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Increasing mean arterial blood pressure in sepsis: effects on fluid balance, vasopressor load and renal function

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Abstract

Introduction: The optimal goal for mean arterial blood pressure (MAP) during the initial resuscitation of severe sepsis and septic shock is unclear. Our objective was to evaluate the effects of two different mean arterial blood pressure targets on needs for resuscitation, organ dysfunction, mitochondrial respiration and inflammatory response in a long-term model of fecal peritonitis.

Methods: Twenty-four anesthetized and mechanically ventilated pigs were randomly assigned (n = 8 per group) to a septic control group (Septic-CG) without resuscitation until death or one of two groups in which resuscitation was performed after 12 hours of untreated sepsis for 48 hours, targeting MAP between 50 and 60 mmHg (Low-MAP) or between 75 and 85 mmHg (High-MAP).

Results: MAP at the end of resuscitation was 56±13 mmHg (mean±SD) and 76±17 mmHg respectively, for Low-MAP and High-MAP groups. One animal each in High- and Low-MAP groups, and all animals in the septic control group died [median survival time: 21.8 hours (inter-quartile range: 16.3-27.5 hours). Norepinephrine was administered to all animals of the High-MAP group [0.38 (0.21-0.56) mcg/kg/min], and to three animals of the Low-MAP group [0.00 (0.00-0.25) mcg/kg/min; p=0.009]. The High-MAP group had a more positive fluid balance (3.3±1.0 ml/kg/h vs. 2.3±0.7 ml/kg/h; p=0.001). Inflammatory markers, skeletal muscle ATP content and hemodynamics other than MAP did not differ between Low- and High-MAP groups. The incidence of acute kidney injury (AKI) after 12 hours of untreated sepsis was, respectively for Low- and High-MAP groups, 50% (4/8) and 38% (3/8), and in the end of the study 57% (4/7) and 0% (p=0.026). In Septic-CG, maximal isolated skeletal muscle mitochondrial Complex I, State 3 respiration increased from 1357±149 pmol/s/mg to 1822±385 pmol/s/mg, (p=0.020). In High- and Low-MAP groups, permeabilized skeletal muscle fibers Complex IV-state 3 respiration increased during resuscitation (p=0.003).

Conclusions: The MAP targets during resuscitation did not alter the inflammatory response, nor affected skeletal muscle ATP content and mitochondrial respiration. While targeting a lower MAP was associated with increased incidence of AKI, targeting a higher MAP resulted in increased net positive fluid balance and vasopressor load during resuscitation. The long-term effects of using lower MAP targets on kidney function and whether the resulting increased fluid balance and vasopressor load using higher MAP targets are of relevance for recovery after initial resuscitation needs to be evaluated in further long term studies.

Introduction

The arterial blood pressure is the driving force to blood flow through the tissues. According to the principle of blood flow autoregulation, if cardiac output is constant, blood flow to tissues does not change until blood pressure falls below a critical value. When this autoregulatory threshold is reached, any additional decrease in arterial blood pressure levels will compromise organ blood flow [1].

The Surviving Sepsis Campaign Guidelines recommend a mean arterial blood pressure (MAP) higher than 65 mmHg during the initial resuscitation of severe sepsis and septic shock [2]. However, there is only weak evidence to support this recommendation.

Few small prospective clinical studies addressed the effects of different MAP levels on tissue perfusion in septic shock [3-6]. Increasing MAP from 65 to 85 mmHg did neither improve systemic oxygen metabolism, splanchnic perfusion nor renal function, had inconsistent effects on local microcirculation, and was associated with higher left ventricular stroke work index and increased exposure to norepinephrine [3,4,6]. Apart from influencing systemic and regional perfusion and organ blood flow distribution [7,8], vasopressors may also affect the balance between oxygen and substrate supply and metabolic needs [9], with the potential to affect mitochondrial function [10], which has been associated with organ failure and poor outcome during sepsis [11-13]. Furthermore, in clinical practice as well as in research, the actual blood pressures achieved are often markedly higher than the targets originally prescribed [14], resulting in an unnecessary and potentially harmful further exposure to fluids and catecholamines [15].

The optimal balance between arterial blood pressure goals and minimal side effects of fluids and drugs needs to be determined [16]. On one hand, aiming higher MAP levels during the initial resuscitation of septic shock patients increases the exposure to fluids, vasopressors and inotropes which has been associated with increased mortality [15]. On the

other hand, targeting lower levels of MAP may increase the incidence of tissue hypoperfusion and contribute to progression of organ failure [17-19].

The aim of this experimental study was to evaluate the impact of two different levels of mean arterial blood pressure [MAP between 50 and 60 mmHg (Low-MAP) or between 75 and 85 mmHg (High-MAP)] on need for resuscitation, sepsis-associated organ dysfunction, mitochondrial respiration and inflammatory response in a long-term model of fecal peritonitis.

Materials and methods

This 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 Bern, Switzerland.

Twenty-four domestic pigs of both sexes [weight: 41.0 ± 3.1kg (mean±SD)] were fasted for 12 hours prior to the experiment, with free access to water. The pigs were sedated with intramuscular ketamine (20 mg/kg) and xylazine (2 mg/kg). Then, a peripheral intravenous catheter was inserted in an ear vein for administration of fluids and drugs. Pigs were orally intubated following anesthesia induction with midazolam (0.5 mg/kg) and atropine (0.02 mg/kg). Anesthesia was maintained with propofol (4 mg/kg/h) and fentanyl (5 µg/kg/h during surgery and 2 µg/kg/h afterwards). When necessary, additional injections of fentanyl (50 µg) or midazolam (5 mg) were administered. The stomach was kept empty by insertion of a large-bore orogastric tube.

Animals were ventilated in a volume-controlled mode with a positive end-expiratory pressure (PEEP) of 5 cm H_2O, a fractional inspiratory oxygen concentration (FiO_2) of 30%, and a tidal volume of 8 ml/kg (Servo-i®; Maquet Critical Care, Solna, Sweden). The respiratory rate was adjusted aiming an arterial carbon dioxide partial pressure ($PaCO_2$) between 35 and 45 mmHg.

Surgical preparation

The right jugular vein was cannulated via a midline cervical incision for a pulmonary artery catheter placement. A double lumen catheter was placed into the left femoral vein. An arterial catheter for blood pressure measurement and blood sampling was placed into the right carotid artery. Four mm ultrasound Doppler flow probes (Transonic® Systems Inc., Ithaca, NY, USA), which had previously been calibrated in vitro, were placed around the left carotid artery and the left femoral artery. With the pig in the supine position, a midline mini-laparotomy was performed and a large-bore intra-peritoneal drain inserted. After preparation, the abdominal incision was closed. Finally, a cystostomy was performed for drainage of urine. Throughout the surgical preparation, Ringer's lactate was infused at 3 mL/kg/h.

Study protocol

After surgical preparation, animals were stabilized for 6 hours and Ringer's lactate and glucose 50% (G50%) were infused (adding up to 1.5 mL/kg/h) and adjusted to keep blood glucose in the range of 3.5 to 5.0 mmol/L. Then, baseline measurements were performed and pigs were randomly assigned (n = 8 per group) to a septic control group (Septic-CG) without resuscitation until death or one of two groups in which resuscitation was performed after 12 hours of untreated sepsis for 48 hours, targeting MAP between 50 and 60 mmHg (Low-MAP) or between 75 and 85 mmHg (High-MAP) (Figure 1). Fecal peritonitis was induced by peritoneal instillation of 2 g/kg body weight of autologous feces dissolved in 250 mL warmed glucose 5% solution. Afterwards, the intra-peritoneal drain remained clamped throughout the experiment.

