

Muscle membrane properties in a pig sepsis model: effect of norepinephrine

Delphine Boërio, PhD¹, Thiago D. Corrêa, MD, PhD², Stephan M. Jakob, MD PhD³, Karin A.

Ackermann, MD¹, Hugh Bostock, PhD⁴, Werner J. Z'Graggen, MD⁵

¹Department of Neurology, Inselspital, Bern University Hospital, University of Bern, Switzerland.

²Department of Intensive Care Medicine, Inselspital, Bern University Hospital, University of Bern, Switzerland and Intensive Care Unit, Hospital Israelita Albert Einstein, São Paulo, Brazil

³Department of Intensive Care Medicine, Inselspital, Bern University Hospital, University of Bern, Switzerland

⁴Sobell Department of Motor Neuroscience and Movement Disorders, Institute of Neurology, University College London, London, UK

⁵Department of Neurosurgery, Inselspital, Bern University Hospital, University of Bern, Switzerland

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Correspondence: W.J. Z'Graggen, Departments of Neurology and Neurosurgery, University Hospital Bern, Inselspital, 3010 Bern, Switzerland, E-mail: werner.zgraggen@insel.ch

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Abstract

Introduction: Sepsis-induced myopathy and critical illness myopathy are common causes of muscle weakness in intensive care patients. This study investigated the effect of different mean arterial blood pressure (MAP) levels on muscle membrane properties following experimental sepsis.

Methods: Sepsis was induced with faecal peritonitis in 12 out of 18 anesthetized and mechanically ventilated pigs. Seven were treated with a high (75-85 mm Hg) and 5 with a low (≥ 60 mm Hg) MAP target for resuscitation. In septic animals, resuscitation was started 12 hours after peritonitis induction, and muscle velocity recovery cycles were recorded 30 hours later.

Results: Muscles in the sepsis/high MAP group showed an increased relative refractory period and reduced early supernormality, compared to the remaining septic animals and the control group, indicating membrane depolarization and/or sodium channel inactivation. The membrane abnormalities correlated positively with norepinephrine dose.

Discussion: Norepinephrine may contribute to sepsis-induced abnormalities in muscle by impairing microcirculation.

Keywords: critical illness myopathy, experimental sepsis, ICU acquired weakness, electrophysiology, electromyography, norepinephrine

Introduction

Intensive care unit (ICU) acquired weakness is a frequent complication in critically ill patients [1-4]. Typically, patients become symptomatic with generalized muscle weakness and failure to wean from mechanical ventilation. ICU-acquired weakness is often caused by sepsis-induced myopathy, critical illness myopathy (CIM), or a combination of these and other entities [5]. Sepsis, systemic inflammatory response syndrome and multiple organ failure have been identified as important risk factors for ICU-acquired muscle pathologies [1]. Multiple mechanisms have been proposed, such as impaired microcirculation with consecutive bioenergetic failure [1, 5], toxic action of cytokines [1, 3, 5], disturbances of electrolyte gradients [6 - 8] and muscle catabolism [9 - 13]. Furthermore, it has been shown that during sepsis muscle membrane potential changes occur [14 - 17]. Using a pig model, we demonstrated that muscle membrane abnormalities consistent with membrane depolarization and/or sodium channel inactivation occurred within 6 hours of sepsis induced by faecal peritonitis [18]. Similar changes were found in patients diagnosed of probable CIM [6].

Muscle membrane properties can be assessed in vivo by recording multi-fiber muscle velocity recovery cycles (MVRC). Using direct muscle stimulation, a cluster of muscle fibers are excited with paired stimuli separated by an inter-stimulus interval (ISI) that is varied between 2 and 1000 ms. The conduction velocity of the second action potential depends on the ISI [19 - 23]. This allows the measurement of muscle relative refractory period (MRRP) and early supernormality (ESN). Both measures are sensitive to muscle membrane potential and muscle ion channel function. In the early phase of septic myopathy and in probable CIM a prolongation of MRRP and a reduction of ESN were found, indicating that muscle membranes are depolarized and/or that muscle sodium channel inactivation was increased [6, 18].

