Research Article

Innate Immunity

Innate Immunity
18(2) 217–230

DOI: 10.1177/1753425911398279
ini.sagepub.com
SAGE

Effects of catecholamines on hepatic and skeletal muscle mitochondrial respiration after prolonged exposure to faecal peritonitis in pigs

Madhusudanarao Vuda, Lukas Brander, Ralph Schröder, Stephan M Jakob, Jukka Takala and Siamak Djafarzadeh

Abstract

Use of norepinephrine to increase blood pressure in septic animals has been associated with increased efficiency of hepatic mitochondrial respiration. The aim of this study was to evaluate whether the same effect could be reproduced in isolated hepatic mitochondria after prolonged *in vivo* exposure to faecal peritonitis. Eighteen pigs were randomized to 27 h of faecal peritonitis and to a control condition (n = 9 each group). At the end, hepatic mitochondria were isolated and incubated for one hour with either norepinephrine or placebo, with and without pretreatment with the specific receptor antagonists prazosin and yohimbine. Mitochondrial state 3 and state 4 respiration were measured for respiratory chain complexes I and II, and state 3 for complex IV using high-resolution respirometry, and respiratory control ratios were calculated. Additionally, skeletal muscle mitochondrial respiration was evaluated after incubation with norepinephrine and dobutamine with and without the respective antagonists (atenolol, propranolol and phentolamine for dobutamine). Faecal peritonitis was characterized by decreasing blood pressure and stroke volume, and maintained systemic oxygen consumption. Neither faecal peritonitis nor any of the drugs or drug combinations had measurable effects on hepatic or skeletal muscle mitochondrial respiration. Norepinephrine did not improve the efficiency of complex I- and complex II-dependent isolated hepatic mitochondrial respiration [respiratory control ratio (RCR) complex I: 5.6 ± 5.3 (placebo) vs. 5.4 ± 4.6 (norepinephrine) in controls and 2.7 ± 2.1 (placebo) vs. 2.9 ± 1.5 (norepinephrine) in septic animals; RCR complex II: 3.5 ± 2.0 (placebo) vs. 3.5 ± 1.8 (norepinephrine) in controls; 2.3 ± 1.6 (placebo) vs. 2.2 ± 1.1 (norepinephrine) in septic animals]. Prolonged faecal peritonitis did not affect either hepatic or skeletal muscle mitochondrial respiration. Subsequent incubation of isolated mitochondria with norepinephrine and dobutamine did not significantly influence their respiration.

Keywords

Dobutamine, faecal peritonitis, haemodynamics, liver, muscle, norepinephrine, respiration

Date received: 23 August 2010; revised: 16 December 2010; accepted: 22 December 2010

Introduction

The contribution of mitochondrial dysfunction to sepsis-related morbidity is controversial. While the presence of mitochondrial dysfunction has been demonstrated in skeletal muscle biopsies collected from septic patients within 24 h of admission to intensive care,[1] and in skeletal muscle and liver specimens in rats in a long-term, fluid-resuscitated, faecal peritonitis model,[2] others found normal skeletal muscle and hepatic mitochondrial complex functions in rat endotoxaemia and faecal peritonitis models lasting 16–24 h.[3,4] Even more controversially, hepatic, but not skeletal, muscle mitochondrial dysfunction has been reported in a 24 h porcine endotoxaemia model.[5]

While some of the controversies may be species-related, type and severity of sepsis model, and specific

Department of Intensive Care Medicine, Inselspital, Bern University Hospital and University of Bern, Bern, Switzerland

Corresponding author:
Siamak Djafarzadeh, Department of Intensive Care Medicine, Bern University Hospital, Inselspital, CH-3010 Bern, Switzerland.
Email: siamak.djafarzadeh@insel.ch

mitochondrial functions assessed, concomitant treatment may also have an influence.[6–10] For example, mitochondrial alterations have been found in muscle biopsies of patients on statin therapy;[7] the volatile anaesthetics halothane, isoflurane and sevoflurane have been shown to inhibit complex I of cardiac mitochondria;[8] the sedative drug propofol seems to impair mitochondrial respiration in isolated perfused guinea pig hearts;[9] and levosimendan, a relatively new inotropic and vasodilatory drug, has been associated with decreased mitochondrial membrane potential in rat liver mitochondria.[10]

Catecholamines such as norepinephrine, dopamine and dobutamine are commonly used to support haemodynamics in patients with sepsis. Whilst norepinephrine increases cardiac output and oxygen delivery during sepsis,[11] this drug also has a direct effect on isolated hepatocytes by activating alpha-adrenergic receptors, increasing cytosol and intra-mitochondrial calcium levels, and stimulating dehydrogenases of the citrate cycle.[12,13] In endotoxaemic pigs, hepatic mitochondrial respiration was more efficient when norepinephrine was infused to increase blood pressure as compared to endotoxaemic animals without norepinephrine.[14] While this occurred in the presence of similar hepatic oxygen delivery and consumption rates in the two groups, an effect of norepinephrine-induced improvement in oxygen availability at the microcirculatory level could not be ruled out.[14]

The aim of the present study was to evaluate whether norepinephrine has a direct effect on isolated porcine hepatic mitochondria previously exposed to prolonged faecal peritonitis. We hypothesized that norepinephrine improves mitochondrial respiration under these circumstances. To address whether such effects are specific for hepatic mitochondria or the drug, we also measured skeletal muscle mitochondrial function under the same conditions and after incubation with dobutamine.

Materials and methods

The study was performed in accordance with the National Institutes of Health guidelines for the care and use of experimental animals and with the approval of the Animal Care Committee of the Canton of Bern, Switzerland.

Norepinephrine, dobutamine, yohimbine, prazosin, atenolol, propranolol and phentolamine were purchased from Sigma (Sigma Chemical Co., St Louis, MO, USA). All other chemicals used were of analytical grade.

Animal preparation

Eighteen domestic pigs weighing 41 (38–44) kg (median [range]) were fasted overnight and intramuscularly premedicated with ketamine hydrochloride 100 mg (Narketan, Vetoquinol AG, Ittigen, Switzerland), azaperone 200 mg (Stresnil, Biokema, Crissier-Lausanne, Switzerland) and atropine 0.5 mg (Atropin Sintetica S.A., Mendresino, Switzerland) before induction of anaesthesia and orotracheal intubation. All animals were ventilated (Servo i^{TM}, Maquet Critical Care, Solna, Sweden) with a time-cycled, volume-targeted mode using a tidal volume of 6–8 ml/kg, a fraction of inspired oxygen of 0.4 and a positive end-expiratory pressure of 5 cm H_2O. Anaesthesia was maintained throughout the experiment with continuous intravenous infusions of ketamine hydrochloride (200–300 mg/h; Ketalar, Pfizer AG, Zurich, Switzerland), midazolam (20–30 mg/h; Roche Pharma AG, Reinach, Switzerland), and fentanyl (25–75 mcg/h; Janssen-Cilac, Baar, Switzerland). Neuromuscular blocking agents were not used. The animals were equally randomized into a non-septic control group and a faecal peritonitis group ($n = 9$ each group). In the peritonitis group, 1 g/kg of autologous faeces dissolved in 200 ml glucose 5% (glucose 5%, Sintetica-Bioren S.A., Couvet, Switzerland) was instilled into the abdominal cavity. Twenty-seven hours after induction of peritonitis, 14–16 g of the left liver lobe was excised before sacrificing the animals with an overdose of potassium chloride. Samples for the study of muscle mitochondria were taken in the last 14 experiments only (control, $n = 7$; faecal peritonitis, $n = 7$). Skeletal muscle samples (8–10 g) were taken at baseline and at the end of the experiment from the sternocleidomastoid muscle in 14 animals and the mitochondria were immediately isolated in order to test the respiratory activity.

Haemodynamic monitoring and fluid management

Mean arterial blood pressure (MAP), cardiac stroke volume, and mixed venous oxygen saturation (SvO_2) (Vigilance II, Edwards Lifesciences, Irvine, CA, USA) were monitored continuously. Global oxygen consumption (VO_2) was calculated using standard formulas.

A combination of Ringer's lactated and glucose 50% solutions was administered continuously throughout the experiment at a combined total rate of 5 ml/kg h^{-1}. Glucose infusion was adjusted to maintain blood glucose levels between 3.5 and 6 mmol/l.

