Intensive Care Medicine Experimental 2014, Volume 2 Suppl 1
http://www.icm-experimental.com/supplements/2/S1

Intensive Care Medicine Experimental
a SpringerOpen Journal

MEETING ABSTRACTS Open Access

ESICM LIVES 2014

Barcelona, Spain. 27 September - 1 October 2014

Published: 26 September 2014

These abstracts are available online at http://www.icm-experimental.com/supplements/2/S1

ORAL PRESENTATIONS

OUTCOME OF CARDIAC ARREST

O1

0020. Microcirculatory perfusion and vascular reactivity are altered in post cardiac arrest patients, irrespective of target temperature management to 33° vs 36° (substudy TTM)

M Koopmans[1*], MA Kuiper[1], R Endeman[2], G Veenstra[1], NAR Vellinga[1], R Vos de[3], EC Boerma[1]
[1]Medical Center Leeuwarden, Intensive Care, Leeuwarden, Netherlands; [2]Onze Lieve Vrouwe Gasthuis, Intensive Care, Amsterdam, Netherlands; [3]Academic Medical Center, Epidemiologic, Biostatistics and Bioinformatics, Amsterdam, Netherlands
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**O1

Introduction: After cardiopulmonary resuscitation (CPR), following an out of hospital cardiac arrest (OHCA) hemodynamic failure is common, due to a combination of heart failure and ischemia reperfusion injury. Comatose post-cardiac arrest patients are treated on the intensive care unit (ICU) with mild therapeutic hypothermia (33°), nowadays referred to as target temperature management (TTM) for an assumed neuroprotective effect.
Objectives: In previous reports both microcirculatory alterations and impaired vascular reactivity have been described in post cardiac arrest patients treated with mild therapeutic hypothermia. As of now it is unknown whether these alterations are related to the temperature management itself. Aim of the present study was to investigate the potential difference in microcirculatory alterations and vascular reactivity in patients after out of hospital cardiac arrest treated with target temperature management of 33°C (TTM33) in comparising to patients treated with 36°C (TTM36).
Methods: Our study was designed as a a priory substudy of the open label randomized controlled TTM trial in 2 Dutch mixed ICU's. Microvascular flow index (MFI) was assessed by Side Stream Darkfield imaging and vascular reactivity by near infrared spectroscopy. Variables, including systemic haemodynamics were recorded at start study (T1), after 12 hours (T2) and after 24 hours (T3).
Results: 22 patients were included, 13 in TTM33 and 9 in TTM36. At T1 MFI between groups did not differ significantly (1.08[0.4-1.9] versus 1.67 [0.7-2.4] respectively, p=0.59) . The difference between groups remained insignificant over time. At T1 tissue oxygenation (StO2) was significantly lower in TTM36 in comparison to TTM33 (58.9±13.5 versus 44.6±15.8, p=0.03). Over time this difference between groups disappeared. However, vascular reactivity, expressed as the descending and ascending slope of StO2 after a standardized ischemic occlusion test was similar between groups.
Conclusions: In this relatively small sample size study microcirculatory blood flow and vascular reactivity did not change between TTM33 and TTM36.
Clinicaltrials.gov nr. NCT01850485.

References

1. Nielsen N, *et al*: TTM Trial Investigators. Targeted Temperature Management at 33 C versus 36 C after cardiac arrest. *The New England Journal of Medicine* 2013, **369(23)**:2197-206.
2. Sakr Y, *et al*: Persistent microcirculatory alterations are associated with organ failure and death in patients with septic shock. *Crit Care Med* 2004, **32(9)**:1825-1831.
3. Fries M, *et al*: Microvascular blood flow during cardiopulmonary resuscitation is predictive of outcome. *Resuscitation* 2006, **71**:248.

MUSCLE AND NEURO: FROM EXPERIMENTAL TO CLINICAL STUDIES IN SEPSIS

O2

0026. Voluntary activation of the sympathetic nervous system and attenuation of the innate immune response in humans

M Kox[1,2,3*], LT van Eijk[1,3], J Zwaag[1,3], J van den Wildenberg[1,3], FCJG Sweep[4], JG van der Hoeven[1,3], P Pickkers[1,3]
[1]Radboud University Medical Center, Intensive Care Medicine, Nijmegen, Netherlands; [2]Radboud University Medical Center, Anesthesiology, Nijmegen, Netherlands; [3]Nijmegen Institute for Infection, Inflammation and Immunity, Nijmegen, Netherlands; [4]Radboud University Medical Center, Laboratory Medicine, Nijmegen, Netherlands
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**O2