One possible pathophysiological mechanism for development of ICU-acquired muscle weakness is reduction of microcirculation, causing bioenergetic failure, eventually leading to

ischemic muscle fibre injury. Under physiological conditions, blood flow autoregulation maintains organ perfusion constant in the face of changing perfusion pressure. If autoregulation is impaired either by a drop of systemic perfusion pressure below a critical value or by failure of regulatory mechanisms, organ ischemia can occur. There is an ongoing debate about optimal blood pressure management during sepsis [24]. The Surviving Sepsis Guidelines recommend vasopressors to achieve and maintain a mean arterial blood pressure (MAP) of at least 65 mm Hg in patients not responding to initial fluid resuscitation [25]. Thereby, vasopressors variably affect systemic, regional and organ perfusion [26 - 28].

The aim of the present study was to evaluate the impact of two different MAP target levels on muscle membrane properties in an experimental model of sepsis induced by faecal peritonitis.

Materials and methods

Here we report data from two different studies in which neurophysiological measurements were carried out. Both studies were performed in accordance with the National Institute of Health guidelines for care and use of experimental animals and with the approval of the Animal Care Committee of the Canton of Bern, Switzerland (No. BE 88/09 and BE 70/10). The course of the disease including hemodynamics and mitochondrial function, and aspects of resuscitation have been published previously [29, 30].

Animal Preparation

Animal preparation was described in detail earlier [29,30]. In brief, 18 domestic pigs (mean weight \pm SD: 39.4 \pm 4.1 kg) were fasted overnight for 12 hours and intramuscularly premedicated with ketamine (20 mg/kg) and xylazine (2 mg/kg) before induction of anaesthesia with midazolam (0.5mg/kg) and atropine (0.02 mg/kg) followed by orotracheal intubation.

Anesthesia was maintained throughout the experiment with continuous intravenous infusions of propofol (4 mg/kg/h) and fentanyl (5 µg/kg/h during surgery and 2 µg/kg/h afterwards). If necessary, additional boluses of midazolam (5 mg) and fentanyl (50 µg) were administered. Neuromuscular blocking agents were not used. Animals were ventilated in a volume-controlled mode with a positive end-expiratory pressure of 5 cm H₂O, a fraction of inspired oxygen of 0.3 and a tidal volume of 8 mL/kg (Servo-i[®], Marquet Critical Care, Solna, Sweden). The respiratory rate was adjusted aiming an arterial CO₂ partial pressure (PaCO₂) of 35 to 45 mmHg. A combination of Ringer's lactate and glucose 50% solutions were infused continuously and adjusted to maintain blood glucose in the range of 3.5 to 5 mmol/L. A period up to six hours was allowed for hemodynamic stabilization. Afterwards, peritonitis was induced in 12 pigs by installation of 2 g/kg of autologous feces dissolved in 200-250 ml (37°C) 5% glucose solution into the abdominal cavity. Six animals served as non-septic controls.

Resuscitation was started 12 hours after untreated sepsis and lasted for 48 hours. Afterwards, the animals were deeply sedated and euthanized with an overdose of potassium chloride. During resuscitation, all animals received intravenous antibiotic treatment with piperacillin-tazobactam (2.25 g) every 8 h and deep venous thrombosis prophylaxis with a continuous infusion of non-fractionated heparin (10'000 IU/24h). In series 1 [29], resuscitation was targeted at MAP ≥60 mmHg [in both septic (n=5) and control animals (n=6)], and in series 2 [30] (n=7) at 75-85 mmHg (sepsis/high MAP group).

Animal monitoring, biological data and resuscitation strategies

The following parameters were monitored continuously: hemodynamics, temperature and respiratory parameters (S/5 critical Care Monitor[®], Datex-Ohmeda, GE Healthcare, Helsinki, Finland), continuous thermodilution cardiac output (L/min) and mixed venous oxygen saturation (SvO₂, Vigilance[®], Edwards Lifescience LLC, Irvine, CA, USA). Data were recorded using a

data acquisition and signal analysis software (Soleasy™, National Instruments Corp., Austin, TX, USA) and in an electronic patient data management system (Centricity; GE GE Healthcare, Helsinki, Finland).

Blood samples including arterial blood gas analysis were taken at the end of the stabilization period and immediately after the electrophysiological investigations. Sodium, potassium, glucose, lactate, pH, bicarbonate and base excess were measured. Arterial blood samples were analyzed in a blood gas analyzer (GEM Premier 3000 analyzer, Bedford, MA, USA), for PaO₂, PaCO₂ (adjusted to central body temperature), pH, lactate, bicarbonate, base excess, sodium and potassium.