Additional fluid boluses and vasoactive drugs were administered according to a haemodynamic protocol slightly modified from one described previously.[15–18] Briefly, a bolus of 50-100 ml Ringer's lactated solution was administered when MAP decreased <50 mmHg. The procedure was repeated maximally twice per hour if MAP remained

<50 mmHg and if the first bolus had resulted in an increase in stroke volume. If stroke volume did not increase, a continuous norepinephrine (Noradrenaline, Sintetica S.A., Mendrisio, Switzerland) infusion was started and increased to a maximum dose of 600 mcg/h.

Measurement of creatine kinase activity and arterial lactate concentrations

Blood samples for the measurement of lactate were taken at baseline and at the end of the experiment from the carotid artery (ABL 835 Radiometer®, Copenhagen, Denmark), and creatine kinase was measured from arterial samples at the end of the experiment (Modular P800, Roche Diagnostics, Mannheim, Germany).

Liver mitochondrial isolation

Isolation of liver mitochondria was performed immediately after tissue harvesting at 4°C using a standard procedure based on differential centrifugation.[19] The samples of liver (15 g) excised at the end of the experiment were rapidly immersed in ice-cold liver isolation buffer (mannitol 220 mmol/l, sucrose 70 mmol/l, morpholinopropane sulphonic acid 5 mmol/l, pH 7.4), minced with scissors and homogenized with an additional 10 ml/g of homogenization media (liver isolation buffer plus EDTA 2 mmol/l) in a Potter Elvehjem homogenizer with a loose-fitting Teflon® pestle (four strokes) (Schütt Homgen Plus; Schütt Labortechnik GmbH, Göttingen, Germany). The homogenate was then centrifuged for 10 min at 700 *g*. The supernatant was collected and centrifuged again for 10 min at 7000 *g*. The supernatant was discarded at this time; the pellet was then resuspended in isolation buffer and centrifuged twice for 10 min at 7000 *g* for further purification of the mitochondria. The pellets were then suspended in buffer at a final concentration of 50–100 mg mitochondrial protein per millilitre.

Skeletal muscle mitochondrial isolation

Skeletal muscle mitochondria were isolated as described by Hoppel et al.[20] The muscle specimens were rapidly immersed in ice-cold isolation buffer (KCl 100 mmol/l, $MgSO_4$ 10 mmol/l, morpholinopropane sulphonic acid 50 mmol/l, ethylenedinitrilotetraacetic acid 1.0 mmol/l, ATP 1.1 mmol/l, pH 7.4), transported to the laboratory and weighed. After several rinses with isolation buffer, the skeletal muscle was minced using scissors and was suspended in 10 volumes (wt/vol) of the same medium and treated with a protease (Sigma-Aldrich, St. Louis, MO, USA) 5 mg/g for 10 min at 4°C with constant stirring. The suspension was diluted with an equal volume of isolation medium supplemented to 0.2% (wt/vol) with defatted bovine serum albumin (BSA) and homogenized in a Potter Elvehjem homogenizer with a loose-fitting Teflon pestle (10 strokes). The supernatant was separated by centrifugation (10 min at 10000 *g*) and the pellet was resuspended in BSA-supplemented isolation medium (10 ml/g tissue). The suspension was centrifuged for 10 min at 350 *g*, the supernatant was filtered through two layers of gauze and the mitochondria were sedimented at 7700 *g* for 10 min. The mitochondria were subjected to two additional washes using 5 ml BSA-supplemented muscle isolation buffer/g tissue and 2.5 ml of 100 mmol/l KCl, 50 mmol/l morpholinopropane sulphonic acid, and 0.5 mmol/l ethylene glycol tetraacetic acid (EGTA), pH 7.4, buffer/g muscle and finally resuspended in ~1.0 ml of 100 mmol/l KCl, 50 mmol/l morpholinopropane sulphonic acid and 0.5 mmol/l EGTA, pH 7.4.

Experimental protocol for hepatic and muscle mitochondrial respiration

Mitochondria from each liver biopsy sample were divided into six aliquots and incubated on ice under different conditions: the first aliquot was incubated with vehicle; the second aliquot was incubated with norepinephrine (10 μM) for 1 h (NE); the third and fourth groups were pre-treated with the α-receptor antagonists prazosin and yohimbine (both at a final concentration of 1μM) for 20 min followed by incubation with norepinephrine for an additional hour; the fifth and sixth groups were incubated with prazosin or yohimbine alone for 80 min. The protocol was started immediately after isolation of the mitochondria, and determination of mitochondrial respiration was completed no more than 90 min after incubation for all groups.

Mitochondria from each muscle biopsy sample were divided into 13 groups and incubated on ice. The first group was incubated with vehicle. The second and third groups were incubated with norepinephrine (10 μM) and dobutamine (DB; 10 μM) for 1 h, respectively. The fourth and fifth groups were pre-treated with antagonists prazosin and yohimbine (both at a final concentration of 1μM) for 20 min, followed by incubation with norepinephrine for an additional hour. The sixth, seventh and eighth groups were pre-treated with the antagonists phentolamine, propranolol and atenolol, respectively (all at a final concentration of 10 μM), for 20 min, followed by incubation with dobutamine for one additional hour. In order to see the effect of antagonists alone, groups nine to thirteen were incubated with prazosin, yohimbine, phentolamine, propranolol and atenolol alone for 80 min. The protocol was started immediately

after isolation of the mitochondria, and determination of mitochondrial respiration was completed no more than 4 h after incubation for all groups.

Determination of mitochondrial oxygen consumption by high-resolution respirometry

Protein concentration was determined using the Quant-iT^{TM} protein assay kit (Qubit fluorometer, Invitrogen, Basel, Switzerland) according to the manufacturer's instructions. Respiratory rates were determined at a final mitochondrial protein concentration of 0.4 mg/ml. Respiration was measured at 37°C in 2 ml glass chambers using the High Resolution Oxygraph (OROBOROS; Oxygraph-2 k, Graz, Austria). The medium used for respiration measurements consisted of 110 mM sucrose, 0.5 mM EGTA, 3.0 mM $MgCl_2$, 80 mM KCl, 60 mM K-lactobionate, 10 mM KH_2PO_4, 20 mM taurine, 20 mM HEPES and 1.0 g/l BSA, pH 7.1.[21] The medium was equilibrated for 30 to 40 min with air in the Oxygraph chambers and stirred at 750 rpm until a stable signal was obtained for calibration at air saturation. The corresponding oxygen concentration was calculated from the digitally recorded barometric pressure and the oxygen solubility at 37°C. The amplified signal was recorded in a computer with online display of the calibrated oxygen concentration and oxygen flux (negative time derivative of oxygen concentration; Dat-Lab software for data acquisition and analysis; OROBOROS, Instruments, Graz, Austria). Oxygen consumption was expressed as pmol/s/mg mitochondrial protein. Oxygen levels were always maintained above 40 nmol/ml. Maximal oxidative capacities were determined in the presence of saturating concentrations of oxygen, ADP (0.25 mmol/l) and specific mitochondrial substrates. For complex I-dependent respiration, substrates were glutamate (10 mmol/l) plus malate (5 mmol/l), which provide nicotinamide adenine dinucleotide (NADH) to the respiratory chain (complex I activation). For measurement of complex II-dependent respiration, first complex I was inhibited with rotenone (0.5 µmol/l) and then succinate (10 mmol/l) was added, which provides flavin adenine dinucleotide (FADH) to the respiratory chain (complex II activation). The coupling of phosphorylation to oxidation was determined by calculating the respiratory control ratio (RCR) as the ratio between ADP-stimulated respiration (state 3) and respiration after ADP depletion (state 4). Complex IV-dependent respiration was measured by adding ascorbate (4 mM) and *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylendiamine (TMPD, 0.5 mM). As TMPD exhibited a wide range of auto-oxidation in the buffer, respiration was finally inhibited with sodium azide (5 mM) and the difference between the oxygen consumption before and after the addition of sodium azide was interpreted as the real complex IV (state 3) respiration.

Statistics

Normal distribution was assessed by the Kolmogorov-Smirnov Test. In case of normal distribution, ANOVA for repeated measurements was applied using one (drug, for hepatic mitochondrial respiration; time, for haemodynamics) or two (drug and time, for muscle mitochondrial respiration) within-group factor(s) and one between-group factor (peritonitis vs. control). When data were not normally distributed (mitochondria), placebo samples at baseline from septic and control animals were compared using the Mann-Whitney test, and placebo samples at baseline and in the end (muscle) in each group separately using Wilcoxon test. Afterwards, the effect of drugs at each time point was assessed using non-parametric ANOVA for repeated measurements (Friedman test). A *P*- value of <0.05 was considered significant. All analyses were performed using the SPSS® 15.0 software package (SPSS Inc., Chicago, IL, USA).