Introduction: Excessive or persistent pro-inflammatory cytokine production plays a central role in a variety of inflammatory conditions. Acute activation of the sympathetic nervous system attenuates innate immunity. However, both the autonomic nervous system and innate immune system are regarded as systems that cannot be voluntarily influenced.
Objectives: To evaluate the effects of a training program on the autonomic nervous system and innate immune response.
Methods: We performed a parallel randomized controlled study in healthy male volunteers. Subjects were randomized to receive either a 10-day training program involving meditation (third eye meditation), breathing techniques (i.a., cyclic hyperventilation followed by breath retention), and exposure to cold (i.a., immersions in ice cold water), or no training. Subjects in both groups (n=12 per group) underwent experimental human endotoxemia (intravenous administration of 2 ng/kg *E. Coli* lipopolysaccharide [LPS]) during which the trained individuals practiced the learned techniques.
Results: Practicing the learned techniques resulted in intermittent respiratory alkalosis and hypoxia resulting in significantly increased plasma epinephrine levels (Figure 1). In the trained group, plasma levels of the anti-inflammatory cytokine IL-10 increased more rapidly after LPS administration, correlated strongly with preceding epinephrine levels (r=0.82, p=0.001), and were higher (Figure 2). Levels of pro-inflammatory mediators TNF-α, IL-6, and IL-8 were lower in the trained group (Figure 2) and correlated negatively with IL-10 levels (r=-0.71, p=0.01; r=-0.59, p=0.045; r=-0.71,

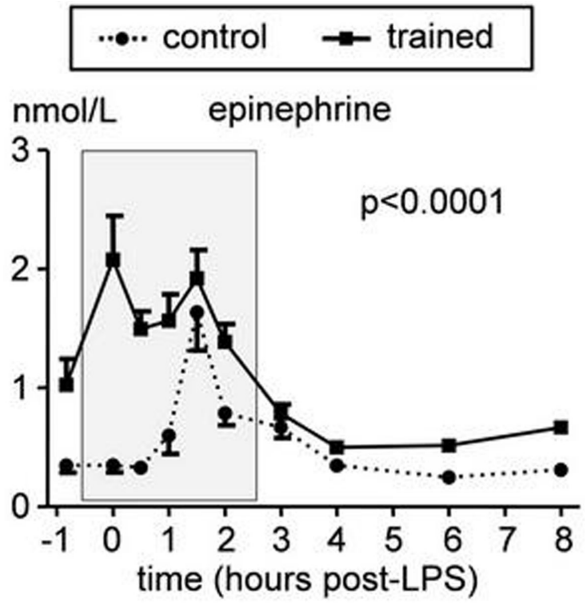

Figure 1(abstract O2) Plasma epinephrine concentrations during experimental endotoxemia in control and trained subjects. Data are expressed as mean ± SEM of 12 subjects per group. Gray box indicates period in which the trained subjects practiced their learned breathing techniques. P values between groups were calculated using repeated measures two-way analysis of variance (ANOVA, interaction term).

p=0.01, respectively). Finally, LPS-induced flu-like symptoms and fever were blunted in the trained group.

Conclusions: Voluntary activation of the sympathetic nervous system results in epinephrine release and subsequent suppression of the innate immune response in humans *in vivo*. These results could have important implications for the treatment of a variety of conditions associated with excessive or persistent inflammation.

Grant acknowledgment: This study was supported by a Serendipity Grant from Reumafonds (http://www.reumafonds.nl).

O3

0027. Kinetic of muscle mass regulation during experimental sepsis

J-C Palao[1,2*], J Morel[1,2], A-C Durieux[2], J Castells[2], S Molliex[1], D Freyssenet[2]
[1]Centre Hospitalier Universitaire de Saint-Etienne, Service d'Anesthésie-Réanimation, Saint-Priest en Jarez, France; [2]Université de Lyon, Laboratoire de Physiologie de l'Exercice, Saint-Etienne, France
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**O3

Introduction: Sepsis induced muscle weakness (SIMW) is associated with an important morbidity of critical care patients. Moreover, respiratory muscles weakness delays weaning from mechanical ventilation.

The down-regulation of Akt pathway and the activation of proteolytic pathways, such as ubiquitin-proteasome or autophagy-lysosome pathways, could play a key role in the pathogenesis of SIMW. However, the kinetic response of these pathways is currently unknown.

Objectives: The aim of this study was to describe the kinetic response of anabolic and proteolytic pathways in a murine model of sepsis. A clear picture of mechanisms involved in SIMW could help to develop strategies to prevent muscle casting.

Methods: Sixteen week-old male mice were divided in a septic group (peritonitis induced by cecal ligature and puncture, CLP) and a sham group (laparotomy without cecal ligature or puncture). Sham-operated mice were pair-fed to septic mice. Following surgery, mice were daily hydrated subcutaneously.