After 12 hours of untreated sepsis (Observation period), animals randomized to the Low-MAP and High-MAP groups were submitted to 48 hours of protocolized resuscitation (Resuscitation period) conducted by trained intensivists (Additional file 1). Alternating boluses of 150 mL Ringer's lactate and 6% hydroxyethyl starch (130/0.4) were administered when signs of hypovolemia became evident. Fluid boluses were repeated as long as the

stroke volume was increased by 10% or more after fluid administration. The maximum dose of hydroxyethyl starch administered during the study was 30 ml/kg. After this maximal dose was reached, only boluses of Ringer's lactate were given. If the mixed venous oxygen saturation (SvO_2) was lower than 50%, dobutamine administration was started at a dose of 5.0 mg/h. This dose was increased by 5.0 mg/h every 30 minutes until the SvO_2 was 50% or higher or until a maximal dose of 20 mg/h was given. If the mean arterial blood pressure was lower than 50 mmHg in Low-MAP group and 75 mmHg in the High-MAP group, norepinephrine was administrated (Additional file 1, Figure S1).

After resuscitation was started, all animals in Low-MAP and High-MAP groups received piperacillin-tazobactam (Tazobac®) 2.25 g, administered every 8 hours, intravenously, and deep vein thrombosis prophylaxis (continuous i.v. infusion of 10,000 IU of non-fractionated heparin/24 hours) (Figure 1). Details on respiratory care and ventilator settings can be found in the Additional file 1. Following 48 hours of resuscitation, study animals were deeply sedated and euthanized with an overdose of potassium chloride.

A basal fluid supply of 1.5 ml/kg/h during the observation period and 3.0 ml/kg/h during the resuscitation period was given as Ringer's lactate and G50%, adjusted to maintain a blood glucose in the range of 3.5 to 5.0 mmol/L (Additional file 1, Figure S2).

Monitoring

Hemodynamics, temperature and respiratory parameters were monitored throughout the study, using a multi-modular patient monitor (S/5 Critical Care Monitor®; Datex-Ohmeda, GE Healthcare, Helsinki, Finland). Thermodilution cardiac output (L/min), mixed venous oxygen saturation (SvO_2) (Vigilance®; Edwards Lifesciences LLC, Irvine, CA, USA), and carotid and femoral artery blood flows (TS 420 double-channel flowmeters, Transonic Systems Inc., Ithaca, NY, USA) were continuously measured. Data was recorded in a software for data acquisition and signal analysis (Soleasy™; National Instruments Corp., Austin, TX, USA),

and in an electronic patient data management system (Centricity Clinisoft®; GE Healthcare, Helsinki, Finland).

Blood sampling

Techniques and time points of blood sampling for determination of blood gases, arterial lactate, electrolytes, hemoglobin, platelets, white blood cell count, serum creatinine, total bilirubin, creatin-kinase, troponin, plasma interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α) are described in Additional file 1.

Mitochondrial function analysis

Right quadriceps muscle samples were taken to assess isolated skeletal muscle, permeabilized skeletal muscle fibers mitochondrial function and for the quantification of skeletal muscle ATP content. Isolation of skeletal muscle, liver mitochondria and mitochondrial oxygen consumption (by high-resolution respirometry) were performed as described previously [20]. Additional tissue sample was taken from the left lobe of the liver at the end of the experiment (Figure 1). Permeabilized muscle fibers preparation is described in detail in Additional file 1.

In septic-CG, respiration of isolated skeletal muscle mitochondria and permeabilized skeletal muscle fibers was assessed at baseline, and before death occurred (End). In low-MAP and high-MAP groups, isolated skeletal muscle mitochondria and permeabilized skeletal muscle fibers mitochondrial respiration were measured at baseline, end of the observation period (EOP; before starting resuscitation) and at the end of the experiment (End). In animals that died earlier, the final samples were taken when the animals were still alive, receiving the maximal norepinephrine dose of 5000 mcg/h, and when mean arterial blood pressure approached 30 mmHg. Skeletal muscle ATP content was quantified using a luciferase-based ATP determination kit (A22066, Molecular Probes, Eugene, OR).

Calculations and acute kidney injury definition

Systemic oxygen delivery (DO_2; mL/min) and consumption (VO_2; mL/min) were calculated using cardiac output and arterial and mixed venous blood gases, according to standard formulas (Additional file 1).

Acute kidney injury (AKI) was defined according to the AKIN criteria as an increase of more than 0.3 mg/dl (26.4 µmol/L) or 150% in serum creatinine from the baseline measurement [21]. Percentage of time below or above a certain blood pressure was calculated using 2-minute median-filtered values.

Statistical analysis

All data are presented as mean values ± standard deviation (SD) or median values with interquartile ranges (IQR) in case of non-normal distribution (tested by the Kolmogorov-Smirnov test). All baseline data, fluids, vasoactive drugs, sedation, fluid balance, creatin-kinase, troponin, liver mitochondrial respiration and skeletal muscle ATP content were compared between groups using a one-way analysis of variance (one-way ANOVA) followed by the Tukey or independent t test. Binary variables were analyzed with Fisher's exact test and Survival proportions between groups were analyzed with the log rank test.

Differences between groups in the evolution over time were assessed by an ANOVA for repeated measurements using *group* as the between-subject factor and *time* as the within-subject factor. This was done first for all three groups, using baseline, end of observation period and end values. Afterwards, the same procedure was repeated for Low- and High-MAP groups only, but including all measurements (baseline, end of observation period, 12, 24, 36 and 48 hours). If a time-group interaction was detected between Low-MAP and High-MAP groups, an independent t test was performed at the end of the experiment (at 48 hours of resuscitation or before death if earlier). In case of non-normal distribution of study variables, time effect was separately measured within each group using the non-parametric Friedman's test. At the end of the observation period and at the end of the experiment, all study groups were compared with Kruskal-Wallis test, and Low-MAP and

High-MAP groups were compared with Mann-Whitney U test, in case of a significant Kruskal-Wallis test. A potential relationship between percentage of time spent below/above different blood pressure levels or administered fluids and end plasma creatinine concentrations/diuresis was evaluated using Spearman's correlation. Paired t-test was performed to compare baseline to end in septic-CG.

A p value of <0.05 was considered significant. The Statistical Package for Social Sciences (SPSS) version 20.0 (SPSS Inc.®; Chicago, IL, USA) was used for statistical analysis.

Results

Mortality

All animals in the septic control group died with a median survival time of 21.8 hours (16.3 - 27.5 hours). One animal each in the Low-MAP and High-MAP groups died during the resuscitation period, respectively, after 38 and 19.5 hours of peritonitis induction (Figure 2).

Treatment intensity

The High-MAP group tended to receive more fluid boluses than the Low-MAP group (p=0.06) and had higher cumulative fluid balance at study end (p=0.001) (Table 1). During the resuscitation period, norepinephrine was administered to 3/8 animals (37.5%) of the Low-MAP and to all animals in the High-MAP Group (p=0.026) (Table 1 and Additional file 2, Figure S1). Dobutamine was administered to 4/8 animals (50.0%) of the Low-MAP and to 3/8 animals (37.5%) of the High-MAP Group (p=1.0). Doses of administered norepinephrine and dobutamine are shown in Table 1.

