuit. Total intravenous anesthesia was provided with midazolam (3 – 5 $\mu gr \cdot Kg^{-1} \cdot min^{-1}$), fentanyl (0.03 – 0.05 $\mu gr \cdot Kg^{-1} \cdot min^{-1}$), and propofol (50 $\mu gr \cdot Kg^{-1} \cdot min^{-1}$), while muscular paralysis was ensured with vecuronium bromide (5 $\mu gr \cdot Kg^{-1} \cdot min^{-1}$) throughout the entire experiment.

Surgical preparation / monitoring installation. Neck vessels were accessed by surgical dissection, and catheters were inserted in the aorta through carotid artery (Bi-lumen central venous 7-Fr catheter; CV-17702. Arrow International, Reading, PA. USA) to monitor aortic arterial pressure and to enable blood sampling for gas analyses and lactate measurements. Moreover, left internal jugular vein was dissected to be used as a port for infusion of resuscitation fluids, while a three-lumen catheter (Three-lumen central venous 7-Fr catheter; CV-25703. Arrow International Reading, PA. USA) was inserted through external right jugular vein to continuously measure central venous pressure and for infusion of norepinephrine and endotoxin. A continuous-cardiac-output (CCO) pulmonary artery catheter (7.5-Fr, Edwards Swan-Ganz CCO; Baxter Edwards Critical Care. Irvine, CA. USA) was inserted through the right internal jugular vein to measure pulmonary arterial pressures,

pulmonary artery occlusion pressure and continuous cardiac output, and to withdraw mixed-venous blood samples. In addition, a thermistor-tipped catheter was inserted through right femoral artery and connected to a transpulmonary – thermodilution cardiac output monitoring system (PulsioFlex - PiCCO; PULSION Medical Systems AG; Münich, Germany) to obtain continuous measurements of cardiac output, stroke volume, pulse pressure and stroke volume variations, and estimations of extra-vascular lung water, end-diastolic global volume and pulmonary vascular permeability. Core temperature was continuously monitored using a thermistor at the tip of the femoral catheter (PulsioFlex - PiCCO; PULSION Medical Systems AG; Münich, Germany). External heating or cooling was used to maintain a central temperature of 36.5 ± 1.0 °C. Continuous electrocardiographic, pulsioximetry, and invasive pressures were recorded throughout the entire experiment (Drägger Infinity Vista XL; Drägger Medical System, Lübeck, Germany). Animals received intravenous lactate Ringer fixed infusion at 3 ml/kg during this surgical preparation phase. Unexpected loses in this period were compensated according to decision of the investigator team.

Afterwards, a midline laparotomy was performed, and abdominal dissection was completed up to expose the abdominal aorta in its supra celiac portion and the superior mesenteric artery. Immediately, ultrasound doppler flow probes (Transonic Systems Inc., Ithaca NY., USA) were placed around these two vessels (supra-celiac abdominal aorta and mesenteric arteries) and connected to ultrasound flowmeter modules (Transonic perivascular flow module TS420; Transonic Systems Inc., Ithaca NY., USA). Through a small ostomy in the antimesenteric jejunal wall, a small laser doppler (LDF) probe was carefully attached to the mucosa, while a second LDF probe was fixed to the jejunal serosa (OxyFlo Pro. Oxford Optronix, UK). Both ultrasound flowmeter and laser doppler signals were continuously recorded in a laptop (HP ProBook 440 G4. Hewlett Packard Development Company, LP; Palo Alto, CA. USA) by using a data acquisition system (PowerLab 4/35; Ad Instruments. Oxford, UK).

A double-lumen catheter (2-lumen central venous 4-Fr bi-lumen catheter; CS-14402. Arrow International. Morrisville, NC. USA) was inserted through the splenic vein up to the confluence with the superior mesenteric vein. Then, splenectomy was

performed after arterial local constriction with epinephrine. An infusion with dextrose 5% at 5 ml/h was provided through this catheter to ensure its permeability during the experiment. A surgical cystostomy was also created and an air-balloon catheter inserted and fixed to the bladder to quantify urinary output. A jejunum loop was exteriorized through the midline incision, and a small segment was opened along its antimesenteric border using electrocautery. After careful hemostasis, the abdominal contents were returned to the cavity, and the abdomen was partially closed, leaving out the jejunostomy loop, which was then covered with moistened compresses and an anti-adherent bag to avoid heat and fluid loss. Such a loop was used to evaluate microcirculatory blood flow at jejunal mucosa at the pre-established time-points. Cables from ultrasound flowmeters and laser doppler probes were exteriorized throughout the midline incision.

General monitoring. Arterial pressure was registered simultaneously at aortic arch and femoral artery during the entire experiment (Drägger Infinity Vista XL; Drägger Medical System, Lübeck, Germany). Cardiac output was measured by transpulmonary thermodilution (PulsioFlex - PiCCO; PULSION Medical Systems AG; Münich, Germany). Calibration of the system was performed each hour during the entire experiment by series of 10 mL boluses of normal saline 0.9% solution at 4 – 6 ºC injected through jugular central venous catheter. The average of three values of cardiac output obtained by such transpulmonary thermodilution was then recorded and calibrated with pulse contour (PulsioFlex - PiCCO; PULSION Medical Systems AG; Münich, Germany) to subsequently obtain continuous measures of cardiac output, stroke volume, and pulse pressure variations. Other transpulmonary thermodilution-derived variables recorded during the experiment included: systemic vascular resistance index (SVRI), global end-diastolic volume index (GEDVI), extravascular lung water index (EVLWI), and the pulmonary vascular permeability index (PVPI). Meanwhile, pulmonary artery catheter was used to monitor mean pulmonary artery, central venous, and pulmonary arterial occlusion pressures, which were measured at the end of expiration and referenced to the mid-chest level. Cardiac output was also continuously measured using the thermodilution principle with a thermal filament on the pulmonary artery catheter (Vigilance II; Edwards Lifesciences LLC. Irvine CA. USA). Pulmonary artery pressure

was continuously recorded and tightly guarded during endotoxin infusion and the rest of the experiment.

Calculation of CO_2 and O_2 variables. Simultaneous arterial, mesenteric, and mixed-venous blood samples were withdrawn to measure blood gases, hemoglobin, and lactate concentrations (GEM 5000 Premier, Instrumentation Laboratory. Bedford, MA. USA.) at each measurement time-point. Oxygen (O_2) and carbon dioxide (CO_2) parameters were calculated according to the following formulas:

- $CaO_2 = (Hg \times SaO_2 \times 1.34) + (PaO_2 \times 0.003)$
- $CvO_2 = (Hg \times SvO_2 \times 1.34) + (PvO_2 \times 0.003)$
- $Da\text{-}vO_2 = CaO_2 - CvO_2$
- $DO_2 = CaO_2 \times CO$
- $VO_2 = (CaO_2 - CvO_2) \times CO$
- $ERO_2 = (CaO_2 - CvO_2) / CaO_2$
- $mes\text{-}ERO_2 = (CaO_2 - CvmesO_2) / CaO_2$
- Mixed-venous-to-arterial CO_2 difference ($P\bar{v}\text{-}aCO_2$) = $PvCO_2 - PaCO_2$
- Mesenteric-venous-to-arterial CO_2 difference ($Pmes\text{-}aCO_2$) = $PmesCO_2 - PaCO_2$

Where CaO_2 and CvO_2 are arterial and venous O_2 contents; PaO_2 and PvO_2 represent arterial and venous partial oxygen pressures, respectively; DO_2 and VO_2 represent oxygen delivery and consumption, respectively; CO represents cardiac output; ERO_2 and $mes\text{-}ERO_2$ represent systemic and mesenteric oxygen extraction ratios, respectively; and $CmesCO_2$ and $CaCO_2$ represent mesenteric-venous and arterial CO_2 content.

Microcirculatory measurements. We used a Sidestream dark-field (SDF) imaging device (Micro Scan; MicroVision Medical, Amsterdam, the Netherlands) to explore microcirculation. This portable video-microscope device uses a stroboscopic green light (around 530 nm wavelength), which is delivered to the tissues by multiple light-emitting diodes (LEDs). This wavelength of light is absorbed by hemoglobin of

red blood cells, allowing their observation as dark cells flowing in the microcirculatory net while the light reflected by superficial layers does not reach the optics. As result of peripheral location of LEDs and the synchronization between light emission and camera frame rate, SDF provides a detailed visualization of open capillaries using a 5x objective and providing an on-screen magnification of x380.

Microcirculation at jejunal mucosa was evaluated by direct application of the SDF device through the surgical-prepared jejunostomy at five different points in an intestinal segment of at least fifteen centimeters, after careful removal of intestinal secretions by warm water and gentle aspiration. Light intensity and focus were manually adjusted until obtain the best quality in each case. Operator of SDF device was a well-trained researcher (G.O.T.) with expertise in acquiring images by SDF technique. At each time of measurements, we collected five sequences of video of 10 - 15 seconds each from different adjacent mucosa or serosa areas using a videocard (MicroVideo; Pinnacle system, Mountain Views, CA, U.S.A.). These sequences of video were stored under a random number and later analyzed by two investigators blinded to the origin of sequences (G.A.G.G. and N.O.). For the analysis, the number of villi in each image were counted and individual villi microcirculation was semi-quantitatively classified according to its predominant blood flow, as either: normal-perfused (continuous blood flow), hypoperfused (intermittent or sluggish blood flow) or non-perfused (stopped blood flow). We quantified the percentage of normal-perfused villi (villi-PPV) in each video-sequence at each time-point of measurement [E1].

The intra- and inter-observer variability for these methods have been studied in the past [E2]. For the current study, the intra and inter-observer variability were determined by two observers, analyzing five sequences per each twenty acquired (N.O. and G.A.G.G.). Coefficient of variability of the determination of one video sequence ranged from 3.8 to 6.0% (intra-observer) and from 3.5 to 6.5% (inter-observer) for the proportion of perfused vessels.

Experimental protocol. Experimental timeline is summarized in Figure 1. A stabilization period of at least 60 min was ensured after surgical preparation, catheter insertion and monitoring installation. Afterward, baseline measurements

(BL) were performed, and animals were randomly allocated to immediate norepinephrine support (i-NE) (n = 6), initial fluid loading (i-FL) (n = 6), or sham (n = 3) groups. I-NE and i-FL groups will be mentioned as experimental groups in some parts of the manuscript. Sequence of randomization was prepared by an independent laboratory staff member during the pre-experimental phase of the study. Such sequence was kept in sealed opaque envelopes, and these were opened only after acquirement of BL measurements. After random allocation, a lipopolysaccharide infusion (Escherichia coli O55:B5 purified by gel-filtration chromatography; Sigma-Aldrich; Saint Louis, MO. USA) was started at 0.5 $\mu gr \cdot kg^{-1} \cdot min^{-1}$ and progressively escalated until 6 $\mu gr \cdot kg^{-1} \cdot min^{-1}$ (over around 4 hours) and maintained for 30 minutes after fulfilling shock criteria (Figure 1). Time of shock (TS) was defined by the combined presence of mean arterial pressure (MAP) less than 60 mmHg for at least 15 mins and arterial lactate concentration ≥ 2.0 mmol/L. Pulmonary pressure was continuously monitored during lipopolysaccharide dose escalation to avoid severe pulmonary hypertension and right ventricle failure [E3]. Lipopolysaccharide infusion was decreased to the immediately preceding dose in the case of mean pulmonary artery pressure (PAPm) ≥ 40 mmHg, increase of central venous pressure (CVP) ≥ 5 mmHg regarding to its baseline value, or when increase of CVP exceed pulmonary occlusion pressure (PAOP) by ≥ 3 mmHg. In the case of sustained PAPm ≥ 40 mmHg or persistently CVP exceeding PAOP by ≥ 3 mmHg for ≥ 1 hour, or when developing sustained hypotension, experimental model should be stopped and discarded.