Mice were weighed daily and muscle strength assessed by grip test. Animals were sacrificed at day 1, 4 or 7 after surgery (n = 8/group). Tibialis anterior, gastrocnemius, quadriceps, soleus, extensor digitorum longus and diaphragm muscles were removed to perform molecular (enzymology, RT-qPCR and western blotting) and histological analyses.

Results: Loss of muscle mass and strength was observed as soon as day 1 following CLP and was maximum at day 4 for all skeletal muscles (Fig 1). In the gastrocnemius muscle, chymotrypsin-like activity of the proteasome was also higher at day 4 (Fig 2), whereas activity of cathepsin B+L, proteins involved in autophagy-lysosomal pathway, was maximum at day 7 (Fig 2). In the diaphragm muscle, chymoptrysin-like and cathespin B+L activities peaked at day 4. Citrate synthase activity, a marker of mitochondrial content, was minimal at day 4 both in gastrocnemius and diaphragm muscles.

Conclusions: Our results confirm a major role for the proteasome in muscle wasting during sepsis. Interestingly, the increase in cathepsin B+L activity at day 7 in gastrocnemius muscle, when muscle mass had already started to recover, suggests that the autophagy pathway could participate to the recovery of skeletal muscle by inducing the clearance of damaged proteins and organelles. Such a late increase in cathepsin B+L activity was not observed in diaphragm muscle. The decrease in citrate synthase activity observed in both muscles may reflect mitochondrial impairment. These results should be completed by the ongoing analysis of other biological markers and histological data.

O4

0030. Effect of exercise training on muscle function in a recovery model of critical illness

A Sigurta[*], S Saeed, M Singer
University College London, Bloomsbury Institute of Intensive Care Medicine, London, UK
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**O4

Introduction: Survivors of critical illness experience significant skeletal muscle weakness and physical disability, which may persist for years [1]. Early mobilization is being encouraged to improve functional outcomes [2]. Decreased mitochondrial function and altered mitochondrial biogenesis are implicated in the pathogenesis of sepsis-induced muscle dysfunction [3]. Mitochondrial biogenesis can be stimulated by physical activity [4].

Objectives: To assess the role of exercise training on muscle function and mitochondrial biogenesis in a long term rat model of critical illness and recovery.

Methods: Peritonitis was induced in male Wistar rats by i.p. injection of the fungal cell wall product, zymosan. Animals were divided into 2 groups: (i) trained animals who underwent daily motorised treadmill sessions from day 2-14, progressively increasing treadmill speed and duration to 30 mins at 30 cm/s; (ii) control animals.

Weight and clinical score were recorded daily. Muscle function was assessed on days 2, 7 and 14 using exercise capacity and forelimb grip strength. On day 14, animals were culled for harvesting of gastrocnemius and soleus muscle that were weighed and then used to measure (by RT-PCR) gene expression assays of the biogenesis factors, PGC-1alpha, NRF and Tfam. Results given as ratios, using HMBS as the housekeeping gene.

Results: All animals lost weight post-zymosan and gained weight thereafter, with trained animals doing so at a greater rate (Fig 1). Exercise capacity increased by approximately 50% in both groups between Day 2 and Day 7, but was only maintained at Day 14 in trained animals. Grip strength was maintained throughout in trained animals but fell by 29.2±14% at Day 7 in controls. Tfam and NRF, but not PGC-1alpha, were higher in gastrocnemius in trained animals, but not in soleus (Fig 2).

Conclusions: Exercise training increases weight gain in this model of critical illness and recovery. Preliminary data shows improved mitochondrial biogenesis in gastrocnemius but not soleus with exercise.

References

1. Herridge MS, *et al*: **Functional disability 5 years after acute respiratory distress syndrome.** *N Engl J Med* 2011, **364**:1293-304.
2. Schweickert WD, *et al*: **Early physical and occupational therapy in mechanically ventilated, critically ill patients: a randomised controlled trial.** *Lancet* 2009, **373**:1874-82.
3. Carré JE, *et al*: **Survival in critical illness is associated with early activation of mitochondrial biogenesis.** *Am J Respir Crit Care Med* 2010, **182**:745-51.
4. Wang L, *et al*: **Similar expression of oxidative genes after interval and continuous exercise.** *Med Sci Sports Exerc* 2009, **41**:2136-44.