Results

Anaesthetics and analgesics

Administered cumulative doses of fentanyl (controls 4.06 ± 1.27 mg, septic animals 4.73 ± 2.11 mg; $p = 0.404$), midazolam (controls 803 ± 136 mg, septic animals 731 ± 168 mg; $p = 0.311$) and ketamine (controls 9.04 ± 1.34 g, septic animals 8.44 ± 3.13 g; $P = 0.585$) were not different between the groups.

Systemic haemodynamic, creatine kinase activity and arterial lactate concentrations

During the experiment, MAP and stroke volume decreased and heart rate increased in septic animals, while MAP increased and stroke volume and heart rate remained unchanged in controls (Figure 1a–c). In five septic animals a median (range) total dose of norepinephrine of 883 (490-2913) mcg was administered during the study. Systemic oxygen consumption was maintained in both groups (Figure 1d). Central venous pressure (CVP; Figure 1e) and pulmonary artery occlusion pressure (PAOP; Figure 1f) remained unchanged in both groups. Fluid balance was $+1047 \pm 1108$ ml in controls and $+4947 \pm 1235$ ml in septic animals ($P < 0.001$).

Creatine kinase activities at the end of the experiment were 622 (465;10154) U/l in controls and 808 (245;1167) U/l in septic animals ($p = 0.967$). Lactate levels in arterial blood changed from 0.76 ± 0.20 mmol/l at baseline to 0.89 ± 0.24 mmol/l at the end of the experiment in control

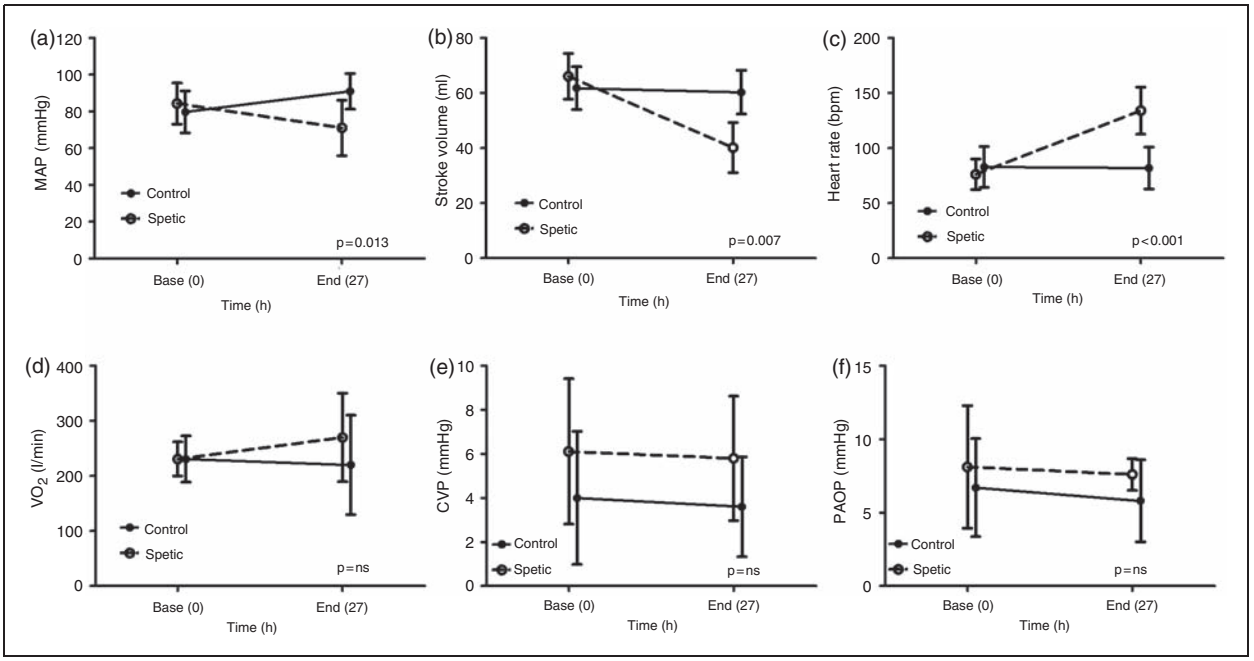

Figure 1. Changes in (a) mean arterial pressure (MAP), (b) stroke volume, (c) heart rate, (d) systemic oxygen consumption (VO_2), (e) central venous pressure (CVP) and (f) pulmonary artery occlusion pressure (PAOP) from before induction of sepsis (baseline) to the end of the protocol in non-septic controls (solid line) and in septic animals (dashed line). *P* value: MANOVA time x group interaction. Data points are group mean values, error bars indicate ± one standard deviation.

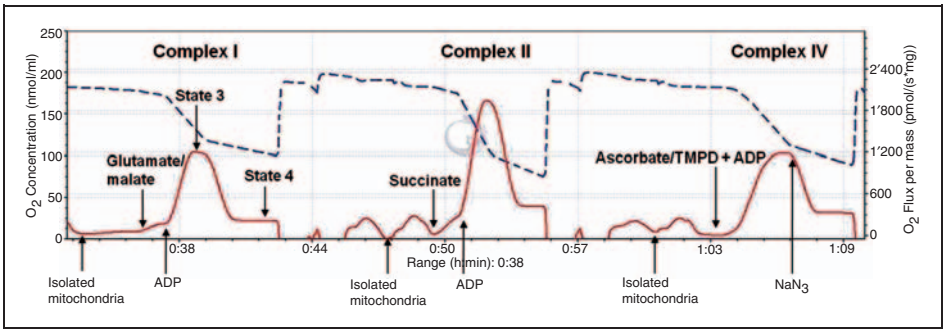

Figure 2. Representative diagram of measurement of respiration rates in isolated liver/muscle mitochondria using high-resolution respirometry (Oxygraph-2 k) and DatLab 4.2® software for data acquisition and analysis. Oxygen consumption is expressed as pmol/second/mg protein. State 3: active respiration after addition of ADP. State 4: respiration after consumption of ADP. The ratio between state 3 and state 4 was calculated as the respiratory control ratio (RCR; state 3/state 4). Oxygen concentration (dashed line) decreases in time as mitochondria use the available oxygen. The oxygen flux (solid line) represents the directly calculated oxygen use (negative time derivative of oxygen concentration).

animals, and from 0.90 ± 0.46 mmol/l at baseline to 1.89 ± 1.01 mmol/l at the end of the experiment in septic animals ($P = 0.074$, time-group interaction MANOVA).

Mitochondrial function

A representative diagram of measurement of respiration rates in isolated hepatic/muscle mitochondria at an experimental density of 0.4 mg of mitochondrial protein/ml using high-resolution respirometry is shown in Figure 2. The oxygen flux (solid line) represents the directly calculated oxygen use and is expressed as pmol/s/mg of mitochondrial protein. Oxygen concentration (dashed line) decreases over time as mitochondria use the available oxygen.

Baseline skeletal muscle mitochondrial respiration from one septic pig was not measured because of technical problems. Similarly, skeletal muscle mitochondrial respiration with antagonists alone was not measured for one septic pig at the end of the experiment. Hepatic mitochondrial respiration is indicated in Tables 1, 2, and 3, and skeletal muscle mitochondrial respiration (Complex I state 3 and 4, Complex II state 3 and state 4, and Complex IV state 3) in Tables 4, 5, and 6. Complex I- and II-dependent skeletal muscle mitochondrial respiratory control ratio data are indicated in

Table 1. Complex I-dependent hepatic mitochondrial respiration

Complex I		Control (N = 9)	Septic (N = 9)
State 3	Placebo	917 ± 421	978 ± 371
	Norepinephrine	914 ± 438	1007 ± 410
	Prazosin + norepinephrine	929 ± 391	1013 ± 576
	Yohimbine + norepinephrine	842 ± 374	973 ± 488
	Prazosin	908 ± 343	1012 ± 381
	Yohimbine	853 ± 292	983 ± 353
State 4	Placebo	302 ± 238	510 ± 290
	Norepinephrine	292 ± 246	446 ± 290
	Prazosin + norepinephrine	288 ± 221	441 ± 329
	Yohimbine + norepinephrine	307 ± 222	437 ± 298
	Prazosin	293 ± 200	503 ± 382
	Yohimbine	311 ± 211	579 ± 363
RCR	Placebo	3.8 [1.3–17.3]	1.7 [1.2–7.6]
	Norepinephrine	3.3 [1.4–15.5]	2.1 [1.4–5.6]
	Prazosin + norepinephrine	3.0 [1.6–15.8]	2.3 [1.3–6.4]
	Yohimbine + norepinephrine	2.6 [1.5–14.7]	2.0 [1.4–8.2]
	Prazosin	3.0 [1.6–17.3]	2.2 [1.2–5.7]
	Yohimbine	2.8 [1.3–15.5]	1.5 [1.2–7.5]

Values are mean ± SD for state 3 and state 4 and median (range) for respiratory control ratio (RCR).
Comparison of complex I-dependent isolated hepatic mitochondrial respiration from control and septic animals after incubation with norepinephrine and antagonists prazosin and yohimbine. State 3 and 4 oxygen consumption is expressed as pmol/s/mg protein. RCR, respiratory control ratio (oxygen consumption of state 3/state 4). No significant differences were found between the placebo and any of the drugs or drug combinations ($P > 0.05$ for all comparisons).

