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# Severity of polymicrobial sepsis modulates mitochondrial function in rat liver

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ABSTRACT

Mitochondrial dysfunction is assumed to be an important contributor to multi organ dysfunction syndrome. Here, the effects of varying degrees of sepsis on hepatic mitochondrial function were investigated. Moderate or more severe sepsis was induced in rats using a colon ascendens stent peritonitis (CASP)-model (16 G and 14 G stent respectively).
Respiratory control ratio (RCR) was significantly higher in the 16 G-group and unchanged in the 14 G-group compared with healthy controls. The ADP/O ratio was similar in all groups.
Our results indicate that different severities of sepsis differently influence the mitochondrial function, which could be a sign of adaptive reaction.



## 1. Introduction

Despite the evolution of new technologies and the development of new drugs, treatment of sepsis and septic shock remains a major challenge. Over the last decade, the overall mortality rate under these conditions remained high (32% in sepsis and 53–72% by sepsis-induced multi organ dysfunction syndrome (MODS) depending on the affected organs) (Marshall et al., 2005; Levy et al., 2012). Microcirculatory and mitochondrial dysfunction are considered to be the main pathophysiological mechanisms in septic shock and MODS (Balestra et al., 2009; Brealey et al., 2002). When circulation becomes unstable, e.g. in septic shock, blood flow is redistributed to maintain the oxygenation of vital organs as heart or brain, while the microcirculation in less essential organs like splanchnic region, kidney and liver is critically reduced. It has recently been suggested that impaired oxygen supply to the liver and not a direct interaction of hepatocytes with inflammatory mediators is responsible for mitochondrial dysfunction (Weidinger et al., 2013). Therapeutic options to improve microcirculation in sepsis include volume resuscitation, catecholamines, and steroids, but these treatment strategies are frequently ineffective. More than 90% of cellular oxygen is consumed by mitochondrial oxidative phosphorylation, which is considered to be the main mechanism of cellular energy generation. Nevertheless, little is known about the role of mitochondrial dysfunction in pathological processes like sepsis. While organ replacement therapies exist for lungs and kidneys, effectively replacing liver function remains a great challenge (Tritto et al., 2012; Fleming, 2011; Brodie and Bacchetta, 2011). Therefore, it is of great importance to understand the pathological processes inducing liver failure in order to prevent hepatic dysfunction. The studies available on hepatic mitochondrial function in sepsis are controversial (Jeger et al., 2013) with impaired (Lowes et al., 2013; Brealey et al., 2004; Larche et al., 2006), unaffected (Trumbeckaite et al., 2013; Mittal et al., 2011; Llesuy et al., 1994), or even improved mitochondrial function (Takeyama et al., 1990; Kantrow et al., 1997; Kozlov et al., 2006, 2007) being reported. One possible reason for this heterogeneity could be the wide spectrum of septic models used, with varying severity of sepsis. However, an examination of different degrees of sepsis severity in one clinically relevant model and under the same experimental conditions has still not been reported. Moreover, different parameters describing mitochondrial function are used and evidence suggests that mitochondrial function changes in the progress of the inflammatory response. It has been shown that mitochondrial respiration improves in the early phase of acute critical illness such as sepsis, and deteriorates during the late phase (Singer et al., 2004). So far, there are only few studies describing time dependent mitochondrial function during sepsis (Brealey et al., 2004). Therefore, a necessary precondition to comparing sepsis severities is the examination at the same time point. Deeper insight into the underlying mechanisms could help to develop therapeutic strategies for sepsis treatment. The aim of the present study was to analyze the effect of sepsis severity on hepatic mitochondrial function in rats.

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## 2. Materials and methods

### 2.1. Animals

The study was approved by the local Animal Care and Use Committee (Landesamt für Natur, Umwelt und Verbraucherschutz, Recklinghausen, Germany) and all experiments were performed in accordance with NIH guidelines for animal care.

31 adult male Wistar rats (353 ± 19 g body weight) were randomized into four groups: group 1 (n = 8): control group (healthy rats), group 2 (n = 7): sham animals (laparotomy with the stent fixed on the outside wall of the gut), group 3 (n = 8): mild CASP (colon ascendens stent peritonitis) with 16 G-stent, group 4 (n = 8): severe CASP with 14 G-stent.

### 2.2. CASP/sham operation

Polymicrobial sepsis was induced by the implantation of a stent into the large intestine (colon ascendens stent peritonitis surgery — CASP) as previously reported (Stübs et al., 2013). Sepsis severity was adjusted by implanting stents with two different diameters (16 G and 14 G).