Resuscitation was then started at TS according to the random allocation: (a) animals assigned to i-FL received 30 $mL \cdot kg^{-1}$ of Ringer Lactate over 60 minutes followed by norepinephrine infusion if persisting MAP < 75 mmHg; (b) animals assigned to i-NE received immediate infusion of norepinephrine targeting a MAP ≥ 75 mmHg. Once MAP target was attained in both groups, successive mini-fluid boluses of 4 $mL \cdot kg^{-1}$ of Lactate Ringer were administered to increase cardiac preload according to fluid responsiveness (i.e., only when pulse pressure [PPV] and stroke volume variations [SVV] were ≥ 15%), targeting an arterial lactate < 2.0 $mmol \cdot L^{-1}$ and/or lactate decrease of at least 10% per 30 mins. Cases in which fluid responsiveness was not expected (i.e., PPV and SVV < 15%), mini-fluid loadings were not administered.

Death before completing initial resuscitation was the other reason to discard the model and to replace it by a new one. Two animals (one originally assigned to each experimental group) died before starting resuscitation: the first one developed profound shock secondary to severe right ventricular dysfunction while the second one developed sudden ventricular fibrillation. Both models were accordingly replaced following the original extended allocation sequence (which assumed up to 30% of potential model losses before to complete the protocol).

Sham animals were subjected to identical monitoring as experimental groups. Timing in sham group was referenced from data acquired during the pre-experimental standardization phase and equipoised from post-surgical stabilization period (Baseline-1, main manuscript) to the median time required to fulfill shock definition (Baseline-2) (Figure 1B, main manuscript). Euthanasia was performed at the end of the experiment according to the local regulations for animal research.

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Supplementary Tables and Figures

Table E1. Systemic and regional hemodynamics, oxygen variables, and lactate levels

	Baseline	Shock	T1	T2	T4	T6	Time*Group Effect p	Between Groups p
Systemic Hemodynamics								
HR, beats.min^{-1}								
i-FL	84 (8)	136 (22)	125 (15)	131 (14)	138 (10)	163 (13)		
i-NE	81 (7)	129 (20)	159 (13)	134 (13)	126 (9)	135 (12)	0.095	0.679
Sham	107 (10)	108 (28)	118 (19)	106 (19)	121 (13)	129 (17)		
MAP, mmHg								
i-FL	96 (7)	54 (4) [c]	78 (6)	86 (3)	86 (2)	87 (5)		
i-NE	91 (7)	54 (3) [b]	74 (5)	86 (6)	85 (2)	84 (4)	< .001	0.063
Sham	103 (10)	103 (5) [b, c]	96 (8)	89 (8)	75 (3)	79 (6)		
CVP, mmHg								
i-FL	7 (8)	8 (1)	10 (1)	7 (1)	10 (1)	10 (1)		
i-NE	9 (1)	8 (1)	8 (1)	9 (1)	9 (1)	10 (1)	0.505	0.396
Sham	11 (1)	11 (2)	10 (2)	11 (2)	11 (2)	11 (2)		
PAOP, mmHg			9 (1)					
i-FL	9 (1)	10 (1)	9 (1)	9 (1)	10 (1)	10 (1)		
i-NE	10 (1)	10 (1)	10 (1)	11 (1)	11 (1)	11 (1)	0.844	0.068
Sham	12 (1)	12 (2)	12 (1)	14 (1)	12 (1)	14 (1)		
Cardiac Output, mL·Kg^{-1}·min^{-1}								
i-FL	79.2 (5.0)	51.5 (6.4)	59.8 (6.7)	53.1 (7.3)	62.8 (11.4)	69.3 (10.5)		
i-NE	97.7 (7.5)	64.2 (7.2)	97.7 (5.6)	86.9 (8.2)	77.4 (12.8)	77.5 (11.7)	0.069	0.035

Sham	67.3 (6.4)	65.3 (8.3)	73.3 (8.7)	90.9 (9.5)	92.3 (14.7)	109.3 (13.5)		
PPV, %								
i-FL	16.8 (3.0)	20.6 (2.2)	16.6 (2.7)	20.6 (2.2)	16.8 (2.3)	17.4 (2.1)		
i-NE	15.5 (2.7)	22.5 (2.1)	23.3 (2.5)	16.0 (2.0)	16.0 (2.0)	15.8 (1.9)	0.266	0.639
Sham	19.7 (3.9)	17.7 (2.9)	16.0 (3.5)	12.3 (2.8)	14.3 (3.0)	15.0 (2.7)		
SVV, %								
i-FL	17.3 (3.4)	17.3 (1.1)	14.8 (2.2)	17.3 (3.0)	17.0 (1.7)	11.8 (2.2)		
i-NE	14.8 (2.8)	13.5 (0.9)	19.2 (1.8)	15.3 (2.4)	15.3 (1.4)	15.0 (1.8)	0.160	0.530
Sham	23.7 (3.9)	21.7 (1.3)	18.0 (2.5)	9.7 (3.4)	15.0 (1.9)	15.0 (2.5)		
GEDVI, $mL \cdot m^2$								
i-FL	648.4 (53.9)	488.4 (42.4) [c]	442.2 (42.9) [a, c]	445.6 (44.4) [a, c]	439.2 (42.6) [a, c]	411.8 (49.2) [a, c]		
i-NE	655.2 (49.3)	535.0 (38.8) [c]	569.8 (39.2) [a]	569.2 (40.6) [a]	592.3 (38.9) [a]	578.5 (44.9) [a]	0.015	0.076
Sham	633.7 (69.7)	630.0 (54.8) [b, c]	653.6 (62.8) [c]	580.7 (57.4) [c]	602.0 (55.0) [c]	602.0 (63.6) [c]		
ELWI, $mL \cdot Kg^{-1}$								
i-FL	14.5 (1.0)	15.3 (1.3)	16.3 (1.2)	16.7 (1.1)	19.9 (1.1) [a, c]	21.4 (1.4) [a, c]		
i-NE	15.5 (0.9)	15.5 (1.2)	16.0 (1.1)	15.5 (1.0)	16.3 (1.0) [a]	16.0 (1.2) [a]	0.004	0.365
Sham	16.3 (1.3)	16.0 (1.4)	16.5 (1.6)	14.3 (1.4)	15.7 (1.5) [c]	15.0 (1.7) [c]		
PVPI								
i-FL	2.7 (0.2)	3.1 (0.3)	3.3 (0.2)	3.3 (0.3)	4.2 (0.2) [a, c]	4.5 (0.3) [a, c]		
i-NE	2.4 (0.1)	3.0 (0.3)	3.1 (0.3)	2.9 (0.2)	2.8 (0.2) [a]	3.0 (0.3) [a]	0.021	0.085
Sham	2.5 (0.2)	2.3 (0.4)	2.7 (0.4)	2.9 (0.3)	2.9 (0.3) [c]	2.9 (0.4) [c]		
Regional / Splanchnic Flow								
Abdominal Aortic Flow, $mL \cdot min^{-1} \cdot Kg^{-1}$								
i-FL	45.4 (3.6)	33.5 (4.3)	40.5 (6.9)	33.4 (4.5)	40.6 (7.3)	43.4 (5.8)		
i-NE	52.5 (3.3)	32.3 (3.9)	51.3 (6.3)	47.4 (4.1)	43.3 (6.7)	47.3 (5.3)	0.219	0.129
Sham	44.5 (4.6)	45.5 (5.6)	52.0 (8.9)	62.4 (5.8)	56.5 (9.5)	62.7 (7.5)		
Mesenteric Flow, $mL \cdot min^{-1} \cdot Kg^{-1}$								
i-FL	11.1 (0.9)	5.8 (0.6) [c]	9.5 (1.1)	8.9 (0.8)	11.0 (1.0) [a]	11.4 (1.1) [a]		
i-NE	11.5 (0.8)	6.3 (0.5) [b]	8.0 (1.0)	9.6 (0.7)	13.2 (0.9) [a, b]	14.2 (1.0) [a, b]	0.011	0.225
Sham	8.0 (1.2)	8.1 (0.8) [b, c]	8.9 (1.4)	8.6 (1.0)	9.0 (1.3) [b]	9.4 (1.4) [b]		

Δ % Aortic Flow, %								
i-FL	100.0 (0.0)	74.2 (7.9)	89.2 (12.5)	73.5 (8.4)	88.0 (13.7)	95.4 (12.1)		
i-NE	100.0 (0.0)	61.7 (7.2)	99.0 (11.4)	91.3 (7.6)	83.9 (12.5)	91.7 (11.1)	0.178	0.019
Sham	100.0 (0.0)	102 (1.0)	114.1 (16.1)	139.3 (10.8)	123.9 (17.7)	140.2 (15.7)		
Δ % Mesenteric Flow, %	100 .0(0.0)	53.5 (5.7) [c]	90.3 (13.2) [c]	82.5 (6.9) [c]	101.8 (10.6)	103.4 (10.7) [a]		
i-FL	100.0 (0.0)	56.0 (5.2) [b]	71.6 (12.1) [b]	84.6 (6.3) [b]	116.6 (9.7)	125.8 (9.8) [a]	0.023	0.256
i-NE	100.0 (0.0)	101.0 (7.4) [b, c]	109.0 (17.2) [b, c]	103.7 (9.0) [b, c]	109.2 (13.7)	116.7 (13.8)		
Sham								
Mesenteric to Aortic Flow ratio, %								
i-FL	24.5 (2.0)	18.5 (2.0)	23.9 (2.0) [a]	27.9 (2.9) [a]	29.6 (3.4) [c]	27.0 (2.4) [c]		
i-NE	22.2 (1.8)	19.7 (1.8)	16.1 (1.8) [a]	21.4 (2.6) [a]	32.5 (3.1) [b]	31.1 (2.2) [b]	0.008	0.003
Sham	18.6 (2.6)	17.7 (2.6)	13.7 (2.6)	12.8 (3.7)	15.2 (4.4) [b, c]	15.2 (3.1) [b, c]		
O_2 and CO_2 variables / Lactate levels								
Systemic DO_2, $mL \cdot Kg^{-1} \cdot min^{-1}$								
i-FL	13.1 (1.3)	9.1 (1.2)	9.4 (1.0) [a, c]	8.8 (1.2) [a, c]	9.8 (1.4) [c]	11.1 (1.2) [c]		
i-NE	15.4 (1.4)	10.8 (1.3)	15.5 (1.1) [a]	13.5 (1.3) [a]	11.4 (1.5)	11.1 (1.3) [b]	0.045	0.039
Sham	11.5 (1.7)	11.7 (1.5)	12.3 (1.3) [c]	13.8 (1.5) [c]	13.2 (1.8) [c]	15.7 (1.5) [b, c]		
Systemic VO_2, $mL \cdot Kg^{-1} \cdot min^{-1}$								
i-FL	5.1 (0.5)	5.7 (0.7)	4.8 (0.7)	5.5 (0.5)	5.5 (0.5)	4.9 (0.5)		
i-NE	5.2 (0.6)	6.1 (0.8)	5.3 (0.8)	5.7 (0.6)	4.7 (0.6)	4.7 (0.6)	0.829	0.958
Sham	5.0 (0.7)	5.0 (0.9)	4.9 (0.9)	5.3 (0.7)	5.0 (0.6)	5.5 (0.7)		
Systemic ERO_2								
i-FL	0.39 (0.03)	0.63 (0.4) [c]	0.51 (0.07) [a, c]	0.63 (0.06) [a, c]	0.58 (0.04) [a, c]	0.45 (0.03) [c]		
i-NE	0.36 (0.03)	0.54 (0.03) [b]	0.34 (0.06) [a,	0.41 (0.06) [a]	0.48 (0.04) [a, b]	0.46 (0.03) [b]	0.044	0.009
Sham	0.42 (0.04)	0.40 (0.05) [b, c]	0.40 (0.09) [c]	0.38 (0.08) [c]	0.38 (0.05) [b, c]	0.35 (0.04) [b, c]		
Splanchnic DO_2, $mL \cdot min^{-1} \cdot 100gr^{-1}$								
i-FL	6.1 (0.7)	3.5 (0.3) [c]	5.0 (0.6)	5.0 (0.6)	5.7 (0.5) [a, c]	6.2 (0.6) [a, c]		