Hemodynamics, oxygen transport, respiratory and acid-base variables

MAP at the end of the resuscitation period was 56±13 mmHg and 76±17 mmHg respectively, for Low-MAP and High-MAP groups (Table 2). During the resuscitation period, achieved blood pressures overlapped to a certain extent between the groups: Low-MAP animals' blood pressure was ≥75 mmHg during 27.5% of the time, while it was <75 mmHg in the High-MAP group during 8.1% of the time (Additional file 2, Table S1). Neither of the other hemodynamic parameters, nor systemic oxygen delivery, consumption, or arterial lactate differed between Low-MAP and High-MAP groups (Table 2). Arterial pH, bicarbonate, base excess, $PaCO_2$, minute ventilation, arterial oxygen partial pressure and fraction of inspired oxygen relationship and PEEP did not differ between Low-MAP and High-MAP groups (Additional file 2, Table S2).

Inflammatory markers and organ function parameters

IL-6 and TNF-alpha plasma levels increased from baseline to the end of observation period in both Low-MAP and High-MAP groups and decreased during the resuscitation period (Table 3). Platelets, white blood cell count, bands, metamyelocytes, total bilirubin, creatin-kinase and troponin did not differ between Low-MAP and High-MAP groups at study end (Table 3). However, animals allocated to Low-MAP exhibited a higher median plasma creatinine level than animals in the High-MAP group (p=0.021; Table 3 and Additional file 2, Figure S2). The incidence of acute kidney injury after 12 hours of untreated sepsis was, respectively for Low- and High-MAP groups, 50% (4/8) and 38% (3/8) (p=1.00). In the end of study, 57% (4/7) in the Low-MAP group and none in the High-MAP group had AKI (p=0.026).

Mitochondrial function

Isolated skeletal muscle mitochondrial function

In Septic-CG, maximal skeletal muscle mitochondrial respiration (Complex I, State 3) increased, [baseline (mean ± SD): 1357 ± 149 pmol/s/mg; end: 1822 ± 385 pmol/s/mg, paired T- test: p=0.020], whereas complex I-state 4 and RCR (Figure 3a), and complex II-

and IV- dependent skeletal muscle mitochondrial respiration did not change (Figure 3 b-c). There were no changes over time or differences between groups Low-MAP and High-MAP in the respiration of isolated muscle mitochondria (Additional file 2, Figure S3).

Isolated liver mitochondrial function

No significant differences between groups were observed for complex I-, II- and complex IV- dependent respirations of isolated liver mitochondria (Figure 4).

Permeabilized skeletal muscle fibers mitochondrial function

Permeabilized skeletal muscle fibers complex I- and II- dependent respiration did not change in the low-MAP and high-MAP groups (Additional file 2, Table S3). However, Complex IV- state 3 respiration increased in low-MAP and high-MAP groups during resuscitation period (time effect: $p=0.003$) without difference between the groups (Additional file 2, Table S3). Permeabilized skeletal muscle fibers respiration did not change in the Septic-CG (Additional file 2, Table S4).

Skeletal muscle ATP content

Skeletal muscle ATP content was preserved in all groups (Figure 5).

Discussion

Blood pressure targets did not modify mitochondrial respiration or markers of inflammation. However, animals in the low blood pressure group had lower SvO_2 and hemoglobin levels, and a higher incidence of acute kidney injury, while animals in the high blood pressure group had increased net positive fluid balance and vasopressor load during resuscitation. If the animals were not resuscitated after 12 hours, they died with increased

plasma levels of inflammatory markers, increased maximal skeletal muscle Complex I mitochondrial respiration, and with preserved skeletal muscle ATP content.

Few clinical studies addressed prospectively the effect of different levels of MAP during the initial phase of severe sepsis and septic shock resuscitation on disease severity and the development of sepsis-associated organ dysfunction [3-6]. The lack of available data supporting a specific MAP target during the initial resuscitation of septic patients is emphasized by the wide range of MAP goals (60-100 mmHg) applied in randomized controlled clinical trials [16]. Furthermore, experimental studies in sepsis either do not include MAP targets in resuscitation algorithms [22-24] or set the MAP target at 65 mmHg [25,26], consistent with the current recommendation of the Surviving Sepsis Campaign [2]. Nevertheless, no clinical or experimental studies have prospectively addressed the effects of lower MAP targets (50 to 60 mmHg) on outcomes.

Jhanji et al. have shown in a case series of sixteen patients with septic shock that increasing MAP from 60 to 90 mmHg using norepinephrine improved systemic oxygen delivery, and cutaneous PtO_2 but did neither affect sublingual microvascular flow nor urinary output [5]. In our study, systemic oxygen delivery increased similarly in low- and high-MAP groups and systemic oxygen consumption was maintained in all groups. However, four out of seven animals (57%) allocated to Low-MAP group in comparison to none in the High-MAP group presented AKI at the end of study. In an experimental sepsis model using either endotoxemia or fecal peritonitis for 22 hours with mean arterial blood pressure >70 mmHg, Benes et al. demonstrated a similar overall incidence of acute kidney injury of 50% [27]. It has been postulated that higher mean arterial blood pressure levels (app. 70 to 80 mmHg) may be necessary to decrease the development of acute kidney injury in septic shock patients [17]. In our study, animals allocated to a MAP between 75-85 mmHg received both higher amount of fluids and norepinephrine in comparison to animals allocated to Low-MAP group. We found a significant correlation between percentage of time spent with blood pressure >60 mmHg and plasma creatinine in the end of the study, but not between the

amount of administered fluids and plasma creatinine. It seems therefore unlikely that more fluid per se would have been beneficial in the low-MAP group.

Data from experimental [24,28] and clinical studies [15,29-37] have shown that therapeutic attempts, including fluids and vasopressors administration to restore hemodynamic stability during sepsis may be associated with poor outcomes. In critically ill septic and non-septic patients, the cumulative positive fluid balance has been associated with worse outcome [29-31,34,36,37], and the administration of catecholamines, particularly at higher dosages, was associated with derangements on regional blood flow [7,8], adverse side effects [33,35] and with increased likelihood to die [15,32,35]. In our experimental model, such effects were not observed or observable. However, whether renal hyperfiltration as a result of high blood pressure and volume load may cause delayed harm to septic kidneys should be addressed in further studies.

Sepsis may result in various changes in mitochondrial enzyme activity and tissue ATP. Muscle complex I activity decreased and complex IV activity increased in patients who subsequently died in sepsis [13], whereas an acute endotoxin challenge increased muscle complex I activity in healthy volunteers [38]. Reduced tissue ATP levels have been observed in various tissues and sepsis models, but not consistently. Quadriceps muscle ATP was reduced in non-survivors of sepsis but not in survivors [13]. In severe sepsis, intercostal muscle ATP was normal, while leg muscle ATP was decreased [39]. In experimental sepsis decreased ATP content has been reported in the rat liver and the kidney but not in the muscle [40,41] and in the liver in porcine bacterial sepsis [42]. In porcine endotoxemia, reduced ATP content in the intestinal mucosa but not muscularis [43], as well as unchanged intestinal wall ATP has been reported [44].