Table 2. Complex II-dependent hepatic mitochondrial respiration

Complex II		Control (N = 9)	Septic (N = 9)
State 3	Placebo	1514 ± 549	1652 ± 586
	Norepinephrine	1518 ± 588	1737 ± 705
	Prazosin + norepinephrine	1502 ± 486	1617 ± 654
	Yohimbine + norepinephrine	1519 ± 548	1687 ± 594
	Prazosin	1548 ± 521	1772 ± 719
	Yohimbine	1444 ± 434	1712 ± 620
State 4	Placebo	571 ± 338	1023 ± 661
	Norepinephrine	587 ± 393	1034 ± 669
	Prazosin + norepinephrine	566 ± 358	923 ± 614
	Yohimbine + norepinephrine	599 ± 349	998 ± 608
	Prazosin	581 ± 341	1013 ± 627
	Yohimbine	608 ± 342	1056 ± 629
RCR	Placebo	3.0 [1.2–6.8]	1.3 [1.2–6.0]
	Norepinephrine	3.4 [1.1–6.4]	1.4 [1.2–3.9]
	Prazosin + norepinephrine	3.0 [1.2–9.4]	1.5 [1.2–10.4]
	Yohimbine + norepinephrine	3.1 [1.3–8.9]	1.4 [1.2–4.7]
	Prazosin	3.4 [1.2–6.7]	1.6 [1.1–4.6]
	Yohimbine	3.3 [1.3–6.6]	1.4 [1.2–5.5]

Values are mean ± SD for state 3 and state 4 and median (range) for respiratory control ratio (RCR).
Comparison of complex II-dependent isolated hepatic mitochondrial respiration from control and septic animals after incubation with norepinephrine and antagonists prazosin and yohimbine. State 3 and 4 oxygen consumption is expressed as pmol/s/mg protein. RCR, respiratory control ratio (oxygen consumption of state 3/state 4). No significant differences were found between the placebo and any of the drugs or drug combinations ($P > 0.05$ for all comparisons).

Table 3. Complex IV-dependent hepatic mitochondrial respiration

Complex IV		Control (N = 9)	Septic (N = 9)
State 3	Placebo	569 [215–1431]	691 [361–1474]
	Norepinephrine	504 [271–1210]	590 [223–1863]
	Prazosin + norepinephrine	579 [328–1205]	766 [246–1529]
	Yohimbine + norepinephrine	500 [336–1666]	665 [350–1071]
	Prazosin	534 [351–1233]	738 [273–1521]
	Yohimbine	558 [351–1299]	516 [239–1158]

Values are median (range).
Complex IV-dependent isolated hepatic mitochondrial respiration from control and septic animals after incubation with norepinephrine and antagonists prazosin and yohimbine. State 3 oxygen consumption is expressed as pmol/s/mg protein. No significant differences were found between the placebo and any of the drugs or drug combinations ($P > 0.05$ for all comparisons).

Table 4. Complex I-dependent muscle mitochondrial respiration

		Control animals		Septic animals	
Complex I		Baseline (N = 7)	End (N = 7)	Baseline (N = 7)	End [§](N ≥ 6)
State 3	Placebo	1017 ± 311	947 ± 440	833 ± 164	928 ± 431
	Norepinephrine	1109 ± 268	1026 ± 469	772 ± 321	965 ± 666
	Prazosin + norepinephrine	1024 ± 307	1141 ± 369	929 ± 172	846 ± 625
	Yohimbine + norepinephrine	1145 ± 293	1156 ± 450	858 ± 181	816 ± 571
	Prazosin	904 ± 304	1035 ± 501	692 ± 290	939 ± 555
	Yohimbine	921 ± 418	1077 ± 544	798 ± 304	788 ± 431
	Dobutamine	1179 ± 309	1052 ± 523	857 ± 308	946 ± 659
	Atenolol + dobutamine	1118 ± 359	1025 ± 402	867 ± 295	891 ± 411
	Propranolol + dobutamine	1198 ± 310	1095 ± 490	785 ± 319	836 ± 456
	Phentolamine + dobutamine	1128 ± 297	1005 ± 462	899 ± 230	852 ± 404
	Atenolol	1114 ± 316	977 ± 562	727 ± 346	992 ± 492
	Propranolol	973 ± 300	967 ± 293	766 ± 351	1023 ± 674
	Phentolamine	1092 ± 189	1011 ± 557	882 ± 269	978 ± 662
State 4	Placebo	410 ± 254	289 ± 240	424 ± 292	424 ± 244
	Norepinephrine	430 ± 246	350 ± 347	316 ± 204	440 ± 291
	Prazosin + norepinephrine	461 ± 288	416 ± 324	929 ± 172	846 ± 625
	Yohimbine + norepinephrine	480 ± 309	429 ± 325	380 ± 221	405 ± 266
	Prazosin	465 ± 277	310 ± 278	364 ± 270	473 ± 264
	Yohimbine	358 ± 188	345 ± 318	322 ± 219	413 ± 162
	Dobutamine	411 ± 259	305 ± 296	374 ± 228	463 ± 344
	Atenolol + dobutamine	433 ± 299	371 ± 316	366 ± 229	395 ± 252
	Propranolol + dobutamine	426 ± 257	381 ± 358	296 ± 182	433 ± 288
	Phentolamine + dobutamine	488 ± 348	365 ± 334	434 ± 275	413 ± 264
	Atenolol	477 ± 408	335 ± 287	367 ± 289	458 ± 220
	Propranolol	437 ± 326	279 ± 177	398 ± 274	511 ± 274
	Phentolamine	460 ± 345	371 ± 345	397 ± 261	488 ± 338

Values are mean ± SD.
Complex I-dependent isolated skeletal muscle mitochondrial respiration in control and septic animals from baseline to end, after incubation with agonists norepinephrine and dobutamine, and with antagonists prazosin and yohimbine for norepinephrine, and atenolol, propranolol, and phentolamine for dobutamine. State 3 and 4 oxygen consumption is expressed as pmol/s/mg protein. No significant differences were found between the placebo and any of the drugs or drug combinations ($P > 0.05$ for all comparisons). [§] (n = 7 for placebo, agonists and for pre-incubation of agonists with antagonists and n = 6 for antagonists alone).