i-NE	5.9 (0.7)	3.4 (0.3) [b]	4.1 (0.5)	4.9 (0.5)	7.0 (0.4) [a, b]	7.7 (0.5) [a, b]	< .001	0.555
Sham	4.8 (0.9)	4.7 (0.4) [b, c]	5.1 (0.7)	4.4 (0.7)	4.4 (0.6) [b, c]	4.7 (0.8) [b, c]		
Splanchnic VO_2, $mL \cdot min^{-1} \cdot 100gr^{-1}$								
i-FL	2.0 (0.4)	2.3 (0.2) [c]	2.2 (0.4)	2.3 (0.3)	2.7 (0.3) [c]	2.6 (0.3) [c]		
i-NE	2.3 (0.3)	2.0 (0.2) [b]	1.7 (0.3)	2.3 (0.3)	2.6 (0.2) [b]	2.7 (0.2) [b]	0.015	0.448
Sham	2.4 (0.5)	1.3 (0.3) [b, c]	2.6 (0.5)	1.6 (0.4)	1.8 (0.3) [b, c]	1.5 (0.3) [b, c]		
Splanchnic ERO_2								
i-FL	0.31 (0.06)	0.66 (0.04) [c]	0.45 (0.08)	0.48 (0.06)	0.47 (0.04)	0.41 (0.04) [c]		
i-NE	0.39 (0.06)	0.60 (0.04) [b]	0.46 (0.07)	0.49 (0.06)	0.39 (0.04)	0.36 (0.04)	0.006	0.212
Sham	0.27 (0.08)	0.26 (0.06) [b, c]	0.52 (0.10)	0.36 (0.08)	0.42 (0.05)	0.31 (0.05) [c]		
Pvmes-aCO_2, mmHg								
i-FL	6.4 (1.7)	21.6 (2.9) [c]	16.0 (2.0) [c]	16.0 (2.0) [a, c]	16.6 (0.7) [a, c]	15.8 (1.1) [a, c]		
i-NE	9.7 (1.6)	22.8 (2.6) [b]	9.5 (1.8)	11.0 (1.0) [a, b]	8.2 (0.6) [a]	7.0 (1.0) [a]	0.001	< .001
Sham	11.2 (2.2)	11.3 (2.6) [b, c]	11.3 (2.6) [c]	7.3 (1.4) [b, c]	8.7 (0.9) [c]	6.7 (1.4) [c]		
Pvmes-aCO_2 : Da-$vmesO_2$	1.22 (0.23)	1.83 (0.25) [c]	2.72 (0.39) [c]	2.15 (0.25) [a, c]	2.29 (0.18) [a, c]	2.55 (0.35) [a, c]		
i-FL	1.53 (0.20)	2.31 (0.23) [b]	1.27 (0.36)	1.58 (0.23) [a]	1.47 (0.16) [a]	1.38 (0.32) [a]	0.012	0.020
i-NE	1.30 (0.29)	1.37 (0.32) [b, c]	1.31 (0.51) [c]	1.22 (0.32) [c]	1.44 (0.23) [c]	1.55 (0.46) [c]		
Sham								
Cvmes-aCO_2 : Da-$vmesO_2$	0.44 (0.13)	1.73 (0.22) [c]	1.83 (0.57)	1.40 (0.34)	1.70 (0.19) [a, c]	1.33 (0.21) [a, c]		
i-FL	0.56 (0.12)	2.17 (0.20) [c]	0.52 (0.51)	0.89 (0.31)	0.77 (0.17) [a]	0.66 (0.19) [a]	0.013	0.085
i-NE	0.43 (0.17)	0.44 (0.29) [b, c]	0.75 (0.74)	0.66 (0.44)	0.77 (0.24) [c]	0.69 (0.27) [c]		
Sham								
Lactate Arterial, $mmol \cdot L^{-1}$	1.6 (0.2)	4.4 (0.9)	4.3 (0.8)	3.3 (0.7)	2.1 (0.2)	1.9 (0.2)		
i-FL	1.7 (0.2)	3.8 (0.8)	3.4 (0.7)	4.2 (0.7)	2.2 (0.2)	1.9 (0.2)	0.186	0.084
i-NE	1.9 (0.2)	1.9 (1.1)	1.6 (1.0)	1.3 (0.9)	1.1 (0.3)	1.0 (0.2)		
Sham								
Lactate mixed-venous, $mmol \cdot L^{-1}$								
i-FL	1.6 (0.3)	4.5 (1.0)	4.6 (0.7)	3.5 (0.8)	2.5 (0.3)	2.4 (0.3)		
i-NE	1.7 (0.2)	4.2 (0.9)	3.7 (0.7)	4.4 (0.7)	2.5 (0.3)	2.3 (0.3)	0.222	0.084
Sham	2.3 (0.3)	1.5 (1.0)	1.5 (1.0)	1.6 (1.0)	0.9 (0.4)	1.2 (0.4)		

Lactate mesenteric-venous, mmol·L^{-1}								
i-FL	2.2 (0.4)	5.2 (0.7) [c]	5.0 (0.7) [c]	3.8 (0.6) [c]	3.0 (0.2) [a, c]	2.6 (0.2) [a, c]		
i-NE	2.1 (0.3)	4.6 (0.6) [b]	3.7 (0.6) [c]	4.3 (0.6) [b]	2.2 (0.2) [a, b]	1.7 (0.1) [a, b]	0.012	0.177
Sham	2.3 (0.5)	2.4 (0.9) [b, c]	1.9 (0.9) [b, c]	1.9 (0.8) [b, c]	1.5 (0.2) [b, c]	1.2 (0.2) [b, c]		
Resuscitation Fluids / Norepinephrine dose								
Norepinephrine, µgr·Kg·min^{-1}								
i-FL	0 (0.0)	0 (0.0)	0.28 (0.14)	0.20 (0.11)	0.29 (0.09) [a]	0.38 (0.08) [a]	0.001	0.063
i-NE	0 (0.0)	0 (0.0)	0.64 (0.13)	0.38 (0.10)	0.10 (0.08) [a]	0.12 (0.07) [a]		
Sham	-	-	-	-	-	-		
Resuscitation Fluids, mL								
i-FL	0.0 (0.0)	0.0 (0.0)	983.3 (19.1) [a]	269.3 (81.4)	160.0 (37.0)	0 (0.0)	< .001	0.002
i-NE	0.0 (0.0)	0.0 (0.0)	452.7 (19.1) [a]	299.7 (81.4)	135.0 (37.0)	48.3 (21.6)		
Sham	-	-	-	-	-	-		
Resuscitation Fluids, mL·Kg^{-1}								
i-FL	0.0 (0.0)	0.0 (0.0)	29.3 (0.6)	8.0 (2.3)	4.8 (1.3)	0.0 (0.6)	< .001	< .001
i-NE	0.0 (0.0)	0.0 (0.0)	13.8 (0.6)	8.9 (2.3)	3.4 (1.3)	1.4 (0.6)		
Sham	-	-	-	-	-	-		

Values are expressed as means (± standard deviation - SD); i-NE denotes immediate norepinephrine group, *n*=6 animals; i-FL denotes initial fluid resuscitation, n=6 animals; and sham, denotes sham group, n=3 animals. T2, T4 and T6: 2, 4 and 6 hours after starting resuscitation.

Abbreviations: HR: heart rate; MAP: mean arterial pressure; CVP: central venous pressure; PAOP: pulmonary artery occlusion pressure; PPV: pulse pressure variation; SVV; stroke volume variation; GEDVI; global end-diastolic volume index; ELWI: extra-vascular lung water index; PVPI: pulmonary vascular permeability index; Δ: delta; DO_2: oxygen delivery; VO_2: oxygen consumption; ERO_2: oxygen extraction ratio

Pairwise comparisons with Bonferroni correction for the effects of treatment at specific times: [a] i-NE vs. i-FL, $p < 0.05$; [b] i-NE vs. Sham, $p < 0.05$; [c] i-FL vs. Sham, $p < 0.05$