Instead of respiratory chain enzyme activities, we assessed the mitochondrial respiration using substrate-induced maximal oxygen consumption. We found no signs of impaired mitochondrial respiration in the muscle isolated mitochondria or in the

permeabilized muscle fibers and the muscle ATP levels remained unchanged even in the untreated septic group with 100 % mortality. In contrast, complex I-dependent state 3 respiration of isolated muscle mitochondria increased in untreated sepsis, and complex IV-dependent state 3 respiration increased in the permeabilized muscle fibers in the resuscitated septic groups. In the presence of unchanged RCR and muscle ATP content, this may be related to increased metabolic activity and increased ADP transport into mitochondria [45]. One mechanism which may help to maintain ATP levels in sepsis is increased phosphocreatine breakdown which provides fuel for various cellular processes [41].

We observed previously that delay in starting resuscitation was associated with decreased muscle ATP levels at the time of death [28], in contrast to the well preserved muscle ATP levels in the present study. Deaths in that study occurred later, after a period of resuscitation, whereas in the present study the deaths occurred earlier, and all except two resuscitated animals survived. The well-preserved muscle ATP levels in the dying animals in the present study are likely to be related to the earlier death.

Resuscitation to a low compared to a high-MAP target had no effect on isolated skeletal muscle mitochondrial respiration, or on isolated liver mitochondrial respiration, suggesting that neither a higher organ perfusion pressure nor increased vasopressor load had an effect. We previously observed that the need to support hemodynamics with norepinephrine in a porcine fecal peritonitis of shorter duration was associated with lower hepatic complex II-dependent state 4 respiration, while state 3 and RCR were not altered [20]. We also found in endotoxemic pigs that norepinephrine improved hepatic mitochondrial respiratory efficiency [46]. Similarly, norepinephrine tended to increase ileal wall mitochondrial complex I activity in an endotoxemic sheep model [47]. The lack of difference in the liver mitochondrial respiration between the groups in the present study suggests that the effect of norepinephrine on the liver is model-dependent or related to the duration of exposure.

Our study has limitations. We evaluated in this study only two ranges of mean arterial blood pressure for the initial resuscitation of severe sepsis and septic shock. Second, respiration rates were not normalized to the citrate synthase activity (mitochondrial content) [48]. Reduced skeletal muscle mitochondrial enzyme activity was observed in patients with sepsis and multiple organ failure if expressed per wet muscle weight but not if normalized to citrate synthase activity, a marker of mitochondrial content [39]. This may suggest decreased mitochondrial mass rather than specific inhibition of the mitochondrial enzymes in sepsis. Consequently, our finding of increased permeabilized muscle fibers complex IV-respiration may be due to variations in mitochondrial content. Third, the final skeletal muscle and liver samples in the animals that died before completing the 48-hour resuscitation period were taken when mean arterial blood pressure approached 30 mmHg, and therefore may potentially reflect a "near death" state rather than unconfounded differences between the study groups. Fourth, we did not evaluate the microcirculation in animals resuscitated with low MAP-target. It is possible that lower MAP is associated with decreased microvascular blood flow [49]. Fifth, we studied a small number of animals per group. Therefore, there is a risk of a type II error, and we cannot exclude true, small, undetected differences between groups. Finally, in this model of fecal peritonitis in young, healthy pigs, MAP thresholds may not have the same importance for tissue oxygenation as in older septic patients.

Conclusions

In this abdominal sepsis model with 100% mortality unless treatment is installed, the MAP targets of 50-60 mmHg vs. 75-85 mmHg during resuscitation did not result in differences in the inflammatory response skeletal muscle ATP content or mitochondrial respiration. While targeting a lower mean arterial blood pressure was associated with increased incidence of acute kidney injury, targeting a higher MAP resulted in increased net positive fluid balance and vasopressor load during resuscitation. The long-term effects of using lower MAP targets

on kidney function and whether the resulting increased fluid balance and vasopressor load using higher MAP targets are of relevance for recovery after initial resuscitation need to be evaluated in further studies.

Key messages

- Targeting a mean arterial blood pressure between 50 and 60 mmHg in septic shock was associated with increased incidence of acute kidney injury.

- Targeting a higher MAP (75 to 85 mmHg) during resuscitation did not ameliorate the inflammatory response and resulted in increased net positive fluid balance and vasopressor load during resuscitation.

- Mean arterial blood pressure targets did not modify the inflammatory response, the skeletal muscle ATP content and the mitochondrial respiration.

- Unresuscitated septic animals died with increased plasma levels of inflammatory markers, increased maximal skeletal muscle Complex I mitochondrial respiration, and with preserved skeletal muscle ATP content.

Abbreviations

AKI: acute Kidney Injury, BL: baseline, CBFI: carotid artery flow index, CVP: central venous pressure, DO_2: systemic oxygen delivery, EOP: 12 hours after peritonitis induction, FBFI: femoral artery flow index, FiO_2: fractional inspiratory oxygen concentration, G50%: glucose 50%, High-MAP: resuscitation targeting MAP between 75 and 85 mmHg, IL-6: plasma interleukin-6, Low-MAP: resuscitation targeting MAP between 50 and 60 mmHg, MAP: mean arterial blood pressure, MPAP: mean pulmonary artery pressure, $PaCO_2$: arterial carbon dioxide partial pressure, PAOP: pulmonary artery occlusion pressure, PEEP: positive end-expiratory pressure, RP: resuscitation period, Septic-CG: septic control group, SvO_2: mixed

venous oxygen saturation, SVRI: systemic vascular resistance index, TNF-α: tumor necrosis factor-α, VO_2: systemic oxygen consumption.

Competing interests

The authors declare have no competing interests

Authors' contributions

TDC, JT and SMJ, devised the study protocol. TDC, MV, SD and SMJ initiated and performed all animal experiments. MV and SD performed mitochondria-related experiments. TDC, MV and SMJ analyzed the data. TDC and MV wrote the first manuscript draft. SMJ, JT and ES critically revised the manuscript. All authors approved the final manuscript.

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Figure 1. Study design for Low and High MAP groups. BL, baseline; PI, peritonitis induction; EOP, end of observation period (before starting resuscitation); End, end of the experiment (at 48 hours of resuscitation or before death if earlier); Black circles, samples from the right quadriceps muscle to assess mitochondrial function and quantification of skeletal muscle ATP content; Black triangle, first dose of intravenous antibiotic (piperacillin/tazobactam, 2.25 g, 8-hourly); Black square, additional tissue samples from the liver for mitochondrial function analysis at the end of the experiment (66 hours or before death, if earlier). Animals allocated to the septic control group (Septic-CG) were followed without resuscitation after peritonitis induction until death.

Figure 2. Kaplan-Meyer Survival Curve.

Figure 3. Isolated skeletal muscle mitochondrial respiration during unresuscitated sepsis. Complex I-, II- and IV- isolated skeletal muscle mitochondrial respiration in septic control animals from baseline to end (represented by connecting lines for each pig, n=8). State 3 and state 4 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). *p, paired t- test: p=0.02.

Figure 4. Isolated liver mitochondrial respiration. Comparison of isolated liver mitochondrial respiration in septic control animals and septic animals resuscitated to low-MAP and high-MAP targets (n=8). State 3 and 4 oxygen consumption is expressed as pmol/second/mg protein. State 3, active respiration after addition of ADP. State 4, respiration after consumption of ADP. RCR, respiratory control ratio (oxygen consumption of state 3/state 4). No significant differences were found between the groups (One-way ANOVA, p>0.05). Horizontal lines represent mean values. Filled circles represent animals that died early.