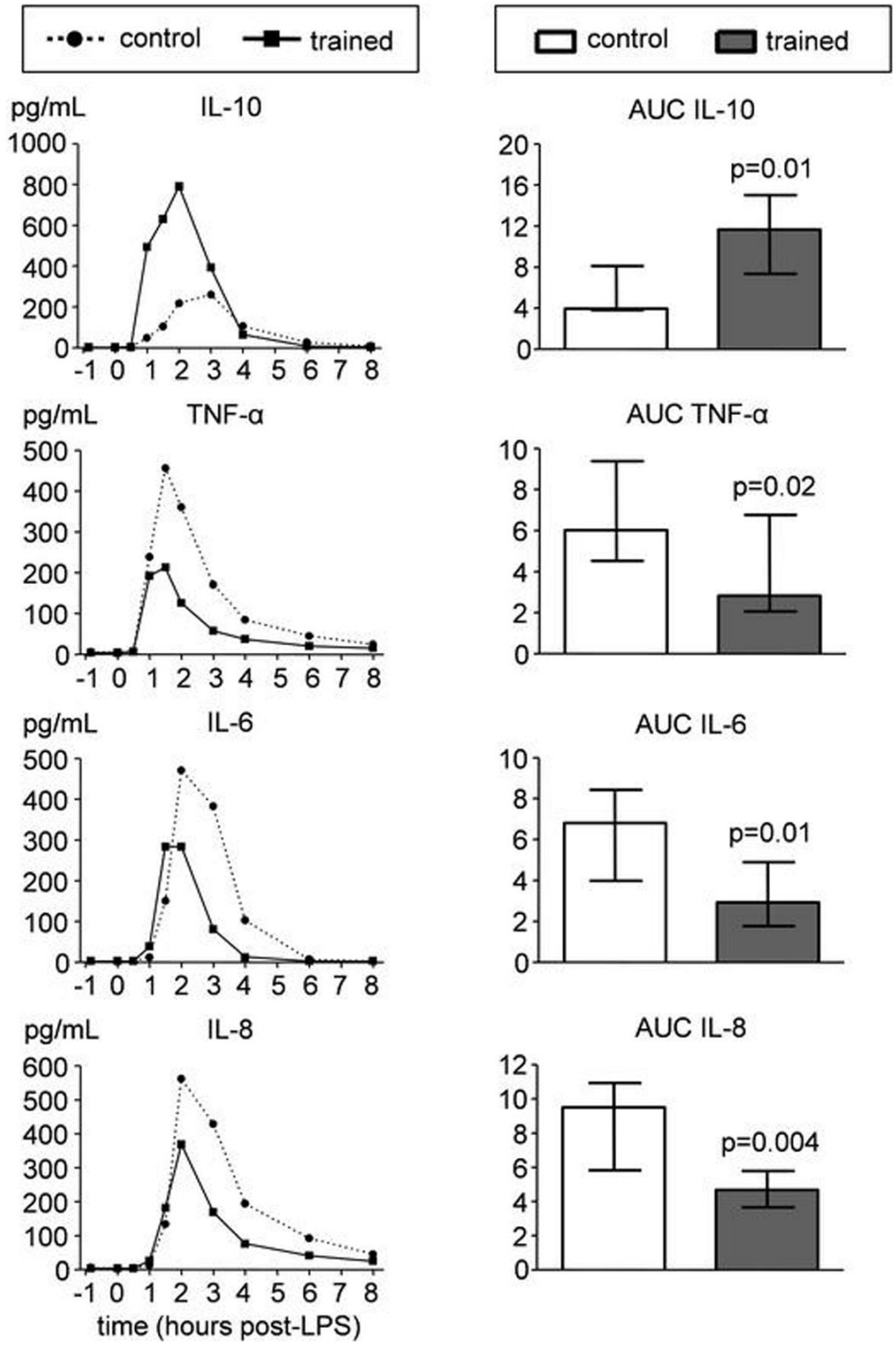

Figure 2(abstract O2) Plasma cytokine concentrations during endotoxemia in control (n=12) and trained (n=12) subjects. Left panels depict median values of anti-(IL-10) and pro-inflammatory (TNF-α, IL-6, and IL-8) cytokines. Right panels depict median ± interquartile range of area under curve (AUC) of cytokines (unit:$\times 10^4$ pg/mL·h). P values were calculated using Mann-Whitney U-tests.