Table E2. Arterial, mixed-venous and mesenteric-venous blood gases

	Baseline	Shock	T1	T2	T4	T6	Time*Group Effect p	Between Groups p
Arterial blood gases								
pH								
i-FL	7.51 (0.02)	7.43 (0.03)	7.41 (0.03)	7.43 (0.03) [a]	7.39 (0.02)	7.39 (0.02)		
i-NE	7.47 (0.02)	7.40 (0.03)	7.33 (0.03)	7.30 (0.03) [a, b]	7.34 (0.02) [b]	7.34 (0.02) [b]	0.028	0.037
Sham	7.45 (0.02)	7.45 (0.03)	7.46 (0.04)	7.45 (0.04) [b]	7.45 (0.03) [b]	7.45 (0.03) [b]		
pCO_2								
i-FL	39.6 (2.6)	35.2 (2.5)	38.4 (2.3)	36.4 (2.8)	39.8 (2.8)	40.4 (3.2)		
i-NE	39.5 (2.3)	40.8 (2.3)	43.8 (2.1)	44.0 (2.6)	42.7 (2.5)	43.7 (2.9)	0.437	0.323
Sham	38.3 (3.3)	38.3 (3.2)	37.3 (3.0)	38.3 (3.6)	37.7 (3.6)	37.6 (4.1)		
pO_2								
i-FL	185.6 (14.2)	141.8 (14.6)	133.6 (15.3)	116.6 (18.4)	110.2 (13.4)	104.8 (9.9)		
i-NE	190.8 (12.9)	160.0 (13.4)	125.2 (14.0)	134.5 (16.8)	130.7 (12.2)	127.3 (9.1)	0.142	0.758
Sham	144.3 (18.3)	144.3 (18.9)	142.7 (19.8)	138.0 (23.8)	130.0 (17.3)	143.0 (12.8)		
BE (arterial)								
i-FL	8.4 (1.8)	-0.8 (1.6)	-0.6 (1.3)	-0.2 (1.1)	-0.9 (0.8)	-0.9 (0.9)		
i-NE	5.1 (1.6)	0.3 (1.5)	-2.6 (1.2)	-5.1 (1.0) [a]	-2.9 (0.7) [a, b]	-2.7 (0.8) [b]	0.004	0.093
Sham	2.7 (2.3)	2.7 (2.1)	2.5 (1.7)	3.0 (1.4) [a]	2.2 (1.1) [b]	2.2 (1.2) [b]		
SO_2								
i-FL	100.0 (0.0)	99.4 (0.4)	99.9 (0.6)	98.6 (1.3)	98.8 (0.5)	98.5 (0.5)		
i-NE	100.0 (0.0)	99.8 (0.3)	98.1 (0.6)	97.7 (1.2)	98.9 (0.5)	98.8 (0.5)	0.365	0.356
Sham	100.0 (0.0)	100.0 (0.5)	100.0 (0.8)	100.0 (1.6)	100.0 (0.8)	100.0 (0.6)		
Mixed-venous blood gases								
pH								

i-FL	7.44 (0.03)	7.34 (0.04)	7.32 (0.03)	7.33 (0.03)	7.31 (0.02)	7.31 (0.03)		
i-NE	7.41 (0.02)	7.33 (0.03)	7.29 (0.03)	7.24 (0.03) [b]	7.27 (0.02) [b]	7.28 (0.02) [b]	0.047	0.079
Sham	7.39 (0.03)	7.39 (0.05)	7.40 (0.04)	7.40 (0.04) [b]	7.40 (0.03) [b]	7.40 (0.03) [b]		
$PvmixCO_2$								
i-FL	45.2 (3.6)	51.0 (3.5)	50.0 (3.1)	50.4 (2.5)	52.2 (3.4)	52.0 (3.2)		
i-NE	48.0 (3.2)	53.3 (3.2)	50.3 (2.8)	52.8 (2.3)	51.2 (3.1)	51.2 (3.1)	0.672	0.518
Sham	47.7 (4.6)	47.7 (4.6)	47.0 (4.0)	46.3 (3.3)	45.3 (4.4)	44.7 (4.1)		
$PvmixO_2$								
i-FL	45.4 (2.3)	37.4 (2.4)	44.4 (3.6)	38.4 (3.1)	40.4 (1.7)	45.2 (2.0)		
i-NE	44.0 (2.1)	42.3 (2.1)	51.0 (3.3)	47.8 (2.9)	43.0 (1.5)	43.8 (1.8)	0.150	0.262
Sham	40.0 (2.9)	40.0 (3.0)	39.7 (4.6)	42.3 (4.0)	42.0 (2.2)	45.0 (2.6)		
BE (vmix)								
i-FL	6.9 (1.4)	0.2 (1.4)	-0.5 (1.1)	0.8 (1.2)	-0.3 (0.9)	-1.1 (0.6)		
i-NE	4.8 (1.3)	-0.2 (1.3)	-2.3 (1.0)	-4.6 (1.1)	-2.5 (0.8)	-2.4 (0.6)	0.029	0.014
Sham	3.9 (1.8)	3.5 (1.8)	3.7 (1.5)	3.0 (1.6)	2.9 (1.1)	2.9 (0.8)		
$SvmixO_2$								
i-FL	61.8 (2.8)	37.0 (3.9)	49.3 (6.5)	36.3 (5.9)	41.6 (4.1)	54.3 (3.4)		
i-NE	65.5 (2.6)	46.8 (3.5)	64.9 (5.9)	58.4 (5.4)	52.3 (3.8)	53.9 (3.1)	0.065	0.007
Sham	58.4 (3.6)	58.4 (5.0)	60.7 (8.3)	62.7 (7.6)	62.5 (5.3)	66.1 (4.4)		
Mesenteric-venous blood gases								
pH								
i-FL	7.44 (0.02)	7.30 (0.03)	7.31 (0.04)	7.34 (0.03)	7.32 (0.02)	7.32 (0.03)		
i-NE	7.39 (0.02)	7.31 (0.03)	7.28 (0.03)	7.25 (0.03) [b]	7.29 (0.02) [b]	7.30 (0.02)	0.014	0.148
Sham	7.37 (0.02)	7.38 (0.04)	7.38 (0.05)	7.38 (0.04) [b]	7.39 (0.03) [b]	7.39 (0.03)		
$PvmesCO_2$								
i-FL	46.0 (3.8)	56.8 (2.5)	54.4 (3.7)	52.0 (2.9)	56.4 (2.8)	54.4 (2.8)		
i-NE	49.2 (3.4)	59.2 (2.3)	53.3 (3.3)	55.0 (2.7)	50.8 (2.5)	50.7 (2.6)	0.135	0.326
Sham	49.7 (4.9)	49.7 (3.1)	48.7 (3.8)	45.7 (3.8)	46.3 (3.6)	44.3 (3.7)		
$PvmesO_2$								
i-FL	50.4 (2.4)	38.4 (2.6)	47.4 (3.4)	44.6 (2.6)	44.2 (2.6)	45.0 (2.7)		
i-NE	45.7 (2.4)	40.7 (2.4)	44.7 (3.1)	42.3 (2.3)	48.3 (2.4)	48.0 (2.4)	0.123	0.816
Sham	40.3 (2.4)	40.2 (3.4)	39.3 (4.4)	46.0 (3.3)	44.0 (3.4)	50.3 (3.4)		
$SvmesO_2$								

i-FL	70.2 (6.4)	35.8 (6.7)	55.1 (8.1)	52.2 (6.4)	52.6 (4.1)	58.0 (4.0)		
i-NE	61.7 (5.9)	48.7 (6.0)	53.8 (7.4)	50.7 (5.9)	61.0 (3.8)	63.7 (3.7)	0.077	0.864
Sham	50.2 (8.3)	50.2 (8.6)	48.9 (10.5)	65.0 (8.3)	58.9 (5.3)	70.2 (5.1)		
BE (vmes)								
i-FL	7.0 (1.6)	0.4 (1.4)	-0.2 (1.4)	0.5 (1.0) [a]	-0.2 (0.8)	-0.5 (0.7) [c]		
i-NE	4.8 (1.5)	0.9 (1.3)	-2.1 (1.3) [b]	-3.4 (1.0) [a, b]	-2.2 (0.7) [b]	-1.9 (0.7) [b]	0.068	0.049
Sham	4.5 (2.1)	4.5 (2.1)	4.5 (1.8) [b]	1.7 (1.4) [b]	2.8 (1.0) [b]	2.1 (0.9) [b, c]		

Values are expressed as means (± standard deviation - SD); i-NE denotes immediate norepinephrine group, *n*=6 animals; i-FL denotes initial fluid resuscitation, n=6 animals; and sham, denotes sham group, n=3 animals. T2, T4 and T6: 2, 4 and 6 hours after starting resuscitation.

Abbreviations: pCO_2: arterial carbon dioxide partial pressure; pO_2: arterial oxygen partial pressure; BE (arterial): arterial base excess; SO_2: arterial oxygen saturation; $PvmixCO_2$: mixed-venous carbon dioxide partial pressure; $PvmixO_2$: mixed-venous oxygen partial pressure; BE (vmix): mixed-venous base excess; $SvmixO_2$: mixed-venous oxygen saturation; $PvmesCO_2$: mesenteric-venous carbon dioxide partial pressure; $PvmesO_2$: mesenteric-venous oxygen partial pressure; BE (vmes): mesenteric-venous base excess; $SvmesO_2$: mesenteric-venous oxygen saturation.

Pairwise comparisons with Bonferroni correction for the effects of treatment at specific times: [a] i-NE vs. i-FL, $p < 0.05$; [b] i-NE vs. Sham, $p < 0.05$; [c] i-FL vs. Sham, $p < 0.05$