Figure 5. Skeletal muscle tissue ATP concentrations in septic controls and animals resuscitated to low-MAP and high-MAP targets. BL, baseline; EOP, end of observation period and END, end of the experiment (at 48 hours of resuscitation or before death if earlier). p>0.05 for all comparisons. The black horizontal bars represent the mean values. Filled circles represent animals that died early.

Table 1. Administered treatment, fluid output and balance during the study.

Variables / Study group	Septic-CG	Low-MAP	High-MAP	p value
Propofol (mg/kg/h)	5.0 ± 1.0	5.8 ± 0.7	5.8 ± 0.5	0.11 [a]
Fentanyl (mcg/kg/h)[†]	4.0 ± 1.6	3.9 ± 0.8	4.4 ± 0.9	0.62 [a]
Midazolam (mg/kg/h)	0.08 ± 0.03	0.06 ± 0.02	0.07 ± 0.04	0.38 [a]
Fluid bolus (ml/kg/h) [††][*]				
Median		0.7	2.0	0.06 [b]
IQR		0.5 - 2.0	1.6 - 2.8	
Norepinephrine				
n/n group (%)		3/8 (37.5)	8/8 (100.0)	0.026 [c]
Median dose (mcg/kg/min)		0.00	0.38	0.009 [b]
IQR		0.00 - 0.25	0.21 - 0.56	
Dobutamine				
n/n group (%)		4/8 (50.0)	3/8 (37.5)	1.00 [c]
Median dose (mcg/kg/min)		0.06	0.00	0.45 [b]
IQR		0.00 - 3.16	0.00 - 1.00	
Total volume received (ml/kg/h)	1.5 ± 0.0	3.6 ± 0.8 [§]	4.7 ± 1.4 [#]	0.001 [a]
Urine output (ml/kg/h)	0.6 ± 0.1	1.0 ± 0.2 [§]	1.1 ± 0.2 [#]	0.001 [a]
Gastric tube output (ml/kg/h)	0.3 ± 0.2	0.3 ± 0.3	0.3 ± 0.2	0.95 [a]
Final balance [‖] (ml/kg/h)	0.6 ± 0.3	2.3 ± 0.7 [§]	3.3 ± 1.0 [#¥]	0.001 [a]
Final balance [‖] (L)	0.5 ± 0.4	5.2 ± 1.1 [§]	6.7 ± 1.4 [#¥]	0.001 [a]

Values are mean ± SD or median (IQR) when indicated. †, sum of basic fentanyl infusion with additional boluses; *, administered during the 48 hours resuscitation period; ††, sum of Ringer's Lactate and hydroxyethyl starch; ‖, final balance = (Ringer's Lactate basal infusion + G50% basal infusion + Ringer's Lactate bolus + hydroxyethyl starch bolus) – (urine output + gastric tube output); a, one-way ANOVA. Tukey post hoc test after ANOVA, comparisons significant at the 0.05 level: ¥, Low-MAP vs. High-MAP; §, Low-MAP vs. Septic-CG; #, High-MAP vs. Septic-CG; b, Mann-Whitney test; c, Fisher's exact test.

Table 2. Systemic and regional hemodynamics and arterial lactate level.

Variables	Group	BL	EOP	RP 12h	RP 24h	RP 36h	RP 48h	END	p value [¶]
MAP	Septic CG	78 ± 9	68 ± 22					29 ± 4	<0.001 [a]
(mm Hg)	Low MAP	65 ± 9	81 ± 16	72 ± 13	67 ± 12	61 ± 6	59 ± 8	56 ± 13	<0.001 [a]
	High MAP	63 ± 7	76 ± 11	86 ± 5	81 ± 7	82 ± 4	82 ± 3	76 ± 17	
Heart rate	Septic CG	104 ± 17	193 ± 41					216 ± 25	<0.001 [a]
(beats/min)	Low MAP	93 ± 11	166 ± 23	165 ± 14	156 ± 23	141 ± 28	122 ± 28	130 ± 46	0.84 [b]
	High MAP	105 ± 23	173 ± 31	163 ± 22	155 ± 31	138 ± 28	117 ± 29	117 ± 29	
MPAP	Septic CG	12 ± 2	16 ± 3					11 ± 2	<0.001 [a]
(mm Hg)	Low MAP	15 ± 3	18± 2	20 ± 5	21 ± 5	23 ± 6	23 ± 5	23 ± 4	0.19 [b]
	High MAP	13 ± 1	18 ± 3	19± 2	21 ± 1	24 ± 2	24 ± 3	27 ± 9	
CVP	Septic CG	1 ± 1	1 ± 1					0 ± 1	<0.001 [a]
(mm Hg)	Low MAP	4 ± 2	2 ± 2	3 ± 2	5 ± 3	7 ± 4	8 ± 4	9 ± 4	0.41 [b]
	High MAP	2 ± 1	1 ± 1	3 ± 2	6 ± 1	7 ± 1	9 ± 2	10 ± 3	
SVRI	Septic CG	692 ± 129	966 ± 197					600 ± 238	0.082 [a]
(mm Hg	Low MAP	589 ± 60	923 ± 203	668 ± 131	451 ± 131	373 ± 56	349 ± 92	319 ± 119	0.19 [b]
L/Kg/min)	High MAP	553 ± 87	1017 ± 304	698 ± 114	525 ± 185	488 ± 109	510 ± 107	475 ± 140	
Cardiac	Septic CG	115 ± 27	70 ± 21					53 ± 19	<0.001 [a]
Index	Low MAP	105 ± 13	88 ± 22	104 ± 13	143 ± 31	145 ± 13	149 ± 19	150 ± 18	0.38 [b]
(ml/kg/min)	High MAP	112 ± 23	77 ± 16	122 ± 29	165 ± 78	160 ± 36	150 ± 31	142 ± 35	
SvO_2	Septic CG	59 ± 8	53 ± 12					35 ± 11	0.001 [a]
(%)	Low MAP	48 ± 2	56 ± 9	58 ± 9	56 ± 9	60 ± 6	61 ± 7	55 ± 17	0.06 [b]
	High MAP	50 ± 8	51 ± 3	61 ± 6	66 ± 3	66 ± 5	67 ± 5	60 ± 20	
DO_2	Septic CG	626 ± 163	592 ± 158					421 ± 113	< 0.001 [a]
(ml/min)	Low MAP	526 ± 118	663 ± 172	682 ± 100	748 ± 99	712 ± 93	687 ± 149	687 ± 149	0.26 [b]
	High MAP	541 ± 92	566 ± 78	749 ± 118	919 ± 444	862 ± 201	744 ± 135	702 ± 173	
VO_2	Septic CG	241 ± 27	267 ± 44					280 ± 91	0.293 [a]
(ml/min)	Low MAP	277 ± 46	284 ± 38	281 ± 28	298 ± 27	282 ± 35	254 ± 39	254 ± 39	0.95 [b]
	High MAP	269 ± 50	273 ± 44	280 ± 49	306 ± 120	276 ± 34	247 ± 30	256 ± 38	
CBFI	Septic CG	6.7 ± 1.3	3.9 ± 0.5					2.8 ± 0.6	<0.001 [a]