Table 5. Complex II-dependent muscle mitochondrial respiration

		Control animals		Septic animals	
Complex II		Baseline (N = 7)	End (N = 7)	Baseline (N = 7)	End [§](N ≥ 6)
State 3	Placebo	715 ± 532	817 ± 456	1098 ± 626	956 ± 405
	Norepinephrine	654 ± 480	718 ± 459	1167 ± 696	1016 ± 459
	Prazosin + norepinephrine	719 ± 586	807 ± 395	1096 ± 550	967 ± 440
	Yohimbine + norepinephrine	769 ± 583	666 ± 319	985 ± 526	992 ± 427
	Prazosin	776 ± 486	990 ± 710	1225 ± 707	1018 ± 258
	Yohimbine	700 ± 578	721 ± 440	1188 ± 614	1044 ± 458
	Dobutamine	670 ± 417	729 ± 349	1205 ± 620	1068 ± 391
	Atenolol + dobutamine	674 ± 542	832 ± 449	1110 ± 707	1004 ± 456
	Propranolol + dobutamine	671 ± 523	865 ± 323	1035 ± 577	1043 ± 434
	Phentolamine + dobutamine	720 ± 571	778 ± 340	1130 ± 648	1165 ± 432
	Atenolol	804 ± 502	854 ± 586	1233 ± 810	1030 ± 363
	Propranolol	797 ± 554	877 ± 675	1155 ± 717	1127 ± 416
	Phentolamine	660 ± 399	705 ± 443	1213 ± 704	987 ± 233
State 4	Placebo	546 ± 342	561 ± 256	582 ± 296	563 ± 291
	Norepinephrine	457 ± 211	422 ± 280	607 ± 331	602 ± 323
	Prazosin + norepinephrine	588 ± 451	606 ± 238	578 ± 270	623 ± 372
	Yohimbine + norepinephrine	627 ± 428	533 ± 222	570 ± 302	628 ± 319
	Prazosin	614 ± 360	619 ± 357	690 ± 413	700 ± 275
	Yohimbine	478 ± 265	503 ± 230	632 ± 331	641 ± 292
	Dobutamine	477 ± 182	438 ± 278	583 ± 294	588 ± 351
	Atenolol + dobutamine	450 ± 277	546 ± 175	575 ± 356	536 ± 252
	Propranolol + dobutamine	417 ± 215	546 ± 225	545 ± 269	603 ± 293
	Phentolamine + dobutamine	470 ± 216	521 ± 220	584 ± 320	651 ± 370
	Atenolol	562 ± 246	512 ± 304	565 ± 318	640 ± 279
	Propranolol	535 ± 283	562 ± 339	609 ± 352	676 ± 284
	Phentolamine	523 ± 335	484 ± 322	628 ± 331	640 ± 294

Values are mean ± SD.

Complex II-dependent isolated skeletal muscle mitochondrial respiration in control and septic animals from baseline to end, after incubation with agonists norepinephrine and dobutamine, and with antagonists prazosin and yohimbine for norepinephrine, and atenolol, propranolol, and phentolamine for dobutamine. State 3 and 4 oxygen consumption is expressed as pmol/s/mg protein. No significant differences were found between the placebo and any of the drugs or drug combinations ($P > 0.05$ for all comparisons). [§] (n = 7 for placebo, agonists and for pre-incubation of agonists with antagonists and n = 6 for antagonists alone).

Figures 3 and 4. Sepsis had no measurable effects on hepatic or skeletal muscle mitochondrial respiration.

In animals treated with norepinephrine (n = 5), hepatic but not skeletal muscle complex II-dependent state 4 was significantly lower than in animals that did not receive norepinephrine (Tables 7 and 8).

Similarly, norepinephrine had no effect on hepatic isolated mitochondrial respiration, either in control animals or in septic animals [complex I-state 3: 917 ±421 pmol/s per mg (placebo) vs. 914 ± 438 pmol/s per mg (norepinephrine) in controls; 978 ± 371 pmol/s per mg (placebo) vs. 1007 ± 410 pmol/s per mg (norepinephrine) in septic animals; complex I-state 4: 302 ±238 pmol/s per mg (placebo) vs. 292 ± 246 pmol/s per mg (norepinephrine) in controls; 510 ± 290 pmol/s per mg (placebo) vs. 446 ± 290 pmol/s per mg (norepinephrine) in septic animals; respiratory control ratio: 5.6 ± 5.3 (placebo) vs. 5.4 ± 4.6 (norepinephrine) in controls; 2.7 ± 2.1(placebo) vs. 2.9 ± 1.5 (norepinephrine) in septic animals (all $P > 0.05$)] (Table 1).

The maximal hepatic mitochondrial respiration (state 3) for complex II was 1514 ± 549 pmol/s per mg (placebo) vs. 1518 ± 588 pmol/s per mg (norepinephrine) in controls; 1652 ± 586 pmol/s per mg (placebo) vs. 1737 ± 705 pmol/s per mg (norepinephrine) in septic animals, state 4 was 571 ± 338 pmol/s per mg (placebo) vs. 587 ± 393 pmol/s per mg (norepinephrine) in controls; 1023 ± 661 pmol/s per mg (placebo) vs. 1034±669 pmol/s per mg (norepinephrine) in septic animals, and the respiratory control ratio was 3.5 ± 2.0 (placebo) vs. 3.5 ± 1.8 (norepinephrine) in controls; 2.3 ± 1.6 (placebo) vs. 2.2 ± 1.1 (norepinephrine) in septic animals (all $P > 0.05$) (Table 2).

Complex IV-dependent hepatic mitochondrial respiration was 708 ± 414 pmol/s per mg (placebo) vs. 623 ± 348 pmol/s per mg (norepinephrine) in controls, and

Table 6. Complex IV-dependent muscle mitochondrial respiration

		Control animals		Septic animals	
Complex IV		Baseline (N = 7)	End (N = 7)	Baseline (N = 7)	End [§](N ≥ 6)
State 3	Placebo	977 ± 591	1081 ± 540	1091 ± 607	1241 ± 500
	Norepinephrine	1059 ± 784	1335 ± 1201	1270 ± 724	1199 ± 475
	Prazosin + norepinephrine	995 ± 545	941 ± 536	1181 ± 652	1381 ± 746
	Yohimbine + norepinephrine	1074 ± 538	976 ± 428	906 ± 601	1160 ± 542
	Prazosin	1123 ± 555	954 ± 503	1163 ± 695	1469 ± 773
	Yohimbine	955 ± 624	1001 ± 401	1138 ± 568	1384 ± 410
	Dobutamine	991 ± 626	1045 ± 753	1103 ± 613	1346 ± 658
	Atenolol + dobutamine	963 ± 636	1108 ± 784	1199 ± 754	1113 ± 413
	Propranolol + dobutamine	1089 ± 529	1313 ± 855	1061 ± 603	1245 ± 495
	Phentolamine + dobutamine	1097 ± 727	895 ± 565	1093 ± 743	1315 ± 719
	Atenolol	861 ± 457	877 ± 436	1156 ± 687	1417 ± 448
	Propranolol	1092 ± 756	1081 ± 676	1150 ± 678	1194 ± 485
	Phentolamine	1120 ± 643	1207 ± 828	1037 ± 596	1492 ± 758

Values are mean ± SD.

Complex IV-dependent isolated skeletal muscle mitochondrial respiration in control and septic animals from baseline to end, after incubation with agonists norepinephrine and dobutamine, and with antagonists prazosin and yohimbine for norepinephrine, and atenolol, propranolol, and phentolamine for dobutamine. State 3 and 4 oxygen consumption is expressed as pmol / s / mg protein. No significant differences were found between the placebo and any of the drugs or drug combinations ($P > 0.05$ for all comparisons). [§] (n = 7 for placebo, agonists and for pre-incubation of agonists with antagonists and n = 6 for antagonists alone).

718 ± 324 pmol/s per mg (placebo) vs. 740 ± 468 pmol/s per mg (norepinephrine) in septic animals ($P > 0.05$) (Table 3).

Neither norepinephrine nor dobutamine, nor any of the drug combinations, had measurable effects on skeletal muscle mitochondrial respiration (Figures 3 and 4, Tables 4–6).

Discussion

This study provided two main findings: 1) 27 h of faecal peritonitis had no effect on respiration of isolated hepatic and skeletal muscle mitochondria; and 2) invitro incubation of isolated hepatic mitochondria with norepinephrine after faecal peritonitis neither improved nor worsened their respiration.

Our results contrast with our previous finding of increased efficiency of hepatic mitochondrial respiration after norepinephrine infusion in endotoxaemic animals,[14] and suggest that norepinephrine has no direct effect on hepatic mitochondrial respiration. This is supported by previous findings on lack of effect of norepinephrine on hepatic mitochondrial respiration in mitochondria previously exposed to endotoxin *in vitro*.[22] As prolonged exposure to faecal peritonitis had no effect on hepatic or skeletal muscle respiration per se, the potential effect of norepinephrine on sepsis-induced deterioration of mitochondrial respiration cannot be determined from the present results. In the present study, *in vivo* administration of norepinephrine to five out of nine septic animals was associated with a significant reduction in isolated liver complex II-dependent state 4 respiration and an insignificant increase in complex II-dependent RCR. Regueira et al. previously reported that a continuous infusion of norepinephrine to endotoxaemic pigs was associated with improved complex I- and II-dependent RCRs.[14] These, and other data,[22] suggest that potential effects of norepinephrine on mitochondrial respiration in sepsis are indirect.