Hemodynamic protocol

Additional fluid boluses and vasoactive drugs were administered according to a hemodynamic protocol already published [29,30]. Briefly, fluid boluses of 150 mL Ringer's lactate and 6% hydroxyethyl starch (130/0.4) were given, when pigs were hypovolemic and stroke volume increased by more than 10% after the preceding administration. The maximal infused dose of hydroxyethyl starch was limited to 30 mL/kg, afterwards only Ringer's lactate was administered. If mixed SvO₂ was lower than 50%, dobutamine administration was started (5 mg/h) and increased by 5 mg/h every 30 min until SvO₂ became $\geq 50\%$ or the maximal dose of 20 mg/h was reached. If MAP was lower than the targeted resuscitation MAP, norepinephrine administration was started.

Recording of muscle multi-fiber velocity recovery cycles

MVRCs were recorded approximately 48 hours after induction of anesthesia (corresponding to 42 hours after induction of peritonitis in the animals with sepsis) using a recently described protocol [18, 21]. In brief, multi-fiber responses to direct muscle stimulation

were recorded on the foreleg from the extensor digitorum muscle. Stimulation currents were delivered through an insulated monopolar needle electrode (TECA, VIASYS Healthcare, Madison, Wisconsin, USA), used as a cathode, while a non-polarizable surface electrode (Red Dot, 3 M Health Care, Borken, Germany) served as anode. The monopolar stimulation needle was inserted perpendicularly into muscle about 15 mm distally to the anode. Stimulus waveforms (rectangular current pulses of 0.05 ms duration) generated by a computer were converted to current with an isolated linear bipolar-current stimulator (DS 5, Digitimer Ltd., Welwyn Garden City, Hertfordshire, UK). Muscle compound action potentials were recorded within the same muscle by means of a 30G EMG electrode (Medtronic, Skovlunde, Denmark) inserted approximately 20 mm proximal to the stimulating needle. A non-polarizable surface electrode served as a ground electrode and was taped on the shoulder of the animal.

The signal was amplified (gain 1000, bandwidth 1.6 Hz to 10 kHz) and digitized (National Instruments NI DAQCARD-6062E, National Instruments Europe Corp., Debrecen, Hungary) using a sampling rate of 20 kHz. Stimulation and recording were controlled by QTRAC software (written by Hugh Bostock, copyright Institute of Neurology, London, UK).

Multi-fiber MVRCs with single conditioning stimuli, two test stimuli 10 ms apart, and with test stimuli alone were recorded. The ISI between the conditioning stimulus and the test stimulus was varied from 1000 to 1.4 ms in 34 steps in an approximately geometric series.

Data analysis has been described in detail in previous papers [21, 23]. From each MVRC we assessed: (1) Relative refractory period, measured as the interpolated ISI at which the response latency first recovered to its unconditioned value, and (2) Early supernormality, measured as the peak percentage reduction in latency at ISIs shorter than 15ms, (3) Late supernormality, measured as the mean percentage reduction in latency at ISIs between 50 and 150 ms, and (4) Extra late supernormality, measured as the increase in late supernormality due to a second conditioning stimulus.

Statistical analysis

Statistical analyses were performed using the QTRAC data analysis software (copyright Institute of Neurology, London, United Kingdom), which was also used to generate the figures.

Hemodynamic data, laboratory results and MVCR parameters of the control, sepsis and sepsis/high MAP groups were compared using the Kruskal-Wallis test. To test for differences between the two treatment groups (sepsis and sepsis/high MAP) the Mann-Whitney U test was used. Correlations between MVRC measures and treatment and other measurements were tested by Spearman's rho.

Results

Characteristics of the three groups (controls, sepsis and sepsis/high MAP) are summarized in Table 1. 42 hours after induction of peritonitis (= 30 hours after initiating the different methods of resuscitation), the sepsis/high MAP group had achieved MAP values of 82 ± 4 mm Hg and the sepsis group MAP values of 71 ± 8 mm Hg ($p = 0.03$). Norepinephrine was administered to all animals in the sepsis/high MAP group and 2/5 animals in the sepsis group. Apart from difference in MAP and a small (0.4°C) difference in core temperature, none of the other non-electrophysiological measurements differed significantly between sepsis and sepsis/high MAP groups (Table 1).

Muscle velocity recovery cycles

Results of electrophysiological investigations are shown in Figure 1 and Table 2. After the 30 hours of resuscitation, MVRCs of animals of the sepsis/high MAP group were shifted to the right and upwards compared to MVRCs of animals of the sepsis group, indicating membrane depolarization and/or sodium channel inactivation. This shift corresponded to a significant

prolongation of the relative refractory period and a significant reduction in early supernormality compared to the sepsis group (Figure 2A, B; Table 2). The late supernormality and extra late supernormality were not significantly affected by the sepsis.