		BL	EOP	RP 12h	RP 24h	RP 36h	RP 48h	END	¶ p value
(ml/kg/min)	Low MAP	6.9 ± 2.2	4.9 ± 2.1	6.4 ± 1.9	8.5 ± 2.1	8.4 ± 1.6	8.0 ± 1.6	7.6 ± 1.8	0.55 [b]
	High MAP	5.4 ± 1.6	4.1 ± 1.2	5.6 ± 1.6	6.7 ± 1.6	6.8 ± 1.8	6.2 ± 1.8	5.8 ± 2.0	
FBFI	Septic CG	3.5 ± 0.7	1.7 ± 0.5					1.2 ± 0.3	<0.001 [a]
(ml/kg/min)	Low MAP	3.9 ± 1.6	1.7 ± 0.8	2.2 ± 0.4	3.4 ± 1.0	3.8 ± 0.6	3.9 ± 0.9	3.7 ± 1.0	0.33 [b]
	High MAP	3.1 ± 0.9	1.4 ± 0.6	2.2 ± 0.7	3.2 ± 1.0	3.2 ± 0.8	3.4 ± 0.7	3.1 ± 1.0	
Arterial	Septic CG	0.7 ± 0.1	1.9 ± 1.2					4.1 ± 2.0	<0.001 [a]
Lactate	Low MAP	1.3 ± 0.6	1.0 ± 0.4	1.2 ± 0.3	1.1 ± 0.4	1.1 ± 0.5	0.9 ± 0.2	1.0 ± 0.4	0.19 [b]
(mmol/L)	High MAP	0.9 ± 0.3	1.0 ± 0.2	1.2 ± 0.5	1.2 ± 0.5	1.1 ± 0.2	1.1 ± 0.3	1.5 ± 1.4	

Values are mean ± SD. BL, baseline; EOP, 12 hours after peritonitis induction (End of observation period for Low-MAP and High-MAP groups); RP, resuscitation period; END, end of the experiment (at 48 hours of resuscitation or before death if earlier); MAP, mean arterial blood pressure; MPAP, mean pulmonary artery pressure; PAOP, pulmonary artery occlusion pressure; CVP, central venous pressure; SVRI, systemic vascular resistance index; SvO_2, mixed venous oxygen saturation; DO_2, systemic oxygen delivery; VO_2, systemic oxygen consumption; CBFI, carotid artery flow index; FBFI, femoral artery flow index; ¶, p value: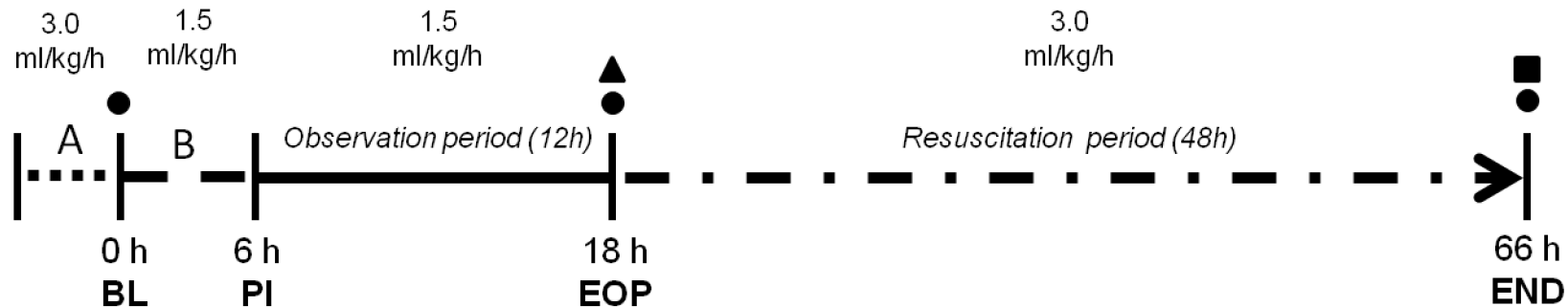 a, time-group interaction with repeated measures ANOVA including BL, EOP and END for all study groups (Septic-CG, Low-MAP and High-MAP); b, time-group interaction with repeated measures ANOVA including BL, EOP, RP 12h, RP 24h, RP 36h and RP 48h for Low-MAP and High-MAP groups.

Table 3. Inflammatory markers and organ function analysis.

Variables	Group	BL	EOP	End	p value [¶]
IL-6 (pg/ml)	Septic-CG	24 ± 44	781 ± 410 *	1785 ± 819	<0.001 [a]
	Low-MAP	20 ± 26	291 ± 165	57 ± 59 [#]	0.61 [b]
	High-MAP	5 ± 10	258 ± 107	95 ± 177	
TNF-alpha (pg/ml)	Septic-CG	102 ± 75	290 ± 145 *	410 ± 160	<0.001 [a]
	Low-MAP	48 ± 16	149 ± 55	93 ± 45 [#]	0.52 [b]
	High-MAP	57 ± 39	130 ± 89	76 ± 65	
Hemoglobin (mg/dL)	Septic-CG	9.4 ± 0.8	15.4 ± 1.6	14.7 ± 1.8	<0.001 [a]
	Low-MAP	9.3 ± 0.6	14.7 ± 0.7	8.4 ± 0.8	0.014 [b]
	High-MAP	9.2 ± 0.4	14.8 ± 1.2	10.0 ± 1.2	0.008 [c]
Platelets (x 10^9/L)	Septic-CG	296 ± 97	131 ± 66 *	98 ± 53	0.18 [a]
	Low-MAP	255 ± 48	176 ± 41	115 ± 42 [#]	0.19 [b]
	High-MAP	323 ± 102	131 ± 66	98 ± 53	
White blood cell count (x 10^9/L)	Septic-CG	14.0 ± 1.6	9.7 ± 3.0 *	10.7 ± 2.8	0.013 [a]
	Low-MAP	18.4 ± 5.2	12.6 ± 4.1	16.9 ± 5.2 [#]	0.014 [b]
	High-MAP	16.9 ± 3.5	18.2 ± 5.0	18.9 ± 4.7	0.46 [c]
Bands (%)	Septic-CG	2.8 ± 4.1	38.1 ± 8.2 *	28.6 ± 19.8	0.021 [a]
	Low-MAP	11.6 ± 15.6	57.2 ± 9.6	23.4 ± 18.2 [#]	0.05 [b]
	High-MAP	0.8 ± 0.7	63.9 ± 8.6	27.4 ± 10.0	0.59 [c]
Metamyelocytes (%)	Septic-CG	0.0 ± 0.0	14.3 ± 12.1 *	12.5 ± 7.5	0.001 [a]
	Low-MAP	0.0 ± 0.0	6.2 ± 3.3	1.9 ± 3.6 [#]	0.93 [b]
	High-MAP	0.0 ± 0.0	6.5 ± 4.1	1.4 ± 3.0	
Creatinine (mg/dl) [Median (IQR)]	Septic-CG	0.5 (0.4-0.8)	1.6 (1.4-1.6) *	2.3 (2.0-2.6)	< 0.03 [d]
	Low-MAP	0.9 (0.8-1.0)	1.2 (1.0-1.7)	1.4 (1.0-2.1) [#]	0.295 [e]
	High-MAP	0.9 (0.8-0.9)	1.1 (0.9-1.2)	0.9 (0.7-1.0)	0.001 [f]
					0.021 [g]
Total bilirubin (µmol/L)	Septic-CG	1.6 ± 1.2	1.0 ± 0.0 *	1.1 ± 0.4	>0.60 [d]
	Low-MAP	1.5 ± 1.1	1.4 ± 1.1	1.6 ± 1.5 [#]	
	High-MAP	1.3 ± 0.5	1.1 ± 0.4	1.4 ± 0.7	
Creatin-Kinase (U/L) [Median (IQR)]	Septic-CG			475 (360-779)	0.045 [f]
	Low-MAP			253 (232-470)	0.28 [g]
	High-MAP			211 (183-332)	
Troponin (µg/L) [Median (IQR)]	Septic-CG			0.015 (0.013-0.032)	0.091 [f]
	Low-MAP			0.008 (0.05-0.028) [#]	
	High-MAP			0.007 (0.006-0.02)	