Whether, how, and to what extent sepsis may alter mitochondrial function is a matter of debate.[23–25] We previously reported well-preserved complex I- and II-dependent mitochondrial respiration in muscle and liver in pigs subjected to 24 h of either endotoxin or faecal peritonitis,[26] despite the presence of organ failure and substantial mortality. Similarly, in a rat caecal ligation and puncture model of sepsis lasting 16 h, the authors found no significant changes in complex I- and complex II-dependent hepatic mitochondrial respiration.[3] Furthermore, hepatic and skeletal muscle mitochondrial function of rats remained unaffected 18–24 h after treatment with either intravenous endotoxin or faecal peritonitis.[4] In contrast, others reported inhibition of complex I-dependent mitochondrial respiration in skeletal muscle biopsies collected from septic patients within 24 hours of intensive care unit admission.[1] The same group demonstrated an association between sepsis

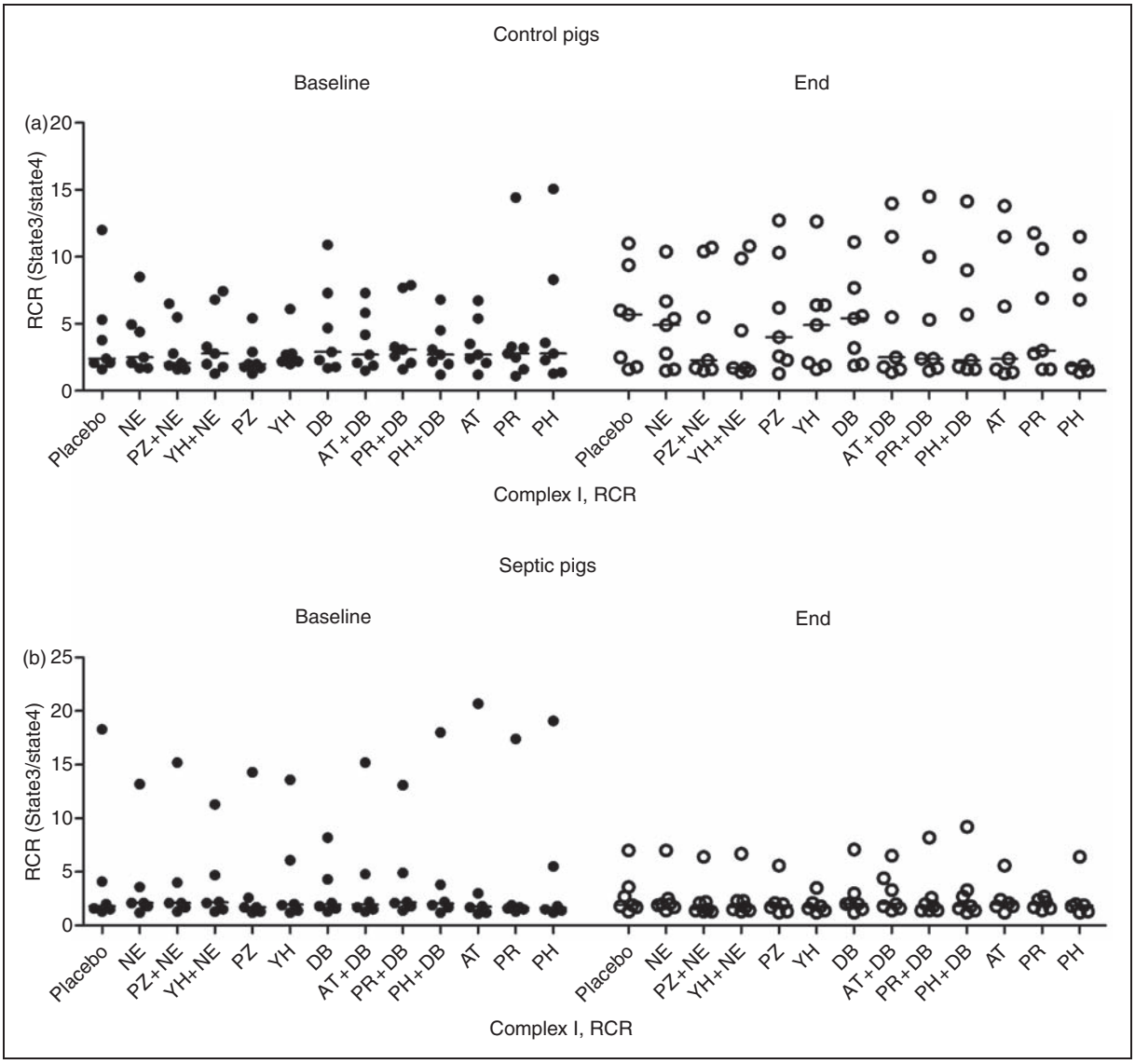

Figure 3. Comparison of complex I-dependent skeletal muscle mitochondrial respiratory control ratio in (a) control and (b) septic animals from baseline (filled circles) to end (open circles), after incubation with agonists norepinephrine and dobutamine, and with antagonists prazosin and yohimbine for norepinephrine and atenolol, propranolol, and phentolamine for dobutamine. No significant differences were found between the placebo and any of the drugs or drug combinations ($P > 0.05$ for all comparisons). Horizontal lines represent median values. NE, norepinephrine; PZ, prazosin; YH, yohimbine; DB, dobutamine; AT, atenolol; PR, propranolol; PH, phentolamine; RCR, Respiratory control ratio (oxygen consumption of state 3/state 4).

and impaired complex I-dependent skeletal muscle and hepatic mitochondrial respiration in a long-term, fluid-resuscitated, faecal peritonitis rat model.[2]

In the present study we used a large animal model of sepsis induced by faecal peritonitis that shares many of the features of clinical sepsis, but did not result in organ failure or mortality. As we measured complex I- and II-dependent hepatic and skeletal muscle mitochondrial respiration by adding physiological substrates to the isolated mitochondria, we cannot exclude sepsis-induced impairment of mitochondrial function by mechanisms not tracked by the methods used in the present study. Such mechanisms may include pyruvate dehydrogenase inhibition,[27] poly-(ADP-ribose) polymerase (PARP-1) activation[28] and decreased tissue mitochondrial density.[29]

There are several potential reasons for the conflicting findings with regard to mitochondrial function in the pathophysiology of sepsis. These include differences in sepsis models (endotoxaemia,[5,30] faecal peritonitis,[2,26] caecal ligation and puncture,[3,31] length of observation period and severity of sepsis), species investigated (humans,[1] pigs,[5,26] rats, [2,3,4] rabbits,[32] guinea pigs[33]), concomitantly administered medication (e.g. anaesthetics), and methods used to prepare