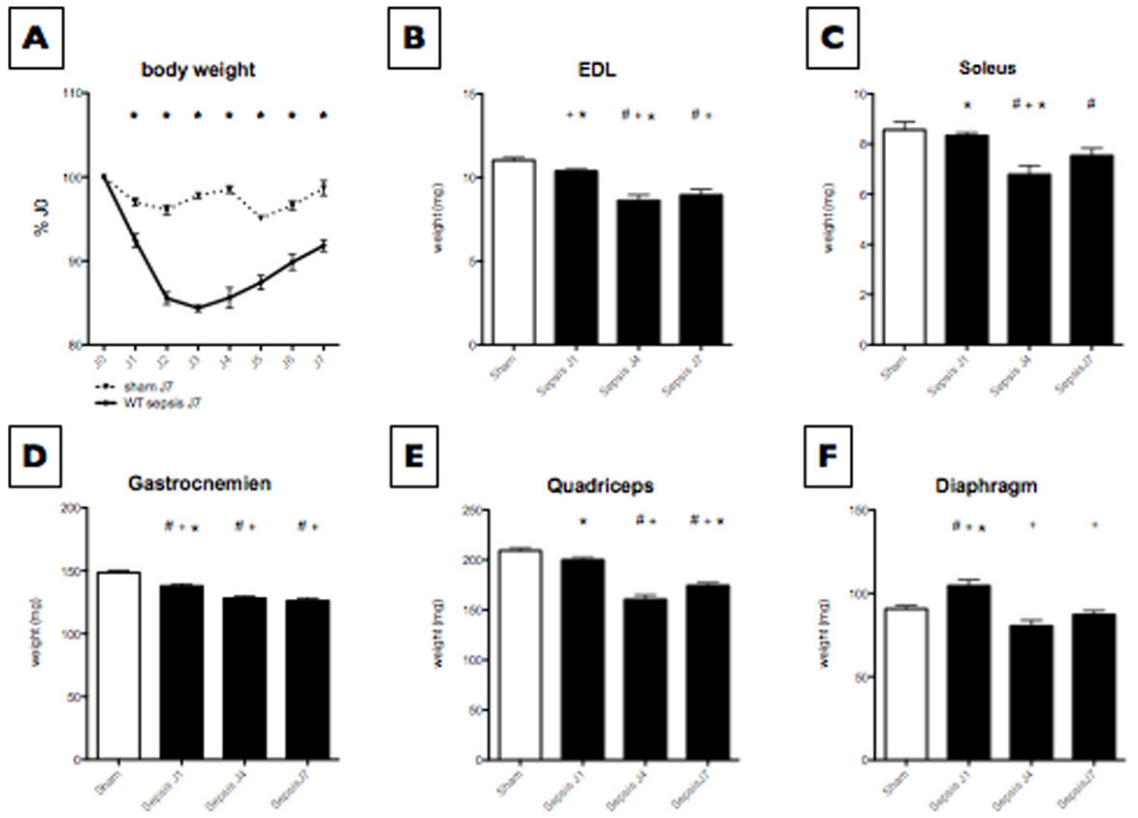

Figure 1(abstract O3) Body and muscles weight. A. Kinetic of animals' body weight (*p<0.05 versus JO). B, C, D, E Skeletal muscles weight (results are mean±SEM. * #' + p<0.05 versus sham, J1, J4 respectively). F Diaphragm weight (results are mean±SEM. * #' + p<0.05 versus sham, J1, J4 respectively).

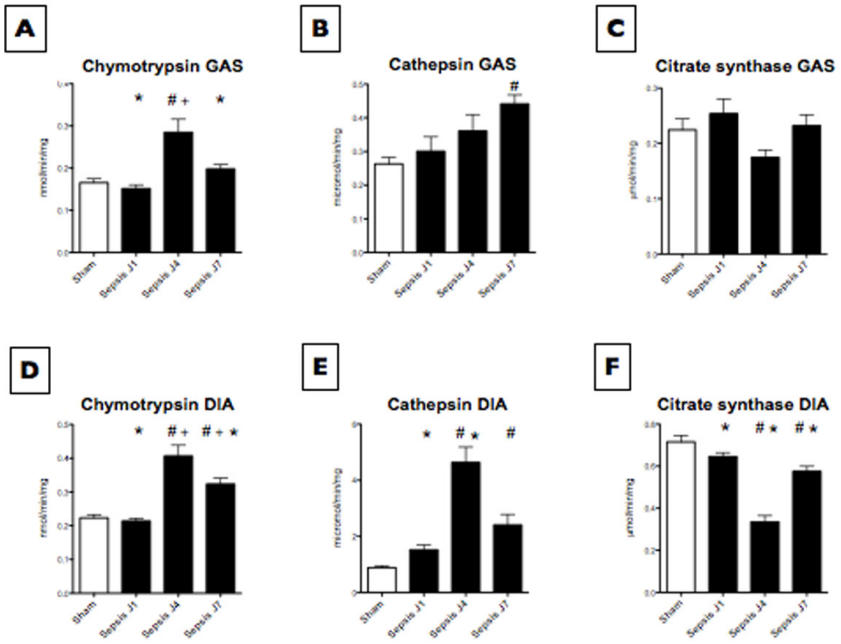

Figure 2(abstract O3) Enzyme activity. Enzyme activity in gastrocnemius (GAS) and diaphragm (DIA). Results are mean±SEM. * #' + p<0.05 versus sham, J1, J4 respectively