Values are mean ± SD or median (IQR) when indicated. BL, baseline; EOP, 12 hours after peritonitis induction (End of observation period for Low-MAP and High-MAP groups); END, end of the experiment (at 48 hours of resuscitation or before death if earlier); #, data available for 7 animals; *, data available for 5 animals, in 1 animal measured after 24 hours of PI and in 4 animals after 18 h of PI; ¶, p value: a, time-group interaction with repeated

measures ANOVA including BL, EOP and END for all study groups (Septic-CG, Low-MAP and High-MAP); b, time-group interaction with repeated measures ANOVA including BL, EOP and END for Low-MAP and High-MAP groups; c, independent t test between Low-MAP and High-MAP groups at the END; d, time effect within each group (Septic-CG, Low-MAP and High-MAP) separately accessed by the non-parametric Friedman's test including BL, EOP and END; e, Kruskal-Wallis test at the EOP; f, Kruskal-Wallis test at the End; g, Mann-Whitney U test between Low-MAP and High-MAP groups at the END.

Additional files

Additional file 1. Word text file containing additional information about the methods, along with related references.

Additional file 2. A Word file containing four tables and three figures. Table S1 indicates the percentage of time spent at different blood pressure ranges; Table S2 describes the acid-base-balance parameters and respiratory system variables; Table S3 gives permeabilized skeletal muscle fibers maximal mitochondrial respiration (state 3) in animals resuscitated to low and high-MAP targets, and Table S4 lists permeabilized skeletal muscle fibers maximal mitochondrial respiration (state 3) in septic control group. Figure S1 shows the mean arterial blood pressure and norepinephrine administration over the study period in animals randomized to Low and High-MAP groups; Figure S2 shows a scatter plot of plasma creatinine of three study groups and Figure S3 shows isolated skeletal muscle mitochondrial respiration in animals resuscitated to Low and high MAP.

Figure 1

Figure 2

a) Complex - I

Complex I - State 3

Complex I - State 4

Complex I - RCR

b) Complex - II

Complex II - State 3

Complex II - State 4

Complex II - RCR

c) Complex - IV

Complex IV - State 3

Figure 3

a) Complex - I

Complex I - State 3

Complex I - State 4

Complex I - RCR

b) Complex - II

Complex II - State 3
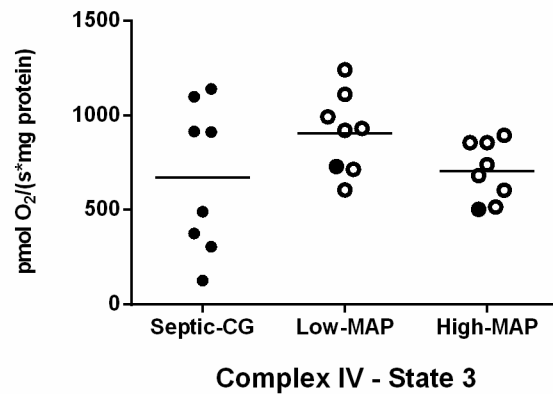
Complex II - State 4