Table E3. Microcirculatory blood flow parameters

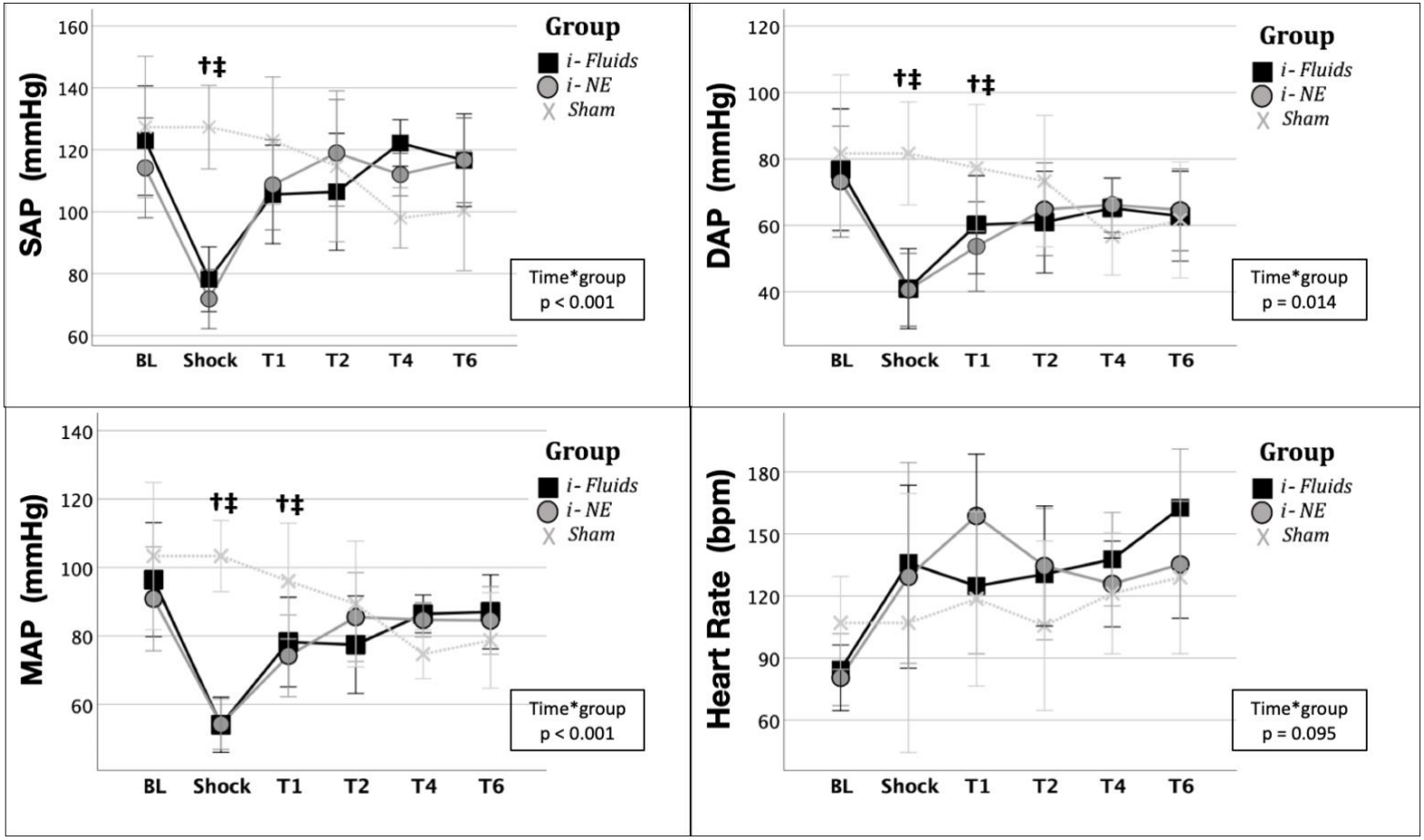
	Baseline	Shock	T1	T2	T4	T6	Time*Group Effect p	Between Groups p
Laser Mucosa, BPU								
i-FL	379.4 (98.3)	151.4 (55.3)	268.8 (73.1)	154.1 (88.3)	247.4 (120.5) [a]	121.4 (128.6) [a]		
i-NE	274.8 (89.6)	163.5 (50.4)	275.3 (66.8)	328.8 (80.6)	522.5 (109.9) [a]	623.8 (117.4) [a]	0.012	0.301
Sham	242.7 (126.8)	242.7 (126.8)	250.3 (94.4)	265.7 (114.0)	276.0 (155.5)	240.7 (166.0)		
Δ % Laser Mucosa, %								
i-FL	100 (0.0)	57.0 (23.1)	108.4 (25.5)	59.6 (19.1)	89.3 (30.5) [a]	43.9 (50.1) [a]		
i-NE	100 (0.0)	70.3 (21.0)	98.6 (23.3)	119.1 (17.4)	189.4 (27.8) [a]	244.2 (45.7) [a]	0.032	0.077
Sham	100 (0.0)	100 (0.0)	103.2 (33.0)	109.9 (24.6)	115.1 (39.3)	99.8 (64.6)		
Laser Serosa, BPU								
i-FL	1,147.4 (373.9)	409.4 (148.8) [c]	611.0 (193.9)	607.8 (396.0)	336.0 (384.3) [a]	230.4 (364.3) [a]		
i-NE	2,136.2 (341.3)	528.0 (135.9) [b]	660.7 (177.0)	1,280.7 (361.5)	1,492.2 (350.5) [a]	1,629.2 (332.6) [a]	0.145	0.092
Sham	903.7 (482.7)	903.7 (482.7) [b, c]	884.3 (250.3)	1.007.7 (511.2)	1,097.7 (495.7)	922.0 (470.4)		
Δ % Laser Serosa, %								
i-FL	100 (0.0)	40.5 (11.7) [c]	70.4 (17.8)	70.1 (32.3)	29.2 (13.7) [a, c]	23.2 (11.4) [a, c]		
i-NE	100 (0.0)	40.8 (10.7) [b]	45.2 (16.3) [b]	88.0 (29.5)	113.8 (12.5) [a]	119.0 (10.5) [a]	0.017	0.040
Sham	100 (0.0)	100 (0.0) [b, c]	102.3 (23.0) [b]	122.8 (41.7)	122.8 (17.7) [c]	105.2 (14.8) [c]		
Jejunal Villi – PPV, %	90.8 (3.6)	5.9 (10.0) [c]		25.0 (12.8) [a, c]		61.6 (10.3) [a, c]		
i-FL	89.3 (3.3)	31.3 (9.1) [b]		83.1 (11.7) [a]		96.8 (9.4) [a]	< .001	0.002
i-NE	96.5 (4.7)	96.5 (12.9) [b, c]		98.0 (16.5) [c]		100.0 (13.3) [c]		
Sham								
Jejunal Villi – MFI, %	2.9 (0.1)	0.3 (0.6) [c]		1.2 (1.1) [a, c]		2.2 (1.0) [a, c]		
i-FL	2.9 (0.1)	1.0 (0.6) [b]		2.8 (0.3) [a]		3.0 (0.0) [a]	< .001	0.002
i-NE	2.9 (0.1)	2.9 (0.1) [b, c]		3.0 (0.1) [c]		3.0 (0.0) [c]		
Sham								

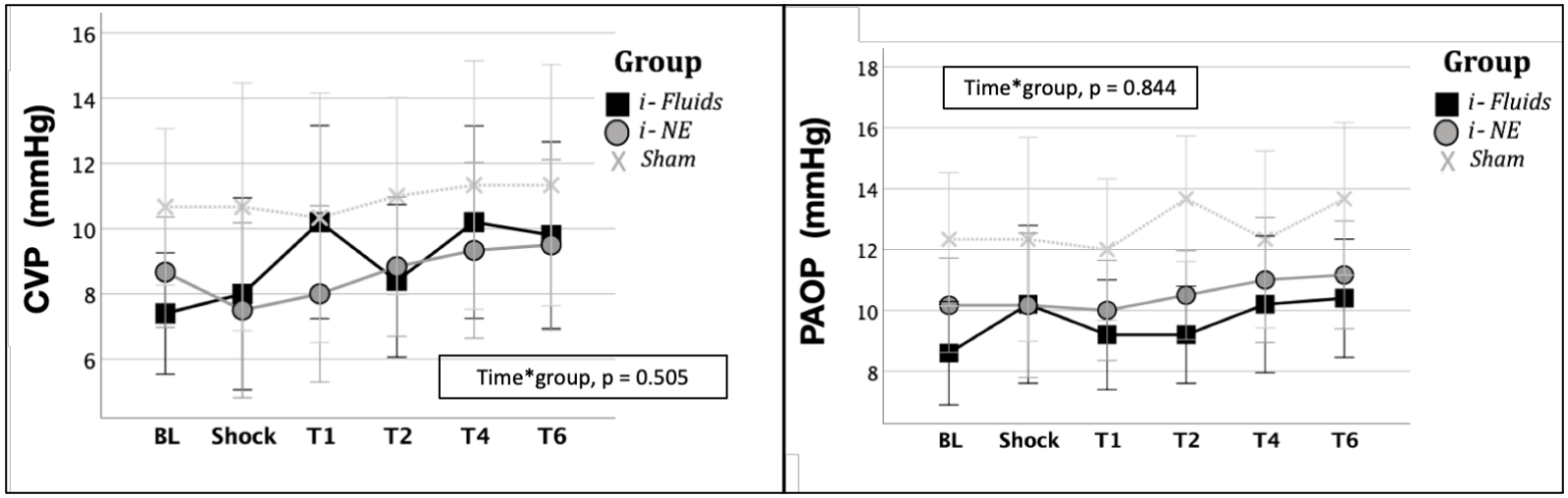
Values are expressed as means (standard deviation - SD); i-NE denotes immediate norepinephrine group, *n*=6 animals; i-FL denotes initial fluid resuscitation, n=6 animals; and sham, denotes sham group, n=3 animals. T2, T4 and T6: 2, 4 and 6 hours after starting resuscitation.

Abbreviations: BPU: blood perfusion units; Δ: delta; DO_2: oxygen delivery; Jejunal villi – PPV: proportion of jejunal-villi with well-perfused vessels; Jejunal villi – MFI: microvascular flow index at jejunal villi

Pairwise comparisons with Bonferroni correction for the effects of treatment at specific times: [a] i-NE vs. i-FL, $p < 0.05$; [b] i-NE vs. Sham, $p < 0.05$; [c] i-FL vs. Sham, $p < 0.05$

Figure E1. Time-course of general hemodynamics



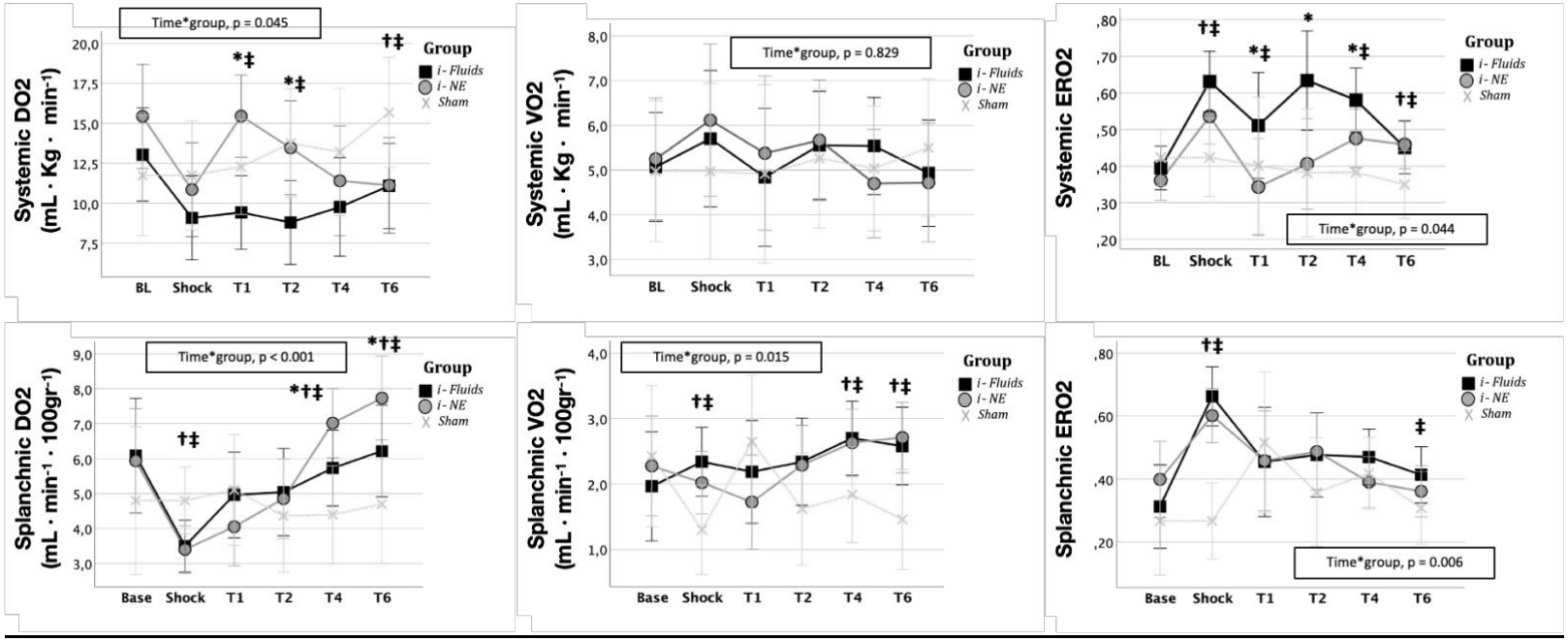


Time course of general hemodynamics. i-NE denotes immediate norepinephrine group, *n*=6 animals; i-FL denotes initial fluid resuscitation, n=6 animals; and sham, denotes sham group, n=3 animals. BL: base-line measurements; Shock: time of shock; T1, T2, T4 and T6: measurements 1, 2, 4 and 6 hours after shock.