To investigate the cause of the differences in relative refractory period and early supernormality between the sepsis/high MAP and sepsis groups, these values were correlated separately with the measurements in Table 1, for the whole group of 12 animals with sepsis (Table 3). This shows that despite the significant differences in muscle membrane properties between the sepsis/high MAP and sepsis groups, there was no significant correlation between individual muscle excitability and MAP measurements. The muscle differences between groups cannot therefore be attributed to MAP itself. The strongest correlations between relative refractory period and peak early supernormality were observed with lactate levels and the total amount of norepinephrine injected (Table 3).

Discussion

After 30 hours of resuscitation, muscle excitability testing demonstrated a significant increase of refractoriness paralleled by a significant reduction in supernormality in the sepsis/high MAP compared to the sepsis group. These changes in muscle excitability measurements correlated positively with the cumulative dose of norepinephrine administered during resuscitation.

Muscle excitability testing provides an assessment of muscle fiber properties that does not depend on nerve function or neuromuscular transmission [19 - 21, 23, 31]. This method was previously used to study the early phase of septic myopathy in a porcine model of peritonitis with consecutive sepsis over a period of 27 hours [18]. Muscle membrane dysfunction was found to occur within 6 hours of experimental sepsis. Furthermore, similar muscle membrane

dysfunction was shown in patients with probable CIM in the chronic state [6], where it provided evidence that membrane depolarization and/or sodium channel inactivation occurs.

We have found similar changes of the two main parameters of muscle membrane excitability measurements (prolongation of refractory period, reduction of early supernormality) during experimental limb ischemia in healthy volunteers [21] and hypothesized that these findings are related to a dysfunction of the Na^+/K^+ pump with consecutive cell membrane depolarization. Impaired microcirculation has already been postulated to be a factor in the development of myopathy in the critical ill [1, 5]. Theoretically, one would expect that an increase of MAP would result in a better organ perfusion if blood flow autoregulation were impaired, or unaffected organ perfusion if autoregulation were sustained. Vasopressors have been shown to variably affect systemic, regional and organ perfusion [26 - 28]. For example, in patients with septic shock, sublingual microcirculatory alterations did not improve in response to an increase of MAP with norepinephrine [32]. Furthermore, linear trend analysis demonstrated reductions in the capillary and in the perfused capillary densities [32]. We found a positive correlation between the cumulative dose of norepinephrine – but not of achieved blood pressure – and muscle membrane alterations, possibly related to a norepinephrine-induced reduction of microcirculation leading to ischemia in the muscle. The strong correlation between muscle membrane changes and lactate, but not pH, support the idea that the membrane changes were related to ischemia, with a resultant switch to anaerobic glycolysis. This hypothesis is also supported by the findings of a recent clinical observational study, which demonstrated that the required amount of catecholamine was a risk factor for the development of impaired direct muscle stimulation [33]. In contrast to our finding of norepinephrine related muscle membrane depolarization, earlier studies have shown in isolated muscles that catecholamines stimulate the Na^+/K^+ pump and cause cell membrane hyperpolarization [34 - 36]. This difference might be explained by the hypothesis, that in vivo the norepinephrine induced hypoperfusion of the

muscle capillaries with consecutive energy depletion and dysfunction of the muscle Na⁺/K⁺ pump overweighs the direct hyperpolarizing effect of norepinephrine on the muscle cell membrane. However, we cannot exclude that muscle membrane depolarization is caused also by other factors e.g. sepsis related inflammatory mediators [1, 3, 5]. Since only the sepsis/high MAP group showed muscle membrane depolarization (and not the sepsis group), we assume that these are of less importance for changes of muscle membrane potential. Furthermore, the strong correlation of muscle membrane excitability parameters with lactate supports the hypothesis that the membrane changes were related to norepinephrine-induced ischemia.