Complex II - RCR

c) Complex - IV

Complex IV - State 3
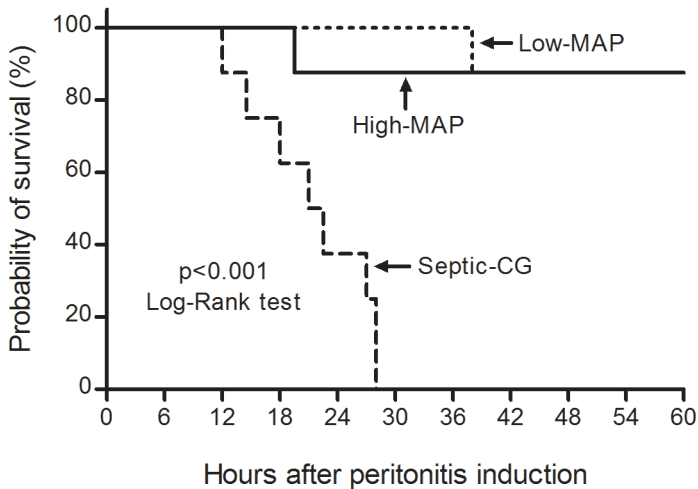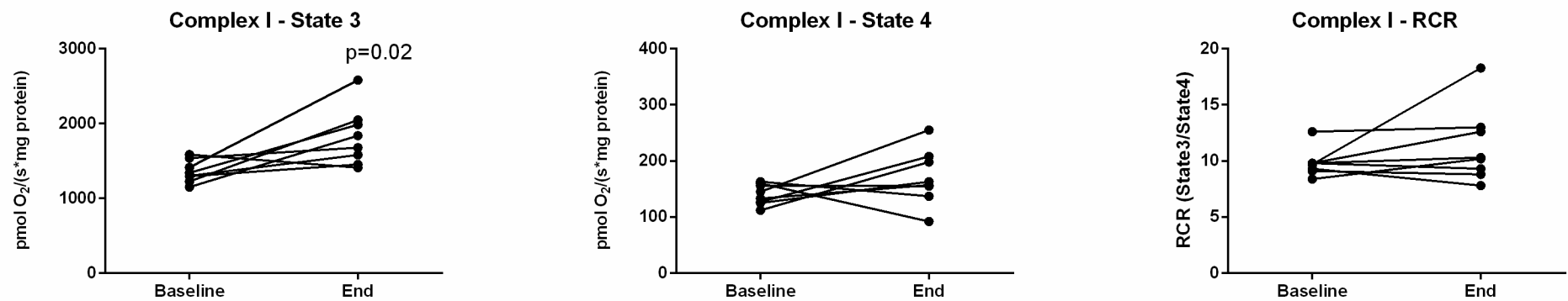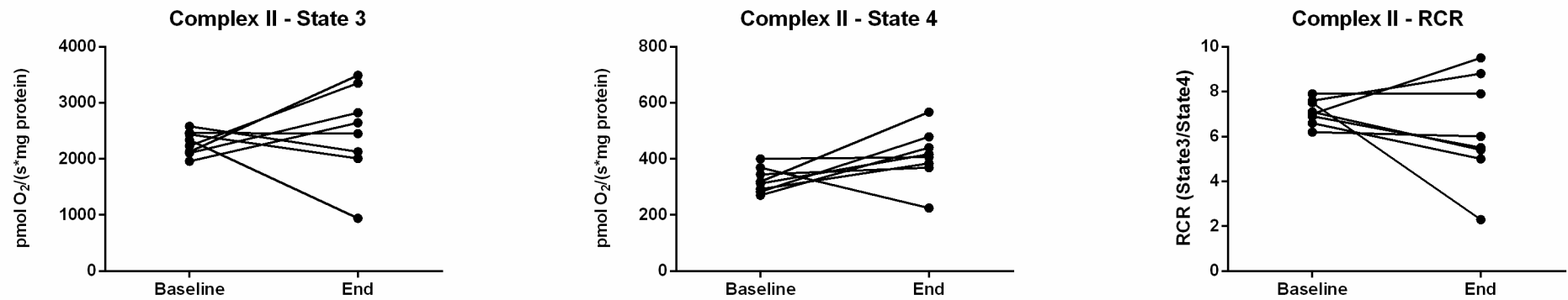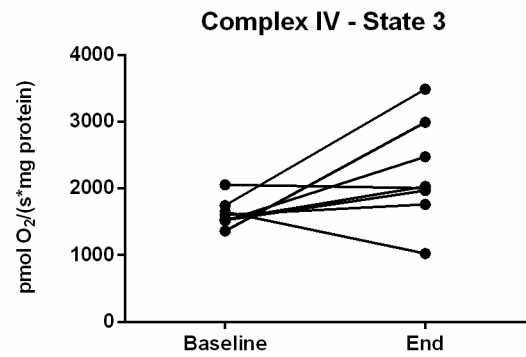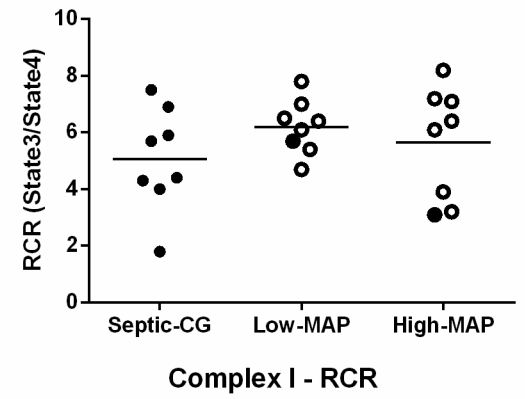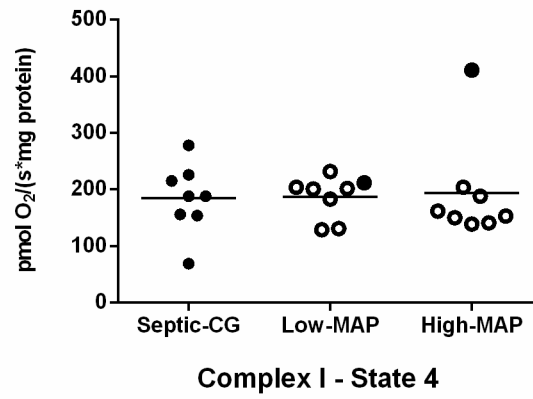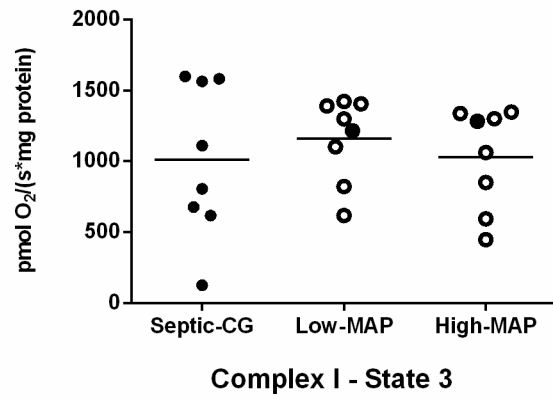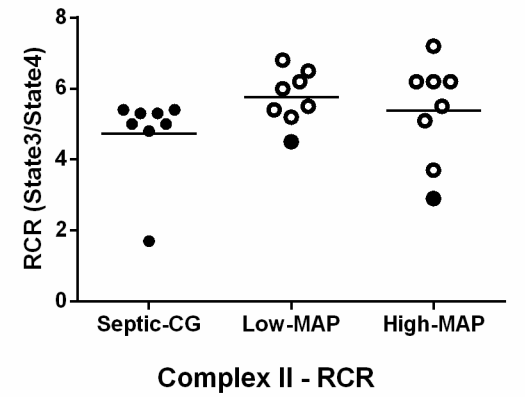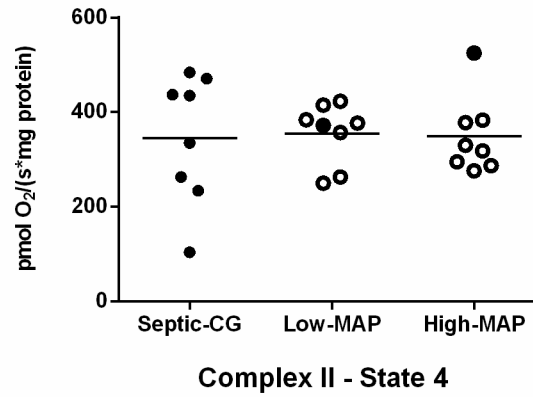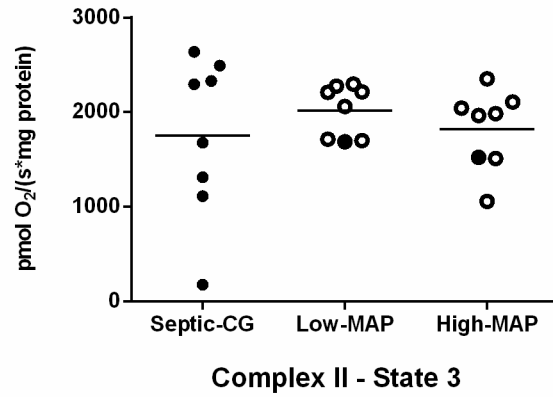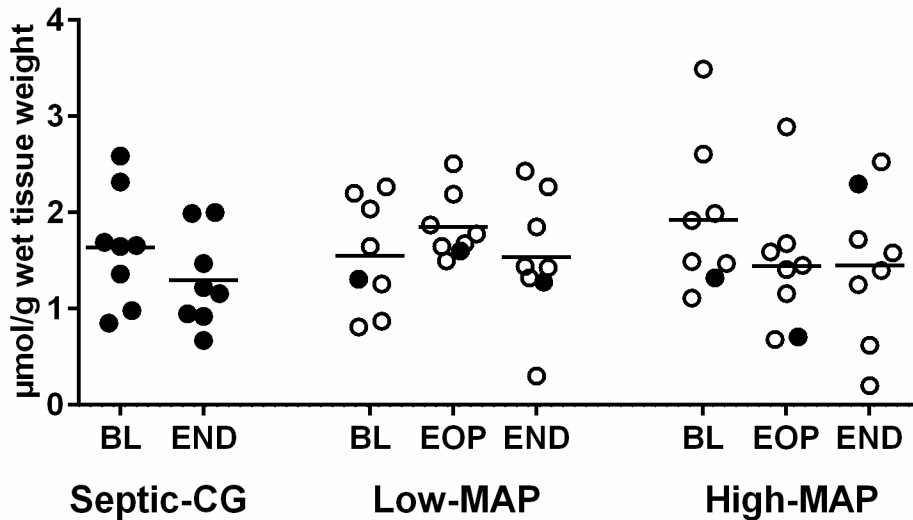
Figure 4

Figure 5

Additional files provided with this submission:

Additional file 1: Additional file 1.docx, 282K
http://ccforum.com/imedia/2092397200906649/supp1.docx
Additional file 2: Additional file 2.docx, 1910K
http://ccforum.com/imedia/6750984579066492/supp2.docx