Time*group interactions by repeated measures ANOVA. Pairwise comparisons with Bonferroni correction for the effects of treatment at specific times:
* i-NE vs. i-FL, $p < 0.05$; † i-NE vs. Sham, $p < 0.05$; ‡ i-FL vs. Sham, $p < 0.05$

Abbreviations: SAP: systolic arterial pressure; DAP: diastolic arterial pressure; MAP: mean arterial pressure; CVP: central venous pressure; PAOP: pulmonary artery occlusion pressure

Figure E2. Time-course of systemic and splanchnic oxygen variables

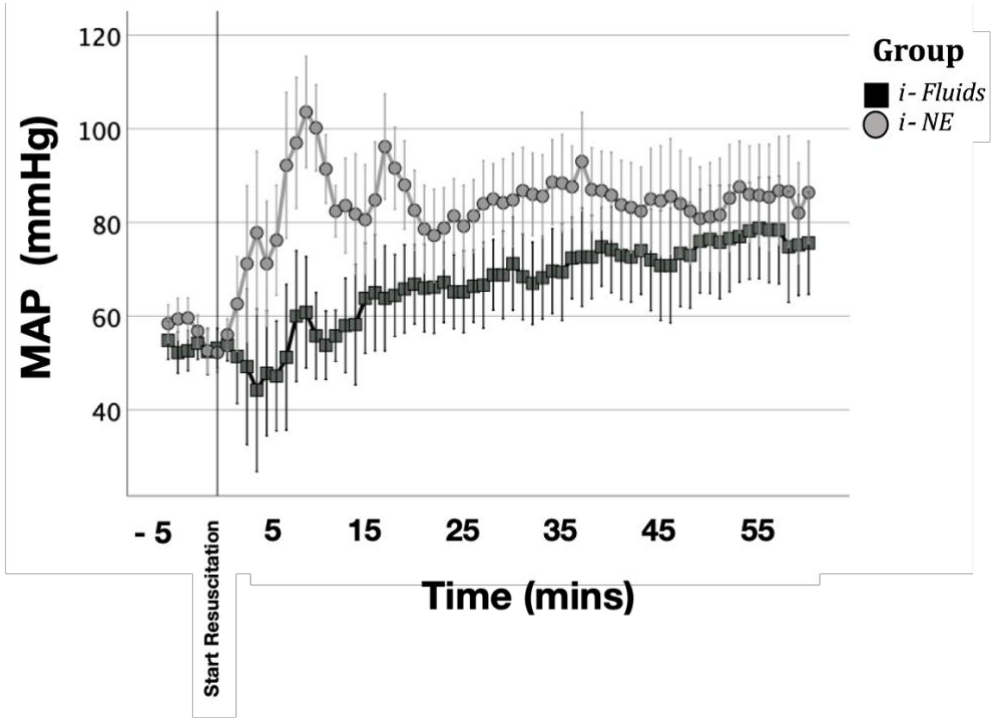


Time course of systemic and splanchnic oxygen variables. i-NE denotes immediate norepinephrine group, *n*=6 animals; i-FL denotes initial fluid resuscitation, n=6 animals; and sham, denotes sham group, n=3 animals. BL: base-line measurements; Shock: time of shock; T1, T2, T4 and T6: measurements 1, 2, 4 and 6 hours after shock.

Time*group interactions by repeated measures ANOVA. Pairwise comparisons with Bonferroni correction for the effects of treatment at specific times:
* i-NE vs. i-FL, $p < 0.05$; † i-NE vs. Sham, $p < 0.05$; ‡ i-FL vs. Sham, $p < 0.05$

Abbreviations: DO_2: oxygen delivery; VO_2: oxygen transport; ERO_2: oxygen extraction ratio

Figure E3. Time course of mean arterial pressure during the first hour of resuscitation

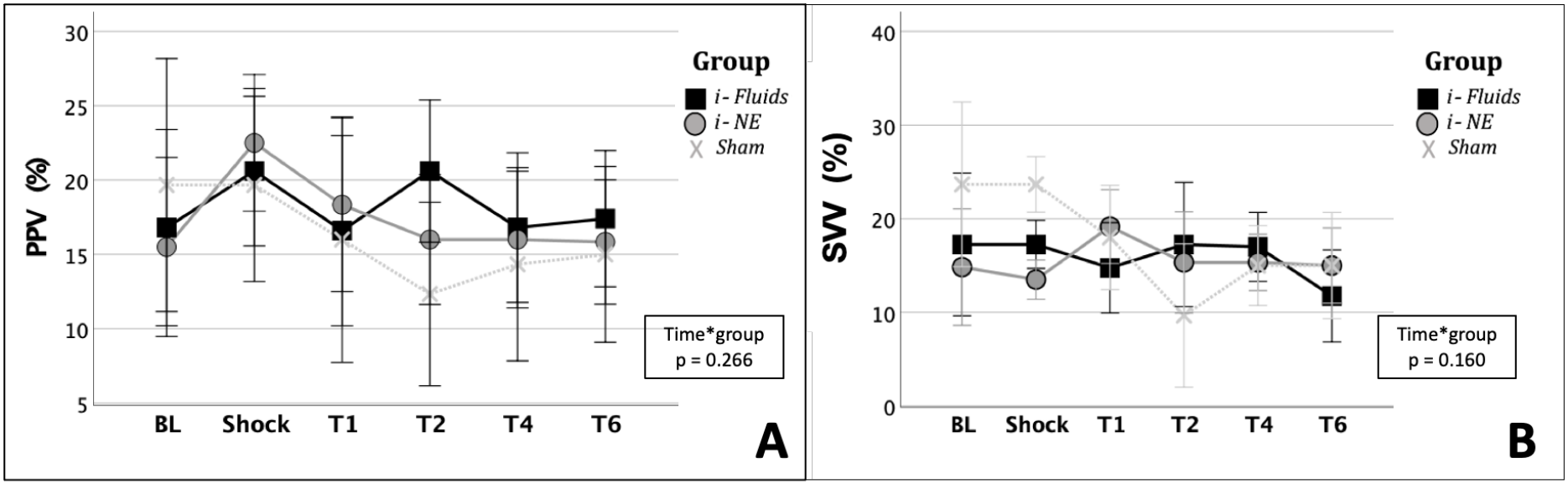


Time course of mean arterial pressure during the first hour of resuscitation. i-NE denotes immediate norepinephrine group, *n*=6 animals; i-FL denotes initial fluid resuscitation, n=6 animals. Abscise represents time in minutes from 5 mins before starting resuscitation and min-to-min during the next 60 minutes (first hour of resuscitation)

Time*group interactions by repeated measures ANOVA, $p < 0.05$

Abbreviations: MAP: mean arterial pressure

Figure E4. Time-course of pulse pressure and stroke volume variations

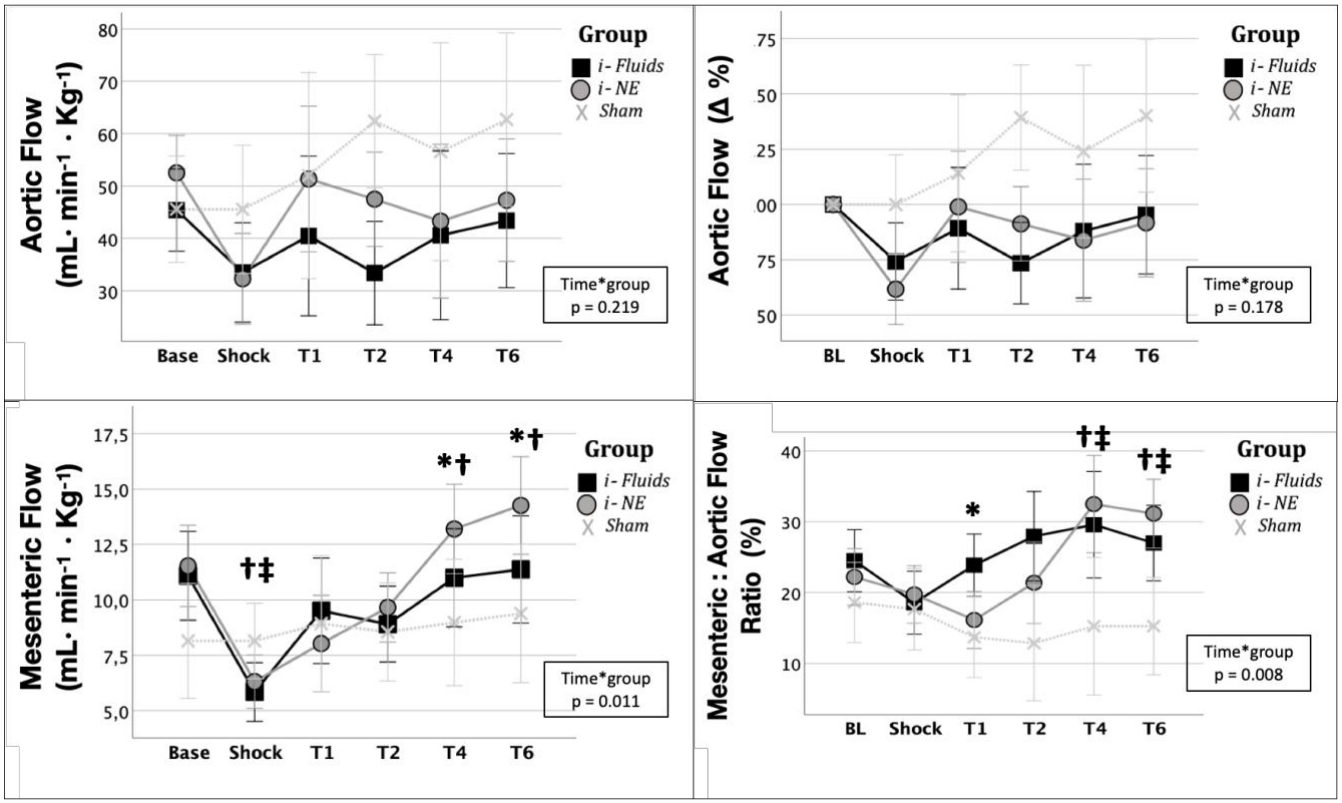


Time course of pulse pressure and stroke volume variations. i-NE denotes immediate norepinephrine group, *n*=6 animals; i-FL denotes initial fluid resuscitation, n=6 animals; and sham, denotes sham group, n=3 animals. BL: base-line measurements; Shock: time of shock; T1, T2, T4 and T6: measurements 1, 2, 4 and 6 hours after shock.