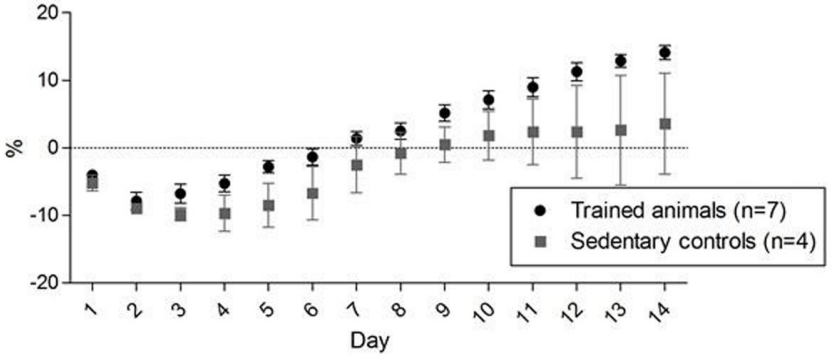

Figure 1(abstract O4) Changes in body weight expressed as percentage from baseline weight. Data shown as mean ± SE.

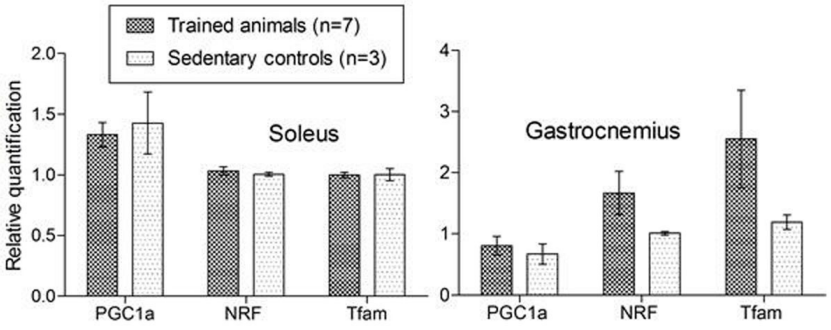

Figure 2(abstract O4) Real-time PCR (mean ± SE) on Day 14 soleus and gastrocnemius muscle.

OXYDATIVE STRESS IN SEPSIS: FROM FUNDAMENTALS TO CLINICS

O5

0032. Relationship between microcirculatory alterations and venous-to-arterial carbon dioxide differences in patients with septic shock

GA Ospina-Tascón[1*], DF Bautista[1], M Umaña[1], WF Bermúdez[1], JD Valencia[1], HJ Madriñan[1], A Bruhn[2], G Hernandez[2], M Granados[1], CA Arango-Dávila[1], D De Backer[3]

[1]Fundación Valle del Lili, Universidad ICESI, Intensive Care Medicine Department, Cali, Colombia; [2]Pontificia Universidad Católica de Chile, Facultad de Medicina, Departamento de Medicina Intensiva, Santiago, Chile; [3]Free University of Brussels, Erasme Hospital, Intensive Care Medicine Department, Brussels, Belgium

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**O5

Introduction: Increased venous to arterial carbon dioxide difference (Pv-aCO_2) have been attributed to low cardiac output states. However, mechanisms conducting to Pv-aCO_2 increases during normal or even high cardiac output conditions as in septic shock are not fully understood. We hypothesized that Pv-aCO_2 could reflect the adequacy of microvascular perfusion during resuscitated septic shock

Objectives: To test the hypothesis that Pv-aCO_2 could reflect the microvascular blood flow during the early phases of resuscitation in septic shock.

Methods: We included 80 patients with a first episode of septic shock admitted to a mixed ICU in a University Hospital over a 12-month period. Time 0 (T0) was set at ICU admission when a pulmonary artery catheter was inserted. Arterial and venous gases analyses were performed at T0 and 6 hours after (T6). We defined Pv-aCO_2 as the difference between the mixed venous and arterial CO_2 partial pressures. A Sidestream Dark-Field (SDF) imaging device (Microvision Medical, Amsterdam, the Netherlands) was used to evaluate the sublingual microcirculation both at T0 and T6. At each assessment, 5 sequences of 20 seconds each were recorded and stored under a random number. An investigator blinded to the sequence order and patient's clinical course, analyzed the sequences semi-quantitatively. The vessels were separated into large and small using a cut-off value of 20 μm in diameter. We evaluated the relation between the percentage of small vessels perfused and the Pv-aCO_2 using linear and non-linear regressions and Spearman Rho test. A $p< 0.05$ was considered as significant.