Our study has some limitations. First, it was not possible to blind the investigators to the treatment regime during the resuscitation phase. Since we used well-defined treatment protocols, we think that this did not cause a bias. Second, we cannot provide microcirculation parameters. However, the numerically lower systemic perfusion in the sepsis/high MAP compared to sepsis group (<80%) together with the massively higher doses of norepinephrine used supports the concept of diminished microcirculation in the former group. The present study was conducted in young pigs - the findings might have been different in adult animals or in patients. Nevertheless, while critical illness polyneuropathy/myopathy is much less frequently described in pediatric compared to adult patients [37], typical findings of the disease have been reported repeatedly in animals younger than our pigs [38 - 40]. Finally, our study findings can only be related to septic myopathy but not to CIM, since the period of observation was too short. We could also neither detect MAP-target-dependent skeletal muscle ATP contents, nor mitochondrial respiration abnormalities [30].

Conclusion

These results suggest that administration of norepinephrine to elevate MAP during resuscitation may contribute to sepsis-induced abnormalities in muscle. This may be due to a

norepinephrine-induced reduction of microcirculation, increasing the risk of ischemic muscle damage. Confirmation in humans is needed.

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List of Abbreviations

CIM: critical illness myopathy

ESN: early supernormality

ICU: intensive care unit

ISI: inter-stimulus interval

MAP: mean arterial pressure

PaCO₂: CO₂ partial pressure

PaO₂: O₂ partial pressure

SvO₂: mixed venous oxygen saturation

MVRC: muscle velocity recovery cycle

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Figure Legends

Fig. 1. Recordings of muscle velocity recovery cycles in pig extensor digitorum. Mean MVRCs for the 7 animals of the sepsis/high MAP group (filled black circles) and the 5 animals of the sepsis group (open black circles) compared with 6 non-septic control animals (open grey circles). *Upper traces:* percentage changes in latency following single conditioning stimulus. *Lower traces:* extra latency changes caused by a second conditioning stimulus, 10 ms earlier.

Fig. 2. Differences between experimental groups 48 hours after induction of anesthesia **A:** muscle relative refractory period, **B:** peak early supernormality. Box and whiskers show median, inter-quartile range and range; asterisks indicate significant differences by Mann-Whitney U-test, * = $P < 0.05$, ** = $P < 0.01$

Table 1: Measurements from the sepsis and sepsis/high MAP pigs, at 42 hours after induction of peritonitis, compared with control animals after same period of anesthesia

Variables / Study groups	Control (n=6)	Sepsis (n=5)	Sepsis/high MAP (n=7)	Kruskal-Wallis Test (P value)	Sepsis/high MAP v. Sepsis (P value)
Core temperature (°C)	39.7 ± 0.7	40.7 ± 0.3	40.3 ± 0.3	0.041	0.048
Hemodynamics					
Mean arterial pressure (mmHg)	77.8 ± 19.2	71.0 ± 7.8	81.9 ± 4.3	0.066	0.030
Heart rate (bpm)	85.7 ± 11.0	137.4 ± 26.0	164.6 ± 36.0	0.003	0.34
Cardiac output (l/min)	5.1 ± 0.9	7.3 ± 1.9	5.8 ± 2.7	0.51	0.20
Stroke volume (mL)	58.0 ± 14.1	55.9 ± 11.4	36.4 ± 15.6	0.22	0.11
Laboratory					
Sodium (mmol/l)	139.2 ± 2.9	136.0 ± 2.9	133.3 ± 2.4	0.017	0.20
Potassium (mmol/l)	3.8 ± 0.3	4.2 ± 0.5	4.9 ± 1.3	0.056	0.20
Arterial lactate (mmol/l)	0.40 ± 0.11	0.86 ± 0.19	1.29 ± 0.57	0.004	0.11
pH	7.51 ± 0.03	7.44 ± 0.05	7.48 ± 0.09	0.019	0.34
Bicarbonate (mmol/l)	30.8 ± 2.5	29.4 ± 1.5	29.2 ± 4.3	0.40	0.76
Base excess (mmol/l)	7.0 ± 2.9	5.8 ± 1.6	5.7 ± 5.0	0.41	0.76
Mean fluid balance (mL/kg/h) ⁺	1.5 ± 0.9	2.8 ± 0.9	3.3 ± 1.3	0.025	0.53
Norepinephrine (mcg)	0 ± 0	984 ± 1968	35,483 ± 36,927	0.005	0.0051

Values are given as mean ± SD. ⁺ Mean fluid balance = (Ringer's lactate basal infusion + glucose 50% basal infusion + Ringer's lactate bolus + hydroxyethyl starch bolus) – (urine output + gastric tube output). Last column gives *P* values comparing sepsis and sepsis/high MAP groups (Mann-Whitney U test). MAP = mean arterial pressure.