Time*group interactions by repeated measures ANOVA. Pairwise comparisons with Bonferroni correction for the effects of treatment at specific times:
* i-NE vs. i-FL, $p < 0.05$; † i-NE vs. Sham, $p < 0.05$; ‡ i-FL vs. Sham, $p < 0.05$

Abbreviations: PPV: pulse pressure variation; SVV: stroke volume variation

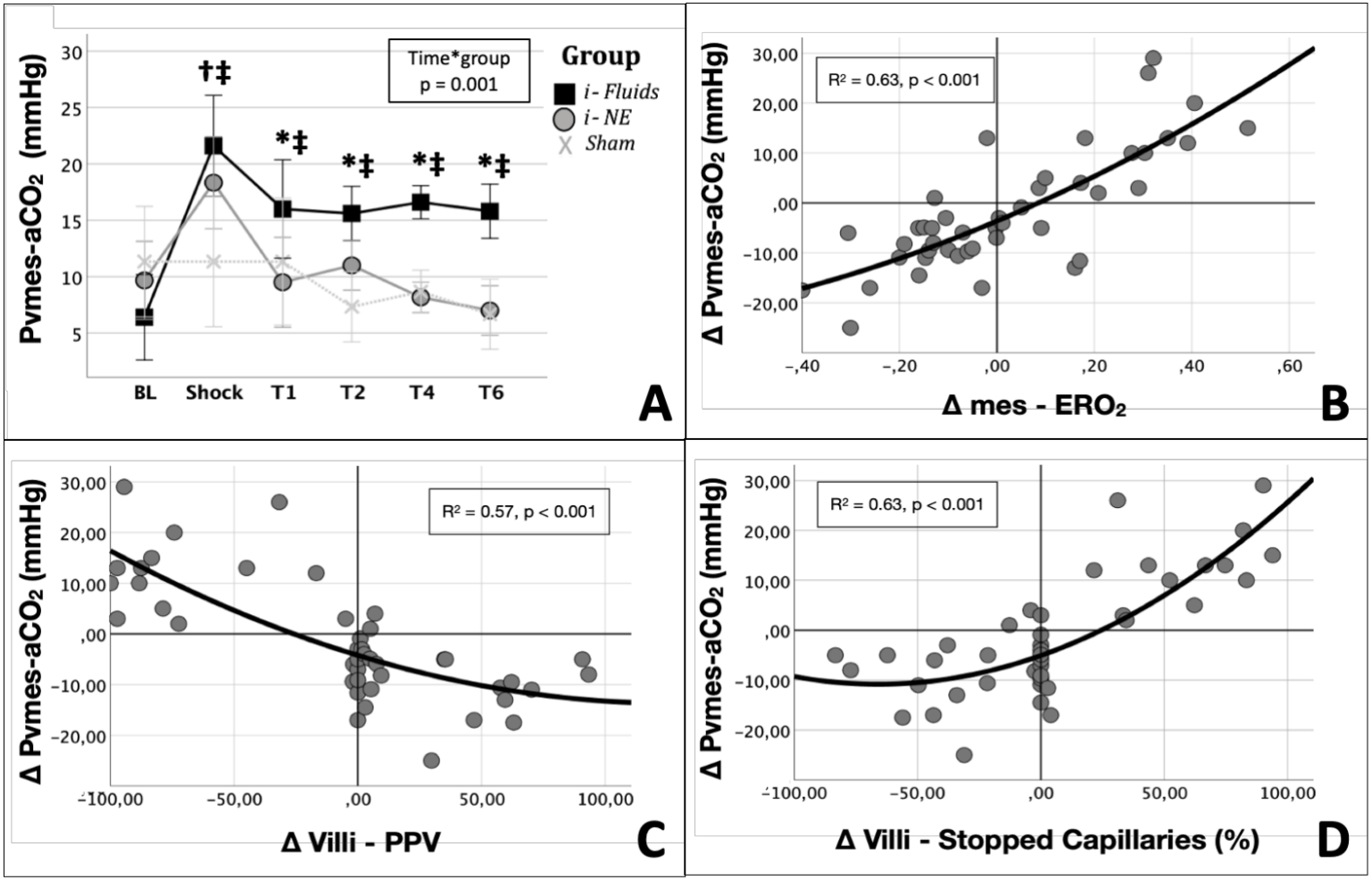
Figure E5. Time-course of abdominal aortic, mesenteric, and mesenteric to aortic flow ratio



Time course of abdominal aortic flow and superior mesenteric artery flows. Measurements are showed in mL·min^{-1}·Kg^{-1} and the % variation regarding to baseline values. i-NE denotes immediate norepinephrine group, *n*=6 animals; i-FL denotes initial fluid resuscitation, n=6 animals; and sham, denotes sham group, n=3 animals. BL: base-line measurements; Shock: time of shock; T1, T2, T4 and T6: measurements 1, 2, 4 and 6 hours after shock.

Time*group interactions by repeated measures ANOVA. Pairwise comparisons with Bonferroni correction for the effects of treatment at specific times:
* i-NE vs. i-FL, $p < 0.05$; † i-NE vs. Sham, $p < 0.05$; ‡ i-FL vs. Sham, $p < 0.05$
Abbreviations: Δ: delta

Figure E6. Mesenteric-to-arterial CO_2 differences, mesenteric ERO_2 and jejunal villi microvascular flow



Time course of venous-to-arterial carbon dioxide differences (Pvmes-aCO_2) (Panel A). Relationships between variations in Pvmes-aCO_2 and mesenteric oxygen extraction ratio (ERO_2) (Panel B); relationships between variations in Pvmes-aCO_2 and proportion of small vessels with continuous flow at jejunal villi (Panel C) and between Pvmes-aCO_2 and proportion of capillaries with stopped flow at jejunal villi (Panel D).

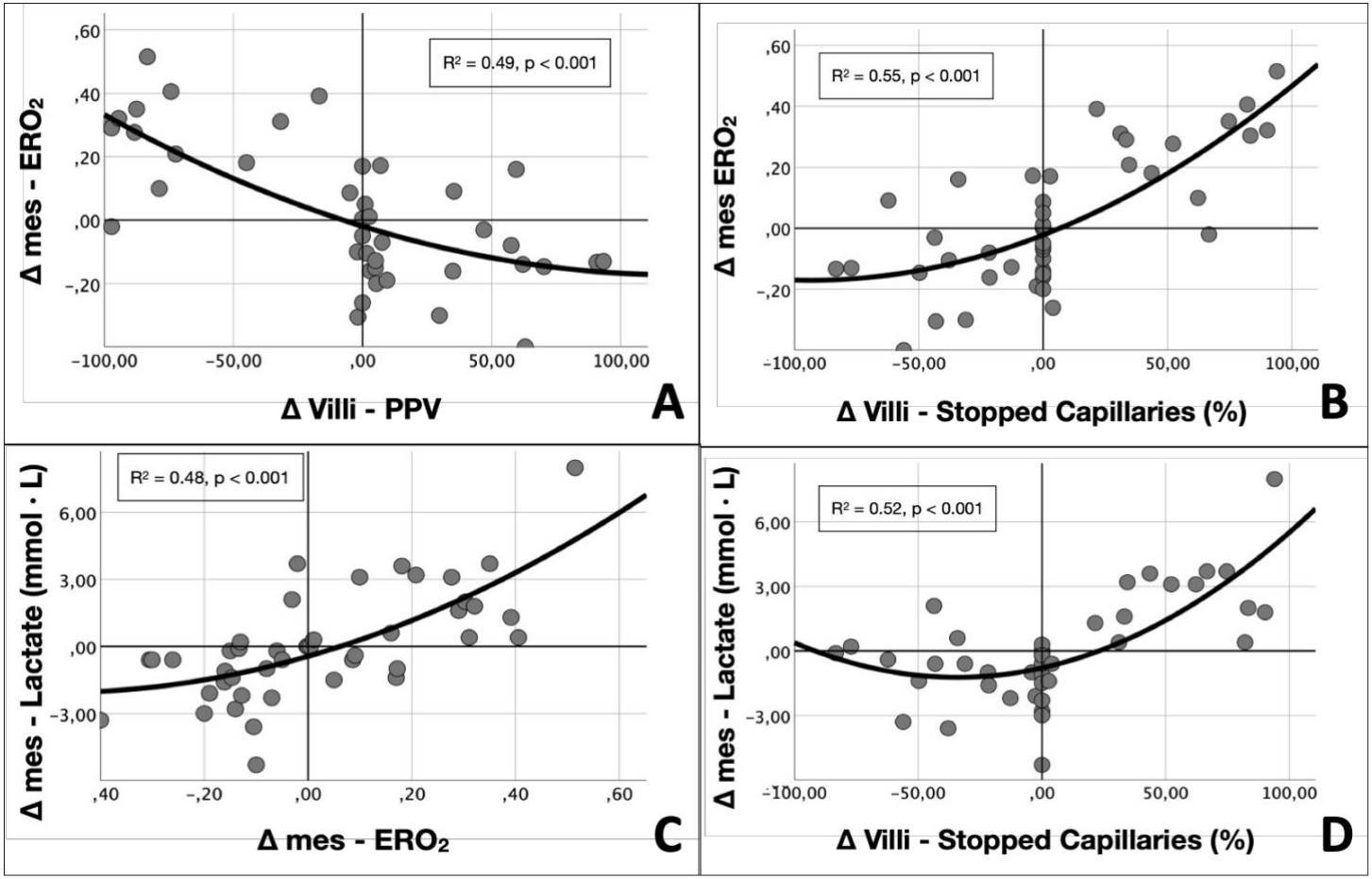
i-NE denotes immediate norepinephrine group, *n*=6 animals; i-FL denotes initial fluid resuscitation, n=6 animals; and sham, denotes sham group, n=3 animals. BL: base-line measurements; Shock: time of shock; T1, T2, T4 and T6: measurements 1, 2, 4 and 6 hours after shock.

Time*group interactions by repeated measures ANOVA. Pairwise comparisons with Bonferroni correction for the effects of treatment at specific times:
* i-NE vs. i-FL, $p < 0.05$; † i-NE vs. Sham, $p < 0.05$; ‡ i-FL vs. Sham, $p < 0.05$

Variation of each parameter was calculated as the simple delta between actual and the immediately precedent value.