Anesthesia was induced and maintained by the volatile anesthetic sevoflurane (3.0 vol.%, $FiO_2$ 0.5) and the opioid buprenorphine (0.05 mg/kg s.c.). An approximately 2 cm median incision of the belly (laparotomy) was performed, the gut was located, and an 8 mm 16 G or 14 G stent was fixed, penetrating the colonic wall by 1 cm. Cautious palpation of the gut and gentle milking of feces into the stent ensured a constant leakage into the belly. Sham animals were anesthetized and laparotomized as stated above but the stent was fixed on the outside wall of the gut without penetrating it. The gut was carefully returned to the abdominal cavity and the abdominal wall was closed. The animals were placed in a warmed cage to recover from anesthesia. 24 h after CASP or sham operation, animals were euthanized with pentobarbital i.p. (120 mg/kg). Blood was obtained by cardiac puncture. A laparotomy was performed and the liver was harvested and immediately placed into 4 °C cold isolation buffer (200 mM mannitol, 50 mM sucrose, 5 mM $KH_2PO_4$, 5 mM morpholinepropanesulfonic acid (MOPS), 0.1% fatty acid-free bovine serum albumin (BSA), 1 mM EDTA, pH 7.15).

The abdominal cavity was examined for signs of inflammation (peritonitis with hemorrhage, fibrin plaques, edema and adhesions in the abdominal cavity). As described previously (Stübs et al., 2013), we used a numeric score sheet (Septic Rat Severity Score: SRSS-System — see Table 1) to determine the severity of sepsis (loss of body weight, appearance, spontaneous behavior, provoked behavior, breathing frequency, expiratory breathing sound, abdominal palpation and condition of droppings). Animals scoring more than 10 points were euthanized. A single investigator performed scoring.

### 2.3. Isolation of mitochondria

Mitochondria were isolated as previously described with minor modifications (Heinen et al., 2008). Briefly, liver tissue was placed in 4 °C cold isolation buffer, minced into 2–3 $mm^3$ pieces, rinsed twice in isolation buffer to remove traces of blood and homogenized. Cell debris was removed by centrifugation at 900 rcf and 4 °C for 10 min. Supernatant was centrifuged at 3250 rcf for 10 min to obtain the mitochondrial pellet. The pellet was resuspended in isolation buffer. Mitochondrial protein concentration was determined by the Lowry method (Lowry et al., 1951) with bovine serum albumin as a standard.

### 2.4. Measurement of mitochondrial respiratory rates

Mitochondrial oxygen consumption was measured at 30 °C using a Clark-type electrode (model 782, Strathkelvin instruments, Glasgow, Scotland). Isolated mitochondria were suspended in respiration medium (KCl 130 mM, $K_2HPO_4$ 5 mM, MOPS 20 mM, EGTA 2.5 mM, $Na_4P_2O_7$ 1 μM, BSA 0.1%, pH 7.15) to yield a protein concentration of 2 mg/ml.

Mitochondrial state 2 respiration was recorded in the presence of either complex I substrates glutamate and malate (both 2.5 mM, G-M) or complex II substrate succinate (10 mM, S). Mitochondrial enzyme glutamate dehydrogenase oxidizes glutamate to α-ketoglutarate, a

**Table 1**
Septic rat severity score.

| Examination | Results | |
|---|---|---|
| Body weight | Weight loss in % | % < 5 ⇒ 0 P |
| | | % 5–15 ⇒ 2 P |
| | | % 15–20 ⇒ 3 P |
| | | % > 20 ⇒ 10 P |
| Appearance | 1. Normal appearance, fur smooth, clean | ⇒0 P |
| | 2. Slight grooming deficiency, rough fur | ⇒1 P |
| | 3. Increasing grooming deficiency, rings around eyes, anus | ⇒2 P |
| | 4. Clear grooming deficiency, crusty eyes, bedding sticks to anus | ⇒3 P |
| Spontaneous behavior | 1. Rat investigates cage, active | ⇒0 P |
| | 2. Rat remains in one place, movement of entire body present | ⇒1 P |
| | 3. Hunched posture, swaying gait | ⇒3 P |
| | 4. Immobile, lateral position | ⇒10 P |
| Provoked behavior | 1. Rat flees when opening cage, strong muscle tonus | ⇒0 P |
| | 2. Rat flees when hand approaches | ⇒1 P |
| | 3. Rat flees when touched | ⇒2 P |
| | 4. No flight reaction | ⇒3 P |
| Breathing frequency | Difference in % | % < 10 ⇒ 0 P |
| | | % 10–20 ⇒ 1 P |
| | | % 20–50 ⇒ 2 P |
| | | % > 50 ⇒ 3 P |
| Expiratory breathing sound | No | ⇒0 P |
| | Yes | ⇒1 P |
| Abdominal palpation | 1. No pain when applying pressure, soft abdomen | ⇒0 P |
| | 2. Slight reaction to abdominal palpation, soft abdomen | ⇒1 P |
| | 3. Clear pain reaction to abdominal palpation, abdominal resistance | ⇒2 P |
| | 4. Clear pain reaction to abdominal palpation, hard abdomen | ⇒3 P |
| Condition of droppings | 1. A lot of normal droppings in cage, defecating during examination | ⇒0 P |
| | 2. A lot of droppings in cage, droppings with blood, runny or mucous | ⇒1 P |
| | 3. Few droppings in cage, independent of the condition | ⇒2 P |
| | 4. No droppings in cage | ⇒3 P |