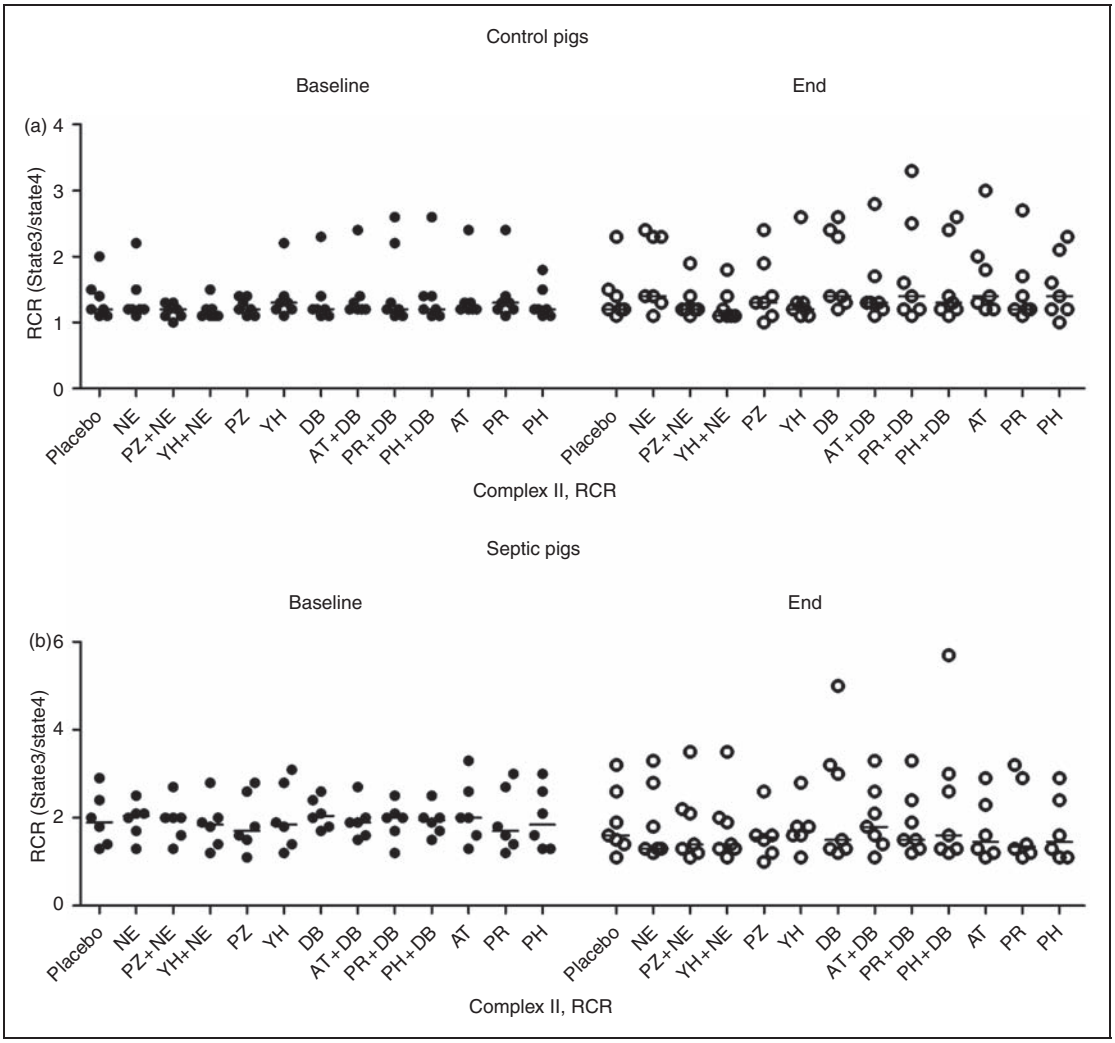

Figure 4. Comparison of complex II-dependent skeletal muscle mitochondrial respiratory control ratio in (a) control and (b) septic animals from baseline (filled circles) to end (open circles), after incubation with agonists norepinephrine and dobutamine, and with antagonists prazosin and yohimbine for norepinephrine and atenolol, propranolol, and phentolamine for dobutamine. No significant differences were found between the placebo and any of the drugs or drug combinations ($P > 0.05$ for all comparisons). Horizontal lines represent median values. NE, norepinephrine; PZ, prazosin; YH, yohimbine; DB, dobutamine; AT, atenolol; PR, propranolol; PH, phentolamine; RCR, Respiratory control ratio (oxygen consumption of state 3/state 4).

Table 7. Effect of norepinephrine on isolated liver mitochondrial respiration

Septic pigs Liver	(−) Norepinephrine (N = 4)	(+) Norepinephrine (N = 5)	*P* (Mann-Whitney test)
Complex I			
State 3	905 [494–1742]	843 [703–1197]	0.81
State 4	633 [282–1031]	324 [93–662]	0.33
RCR	1.6 [1.4–1.8]	3.7 [1.2–7.6]	0.46
Complex II			
State 3	1856 [999–2814]	1561 [1000–1827]	0.62
State 4	1520 [807–2174]	553 [165–1314]	0.05
RCR	1.2 [1.2–1.3]	2.8 [1.2–6.0]	0.06
Complex IV			
State 3	675 [361–1474]	691 [366–793]	0.81

Isolated liver mitochondrial respiration: comparison between placebo samples from septic animals which received or did not receive norepinephrine. Data are expressed as median [range]. RCR, respiratory control ratio.

Table 8. Effect of norepinephrine on isolated skeletal muscle mitochondrial respiration

Septic pigs Muscle	(−) Norepinephrine (N = 3)	(+) Norepinephrine (N = 4)	*P* (Mann-Whitney test)
Complex I			
State 3	868 [417–1116]	949 [577–1621]	0.48
State 4	250 [124–594]	559 [170–715]	0.29
RCR	1.9 [1.7–7.0]	2.3 [1.3–3.6]	0.72
Complex II			
State 3	1012 [356–1444]	901 [625–1452]	0.10
State 4	567 [186–689]	668 [253–909]	0.48
RCR	1.9 [1.5–2.6]	1.5 [1.1–3.2]	0.48
Complex IV			
State 3	1440 [523–1664]	1203 [802–1849]	0.72

Isolated skeletal muscle mitochondrial respiration: comparison between end placebo samples from the septic animals that received or did not receive norepinephrine. Data are expressed as median [range]. RCR, respiratory control ratio.

samples and assess mitochondrial function (isolated mitochondria vs. intact tissue samples, mitochondrial isolation procedures—Percoll gradients[31,34] vs. differential centrifugation[5,26,35]—spectrophotometric and reverse-phase HPLC techniques[2] vs. oxygen consumption assessment by Clark-type oxygen electrodes[3,22] or high-resolution oxygraph,[14] etc.).

While it has been shown that norepinephrine may activate hepatocellular receptors and increase intramitochondrial calcium levels and enzyme activities,[12,13] our results do not provide evidence for a direct effect of norepinephrine on (isolated) mitochondria. It should be mentioned that in the present study, the norepinephrine incubation time was short when compared to an infusion of norepinephrine for several hours. We also explored effects of norepinephrine and dobutamine on skeletal muscle mitochondria and found no influence of either drug on any of the complexes of the mitochondrial respiratory chain. However, in a recent *in vitro* study, catecholamines (dopamine, dobutamine, norepinephrine) were shown to impair the efficiency of glutamate-dependent native liver mitochondrial respiration without significant alterations in state 3 and state 4 respiration rates and any of the other complexes.[22] The use of higher doses of catecholamines and—to a lesser extent—a different technique for the measurement of respiration (Clark-type electrode) may offer some explanations for the different findings. On the other hand, *in vitro* incubation of catecholamines (dopamine, dobutamine) did not alter native muscle mitochondrial respiration.[36]

Ketamine, midazolam and fentanyl may have inhibitory effects on mitochondrial complex activity and oxidative metabolism.[37–39] However, in the present study, cumulative administered doses of anaesthetics and analgesics were not different between controls and septic animals.

Limitations of our study are the relatively mild disease we produced in our animals, the lack of dose responses, and the short duration of incubation for norepinephrine and dobutamine. Further, we evaluated complex functions in isolated mitochondria only. It is conceivable that investigating whole cells or muscle fibres and exploring other components of mitochondrial functions would have revealed hidden effects of norepinephrine. In the present study, cardiac stroke volume decreased in septic animals, whereas it remained stable in controls. Myocardial depression and decreased response to volume administration have been described in septic patients.[40,41] Although fluid was administered based on the stroke volume response using an established haemodynamic protocol with resulting large positive fluid balance, we cannot exclude that an even more aggressive fluid resuscitation strategy would have resulted in less reduction of stroke volume in septic animals. However, as shown previously, high loading with fluids from the start of experimental sepsis—while preventing a decrease in stroke volume—increases mortality.[26]

We conclude that 27 h of faecal peritonitis—resulting in sepsis without shock or organ failure—did not affect hepatic and skeletal muscle respiration of isolated mitochondria and that consecutive incubation with norepinephrine and dobutamine neither improved nor caused deterioration in the efficiency of mitochondrial respiration.

Acknowledgements

The authors are grateful to Jeannie Wurz for editorial support.

Funding

The study was supported by grants from the Swiss National Science Foundation (SNF; 3200B0-113478/1) awarded to LB and from the Stiftung für die Forschung in Anästhesiologie und Intensivmedizin, Bern (07/2008), awarded to RS and LB.

Declaration of conflicting interests

The Department of Intensive Care Medicine has, or has had in the past, research contracts with Abbott Nutrition International, B. Braun Medical AG, CSEM SA, Edwards Lifesciences Services GmbH, Kenta Biotech Ltd, Maquet Critical Care AB, Omnicare Clinical Research AG, and Orion Corporation; and research & development/consulting contracts with Edwards Lifesciences SA and Maquet Critical Care AB. The money is/was paid into a departmental fund; no author receives/received individual fees.