Abbreviations: Δ: delta; villi-PPV: percentage of small vessels with normal flow (i.e., continuous flow) at jejunal villi; Δ villi – stopped capillaries: variation in the proportion of capillaries with stopped flow at jejunal villi

Figure E7. Relationships among microcirculatory blood flow, mesenteric oxygen extraction ratio (ERO_2) and mesenteric lactate levels

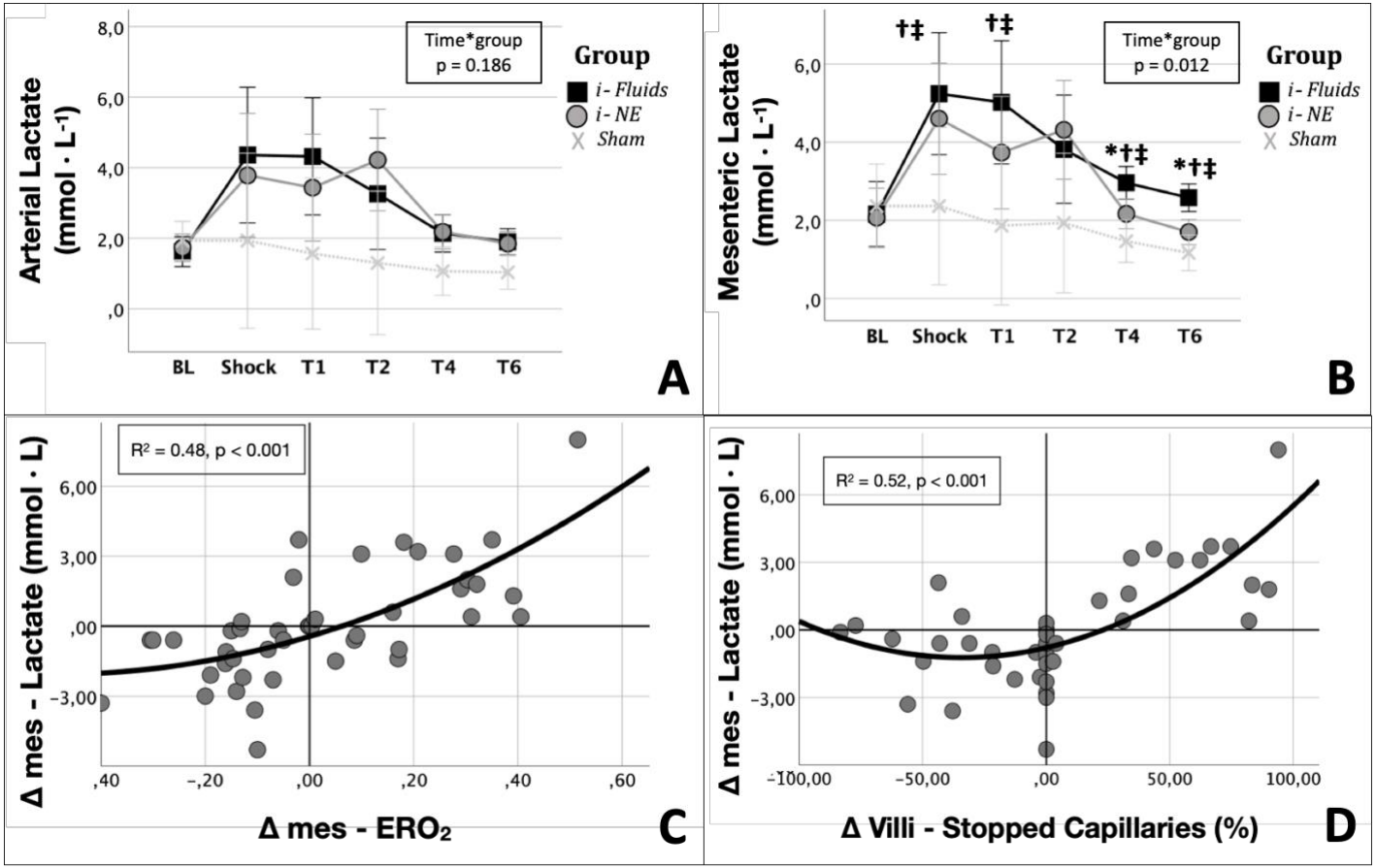


Relationships between microvascular blood flow and mesenteric oxygen extraction ratio (ERO_2) (Panels A and B). Relationships between ERO_2 and mesenteric lactate levels (Panel C) and these in turn, with the proportion of capillaries with stopped flow at jejunal villi (Panel D).
i-NE denotes immediate norepinephrine group, *n*=6 animals; i-FL denotes initial fluid resuscitation, n=6 animals; and sham, denotes sham group, n=3 animals. BL: base-line measurements; Shock: time of shock; T1, T2, T4 and T6: measurements 1, 2, 4 and 6 hours after shock.

Variation of each parameter was calculated as the simple delta between actual and the immediately precedent value.

Abbreviations: ERO_2: oxygen extraction ratio; Δ mes-lactate: variation of mesenteric lactate levels

Figure E8. Arterial and mesenteric lactate levels and their relationship with ERO_2 and microcirculatory blood flow



Time course of arterial lactate (Panel A) and mesenteric lactate levels (Panel B); relationships between mesenteric oxygen extraction ratios (mes-ERO_2) and mesenteric lactate levels (Panel C) and between those last and the proportion of capillaries with stopped flow at jejunal villi (Panel D).

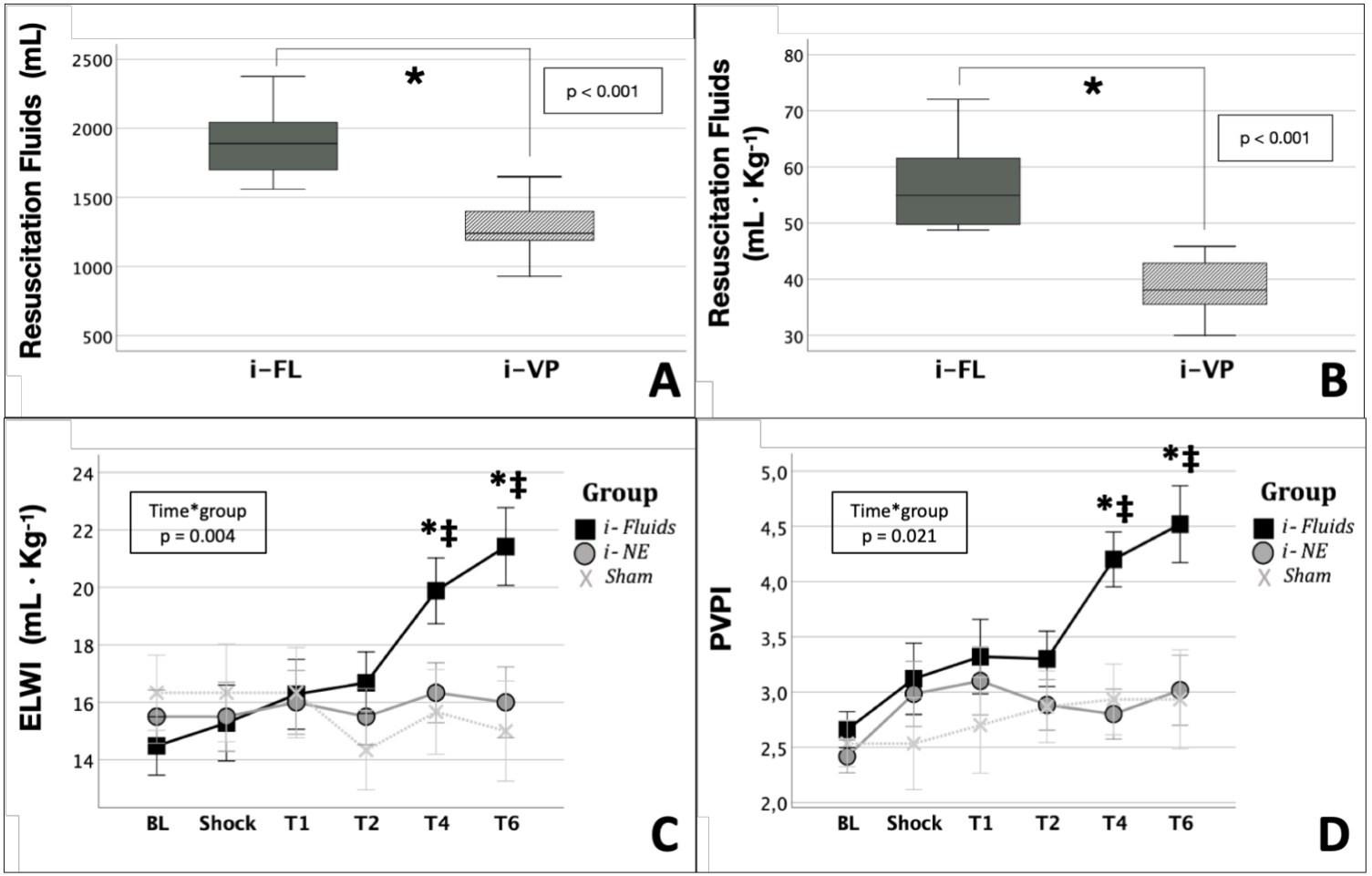
i-NE denotes immediate norepinephrine group, *n*=6 animals; i-FL denotes initial fluid resuscitation, n=6 animals; and sham, denotes sham group, n=3 animals. BL: base-line measurements; Shock: time of shock; T1, T2, T4 and T6: measurements 1, 2, 4 and 6 hours after shock.

Time*group interactions by repeated measures ANOVA. Pairwise comparisons with Bonferroni correction for the effects of treatment at specific times:
* i-NE vs. i-FL, $p < 0.05$; † i-NE vs. Sham, $p < 0.05$; ‡ i-FL vs. Sham, $p < 0.05$

Variation of each parameter was calculated as the simple delta between actual and the immediately precedent value.

Abbreviations: Δ mes-lactate: variations in mesenteric lactate levels; Δ villi – stopped capillaries: variation in the proportion of capillaries with stopped flow at jejunal villi

Figure E9. Total volume of resuscitation fluids, extra-vascular lung water and pulmonary permeability



Total volume of resuscitation fluids in mL (Panel A) and mL·Kg^{-1} (Panel B). Time course of extra-vascular lung water (ELWI) (Panel C) and pulmonary permeability index (PVPI) (Panel D) as assessed by transpulmonary thermodilution.
i-NE denotes immediate norepinephrine group, *n*=6 animals; i-FL denotes initial fluid resuscitation, n=6 animals; and sham, denotes sham group, n=3 animals. BL: base-line measurements; Shock: time of shock; T1, T2, T4 and T6: measurements 1, 2, 4 and 6 hours after shock.

Time*group interactions by repeated measures ANOVA. Pairwise comparisons with Bonferroni correction for the effects of treatment at specific times:
* i-NE vs. i-FL, $p < 0.05$; † i-NE vs. Sham, $p < 0.05$; ‡ i-FL vs. Sham, $p < 0.05$

Abbreviations: ELWI: extra-vascular lung water index; PVPI: pulmonary permeability index

NOTE: Please save this file locally before filling in the table, DO NOT work on the file within your internet browser as changes will not be saved. Adobe Acrobat Reader (available free here) is recommended for completion.

The ARRIVE guidelines 2.0: author checklist

The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

Item		Recommendation	Section/line number, or reason for not reporting
Study design	1	For each experiment, provide brief details of study design including: a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated. b. The experimental unit (e.g. a single animal, litter, or cage of animals).	
Sample size	2	a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used. b. Explain how the sample size was decided. Provide details of any *a priori* sample size calculation, if done.	
Inclusion and exclusion criteria	3	a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established *a priori.* If no criteria were set, state this explicitly. b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so. c. For each analysis, report the exact value of *n* in each experimental group.	
Randomisation	4	a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence. b. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	
Blinding	5	Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	
Outcome measures	6	a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	
Statistical methods	7	a. Provide details of the statistical methods used for each analysis, including software used. b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	
Experimental animals	8	a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	
Experimental procedures	9	For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: a. What was done, how it was done and what was used. b. When and how often. c. Where (including detail of any acclimatisation periods). d. Why (provide rationale for procedures).	
Results	10	For each experiment conducted, including independent replications, report: a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). b. If applicable, the effect size with a confidence interval.	