Table 2: Muscle excitability measurements from the sepsis and sepsis/high MAP pigs, at 42 hours after induction of peritonitis, compared with control animals after same period of anesthesia

Variables / Study groups	Control (n=6)	Sepsis (n=5)	Sepsis/high MAP (n=7)	Kruskal-Wallis Test (<i>P</i> value)	Sepsis/high MAP v. Sepsis (<i>P</i> value)
Relative refractory period (ms)	1.94 ± 0.38	2.35 ± 0.27	5.54 ± 2.28	0.005	0.0025
Peak early supernormality (%)	9.9 ± 2.5	6.5 ± 2.2	3.1 ± 2.1	0.006	0.030
Late supernormality (%)	1.6 ± 0.6	1.4 ± 1.1	1.3 ± 0.8	0.73	0.76
Extra late supernormality (%)	2.0 ± 0.8	1.5 ± 1.1	1.7 ± 1.0	0.46	0.79

Values are given as mean ± SD. Last column gives *P* values comparing sepsis and sepsis/high MAP groups (Mann-Whitney U test). MAP = mean arterial pressure.

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Table 3: Correlations between muscle velocity recovery cycle parameters and other measurements for the pooled septic animals (n=12)

Parameters	Relative refractory period (ms)		Peak early supernormality (%)	
	Rho	<i>P</i>	Rho	<i>P</i>
Core temperature (°C)	-0.559	0.057	0.371	0.23
Mean arterial pressure (mmHg)	0.371	0.23	-0.150	0.64
Sodium (mmol/l)	-0.547	0.063	0.579	0.047
Potassium (mmol/l)	0.605	0.036	-0.451	0.14
Lactate (mmol/l)	0.762	0.0040	-0.790	0.0023
pH	-0.124	0.70	0.253	0.43
Bicarbonate (mmol/l)	-0.357	0.25	0.462	0.13
Base excess (mmol/l)	-0.327	0.30	0.414	0.18
Mean fluid balance (mL/kg/h)	0.524	0.078	-0.720	0.0081
Norepinephrine (mcg)	0.888	0.00014	-0.671	0.016

For each muscle velocity recovery cycle measurement, first column is Spearman's rank correlation coefficient Rho, and second column is *P* value for Rho.

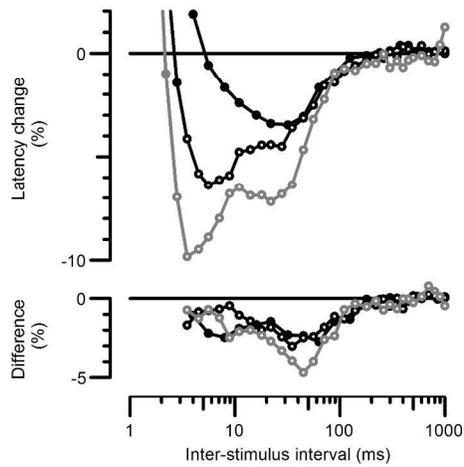


Figure 1: Recordings of muscle velocity recovery cycles in pig extensor digitorum. Mean MVRs for the 7 animals of the sepsis/high MAP group (filled black circles) and the 5 animals of the sepsis group (open black circles) compared with 6 non-septic control animals (open grey circles). Upper traces: percentage changes in latency following single conditioning stimulus. Lower traces: extra latency changes caused by a second conditioning stimulus, 10 ms earlier.

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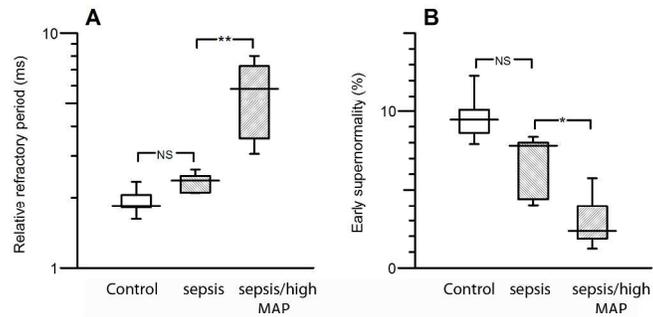


Figure 2: Differences between experimental groups 48 hours after induction of anesthesia A: muscle relative refractory period, B: peak early supernormality. Box and whiskers show median, inter-quartile range and range; asterisks indicate significant differences by Mann-Whitney U-test, * = $P < 0.05$, ** = $P < 0.01$

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