References

1. Brealey D, Brand M, Hargreaves I, et al. Association between mitochondrial dysfunction and severity and outcome of septic shock. *Lancet* 2002; 360: 219–223.
2. Brealey D, Karyampudi S, Jacques TS, et al. Mitochondrial dysfunction in a long-term rodent model of sepsis and organ failure. *Am J Physiol Regul Integr Comp Physiol* 2004; 286: R491–R497.
3. Taylor DE, Ghio AJ and Piantadosi CA. Reactive oxygen species produced by liver mitochondria of rats in sepsis. *Arch Biochem Biophys* 1995; 316: 70–76.
4. Geller ER, Jankauskas S and Kirkpatrick J. Mitochondrial death in sepsis: A failed concept. *J Surg Res* 1986; 40: 514–517.
5. Porta F, Takala J, Weikert C, et al. Effects of prolonged endotoxaemia on liver, skeletal muscle and kidney mitochondrial function. *Crit Care* 2006; 10: R118.
6. Sobel B, Jequier E, Sjoerdsma A and Lovenberg W. Effect of catecholamines and adrenergic blocking agents on oxidative phosphorylation in rat heart mitochondria. *Circ Res* 1966; 19: 1050–1061.
7. Gambelli S, Dotti MT, Malandrini A, et al. Mitochondrial alterations in muscle biopsies of patients on statin therapy. *J Submicrosc Cytol Pathol* 2004; 36: 85–89.
8. Hanley PJ, Ray J, Brandt U and Daut J. Halothane, isoflurane and sevoflurane inhibit NADH: ubiquinone oxidoreductase (complex I) of cardiac mitochondria. *J Physiol* 2002; 544: 687–693.
9. Schenkman KA and Yan S. Propofol impairment of mitochondrial respiration in isolated perfused guinea pig hearts determined by reflectance spectroscopy. *Crit Care Med* 2000; 28: 172–177.
10. Kopustinskiene DM, Pollesello P and Saris NE. Levosimendan is a mitochondrial K_{ATP} channel opener. *Eur J Pharmacol* 2001; 428: 311–314.
11. Ruokonen E, Takala J and Uusaro A. Effect of vasoactive treatment on the relationship between mixed venous and regional oxygen saturation. *Crit Care Med* 1991; 19: 1365–1369.
12. Brown GC. Control of respiration and ATP synthesis in mammalian mitochondria and cells. *Biochem J* 1992; 284: 1–13.
13. Binet A and Claret M. Alpha-adrenergic stimulation of respiration in isolated rat hepatocytes. *Biochem J* 1983; 210: 867–873.
14. Regueira T, Bänziger B, Djafarzadeh S, et al. Norepinephrine to increase blood pressure in endotoxaemic pigs is associated with improved hepatic mitochondrial respiration. *Critical Care* 2008; 12: R88.
15. Ali SZ, Bracht H, Krejci V, et al. The immediate and sustained effects of volume challenge on regional blood flows in pigs. *Anesth Analg* 2008; 106: 595–600.
16. Hiltebrand LB, Krejci V, Jakob SM, Takala J and Sigurdsson GH. Effects of vasopressin on microcirculatory blood flow in the gastrointestinal tract in anesthetized pigs in septic shock. *Anesthesiology* 2007; 106: 1156–1167.
17. Regueira T, Djafarzadeh S, Brandt S, et al. Effects of major abdominal surgery and anesthesia on mitochondrial respiration. *Shock* 2008; 29: 60–61.
18. Taniguchi Y, Bracht H, Porta F, et al. Thermodilution and esophageal Doppler ultrasound in the assessment of blood flow changes induced by endotoxin and dobutamine. *J Trauma* 2008; 65: 175–182.
19. Johnson D and Lardy HA. Isolation of liver or kidney mitochondria. In: Estabrook RW and Pullman ME (eds) *Methods in Enzymology*. New York: Academic Press, 1967, pp.94–96.
20. Hoppel CL, Kerr DS, Dahms B and Roessmann U. Deficiency of reduced nicotinamide dinucleotide dehydrogenase component of complex I of mitochondrial electron transport. *J Clin Invest* 1987; 80: 71–77.
21. Gnaiger E, Rieger G, Kuznetsov A, et al. Mitochondrial ischemia-reoxygenation injury and plasma membrane integrity in human endothelial cells. *Transplant Proc* 1997; 29: 3524–3526.
22. Porta F, Bracht H, Weikert C, et al. Effects of endotoxin and catecholamines on hepatic mitochondrial respiration. *Inflammation* 2009; 32: 315–321.
23. Brealey D and Singer M. Mitochondrial dysfunction in sepsis. *Curr Infect Dis Rep* 2003; 5: 365–371.
24. Singer M. Mitochondrial function in sepsis: Acute phase versus multiple organ failure. *Crit Care Med* 2007; 35: S441–S448.
25. Crouser ED. Mitochondrial dysfunction in septic shock and multiple organ dysfunction syndrome. *Mitochondrion* 2004; 4: 729–741.
26. Brandt S, Regueira T, Bracht H, et al. Effect of fluid resuscitation on mortality and organ function in experimental sepsis models. *Critical Care* 2009; 13: R186.
27. Vary TC. Increased pyruvate dehydrogenase kinase activity in response to sepsis. *Am J Physiol Endocrinol Metab* 1991; 260: E669–E674.
28. Goldfarb RD, Marton A, Szabo E, et al. Protective effect of a novel, potent inhibitor of poly(adenosine 5′-diphosphate-ribose) synthetase in a porcine model of severe bacterial sepsis. *Crit Care Med* 2002; 30: 974–980.
29. Crouser ED, Julian MW, Huff JE, Struck J and Cook CH. Carbamoyl phosphate synthase-1: A marker of mitochondrial damage and depletion in the liver during sepsis. *Crit Care Med* 2006; 34: 2439–2446.
30. Kozlov AV, Staniek K, Haindl S, et al. Different effects of endotoxic shock on the respiratory function of liver and heart mitochondria in rats. *Am J Physiol Gastrointest Liver Physiol* 2006; 290: G543–G549.
31. Taylor DE, Kantrow SP and Piantadosi CA. Mitochondrial respiration after sepsis and prolonged hypoxia. *Am J Physiol Lung Cell Mol Physiol* 1998; 275: L139–L144.
32. Trumbeckaite S, Opalka JR, Neuhof C, Zierz S and Gellerich FN. Different sensitivity of rabbit heart and skeletal muscle to endotoxin-induced impairment of mitochondrial function. *Eur J Biochem* 2001; 268: 1422–1429.
33. Akira K, Emiko K, Hironori I and Akitomo M. ONO-5046, an elastase inhibitor, attenuates liver mitochondrial dysfunction after endotoxin. *Crit Care Med* 1998; 26: 138–141.
34. Kantrow SP, Taylor DE, Carraway MS and Piantadosi CA. Oxidative metabolism in rat hepatocytes and mitochondria during sepsis. *Arch Biochem Biophys* 1997; 345: 278–288.
35. Crouser ED, Julian MW, Blaho DV and Pfeiffer DR. Endotoxin-induced mitochondrial damage correlates with impaired respiratory activity. *Crit Care Med* 2002; 30: 276–284.
36. Porta F, Takala J, Weikert C, Kaufmann P, Krahenbuhl S and Jakob SM. Effect of endotoxin, dobutamine and dopamine on muscle mitochondrial respiration in vitro. *J Endotoxin Res* 2006; 12: 358–366.
37. Markham A, Cameron I and White SJ. The effect of ketamine hydrochloride, a non-barbiturate parenteral anaesthetic on oxidative phosphorylation in rat liver mitochondria. *Biochem Pharmacol* 1981; 30: 2165–2168.

38. Colleoni M, Costa B, Gori E and Santagostino A. Biochemical characterization of the effects of the benzodiazepine, midazolam, on mitochondrial electron transfer. *Pharmacol Toxicol* 1996; 78: 69–76.
39. Zamparelli M, Eaton S, Quant PA, McEwan A, Spitz L and Pierro A. Analgesic doses of fentanyl impair oxidative metabolism of neonatal hepatocytes. *J Pediatr Surg* 1999; 34: 260–263.
40. Weisel RD, Vito L, Dennis RC, et al. Myocardial depression during sepsis. *Am J Surg* 1977; 133: 512–521.
41. Ognibene FP, Parker MM, Natanson C, Shelhamer JH and Parrillo JE. Depressed left ventricular performance. Response to volume infusion in patients with sepsis and septic shock. *Chest* 1988; 93: 903–910.