Results: We found significant but very weak relationships between general hemodynamics or oxygen derived parameters with Pv-CO_2.Pv-aCO_2 was inversely related to the percentage of small vessels perfused both at T0 and T6 (T0: R^2:0.515, $p< 0.001$; T6: R^2:0.453, $p< 0.001$).

Conclusions: Microvascular blood flow is a key determinant of Pv-aCO_2 during normodynamic septic shock. Pv-aCO_2 could track microvascular alterations during early phases of septic shock.

O6

0033. Hypothermia protects brain mitochondrial function from hypoxia in sepsis

KI Chisholm[1*], AL Davies[1], M Singer[2], A Dyson[2], KK Ida[1,3], I Tachtsidis[4], MR Duchen[5], KJ Smith[1]

[1]University College London, Institute of Neurology, London, UK; [2]University College London, Bloomsbury Institute of Intensive Care Medicine, London, UK; [3]University of São Paulo, Medical School, Anaesthesiology LIM-8, São Paulo, Brazil; [4]University College London, Medical Physics and Bioengineering, London, UK; [5]University College London, Cell and Developmental Biology, London, UK

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**O6

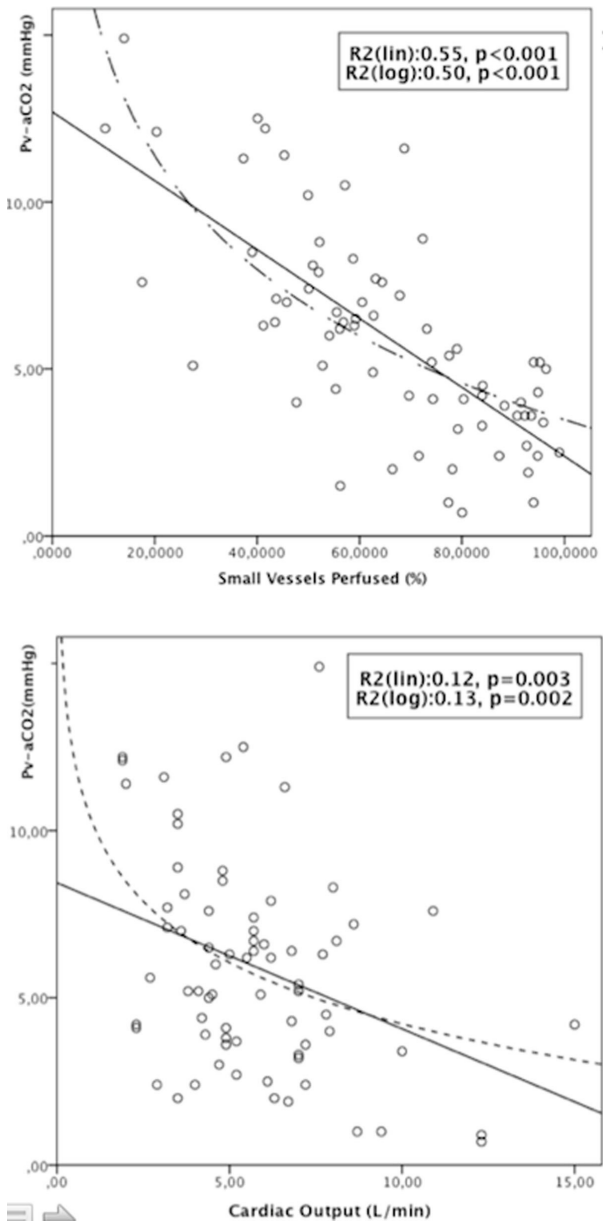

Fig. 1(abstract O5) Pv-aCO2 vs. SVP and CO at T0

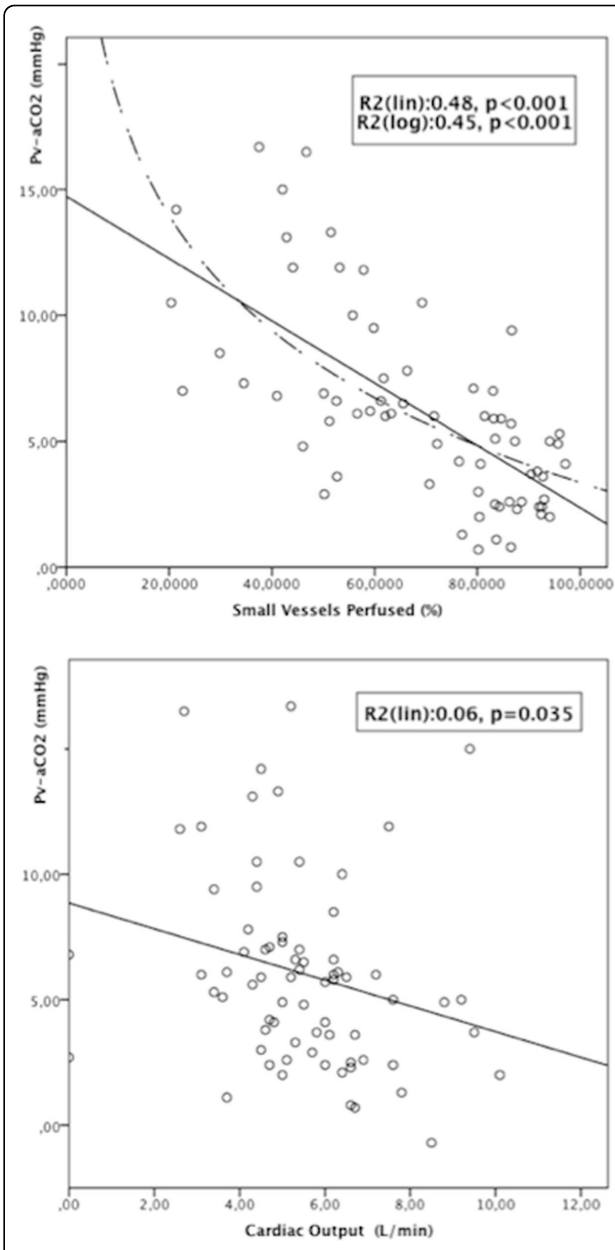

Fig. 2(abstract O5) Pv-aCO2 vs. SVP and CO at T6

Introduction: Hypothermia reduces metabolic requirements and thereby the oxygen supply-demand imbalance arising from hypoxia. Hypothermia has been an effective treatment in several critical care conditions, but the effects of hypothermia on brain mitochondrial dysfunction in sepsis are poorly understood.

Objectives: To examine the relationship between spontaneous hypothermia in a murine model of sepsis, and changes in inspired oxygen, systemic oxygenation and mitochondrial function in the cerebral cortex.

Methods: Mice (C57/bl6) were injected intraperitoneally with lipopolysaccharide (LPS) (5 mg/kg; n = 15) or saline (0.01 ml/g; n = 6). Six hours later, the cerebral cortex was exposed and mitochondrial function assessed using the inherent fluorescence of flavin adenine dinucleotide (FAD). The mice spontaneously breathed a sequence of 21% oxygen (room air), 100%, 21%, 15%, 21% and 10% for 5 minutes each. Seven of the endotoxic mice were maintained at their original body temperature post-LPS injection, while eight were maintained at 37°C using a homeothermic heating mat. Systemic oxygenation was assessed using pulse oximetry. Statistical analysis was performed by selecting areas around the veins and arteries in order to ratio the fluorescence intensity of arteries over veins. The difference in the ratio between groups was compared using a one way ANOVA followed by independent sample t-tests on IBM SPSS 22.

Results: Hyperoxia (100% O_2) had little influence on FAD fluorescence. In contrast, hypoxia (≤15% O_2) resulted in loss of FAD signal (i.e. increased reduced state of the FAD/FADH pool) except for a prominent preservation of signal in a ´halo´ around arteries/arterioles as seen in Figure 1. The threshold for the selective loss of FAD fluorescence appeared ≤10% O_2 in control mice (maintained at 37.0°C). Loss occurred at higher inspired oxygen (15% O_2) in normothermic endotoxic mice, than in mice at their spontaneous hypothermic temperature (32.4°C ± 2.0). Arterial oxygen saturation was similar in normothermic control and endotoxic mice breathing 15% O_2 (endotoxic mice at 37°C = 56.1% ± 6.8,

Table 1(abstract O5) General hemodynamics and Oxygen-derived parameters

	T0	T6
SvO2, (%)	68.8 (61.75-75.0)	69.7 (64.4-75.9)
Cardiac Index, (L/min/m2)	3.3 (2.4-4.0)	3.2 (2.7-3.8)
iDO2, (ml/min/m2)	389.0 (293.4-500.3)	399.0(322.3-468.2)
iVO2, (ml/min/m2)	116.8 (87.3-150.4)	182.8 (106.3-240.4)
Pv-aCO2, (mmHg)	5.2 (3.6-7.2)	5.8 (3.5-7.7)

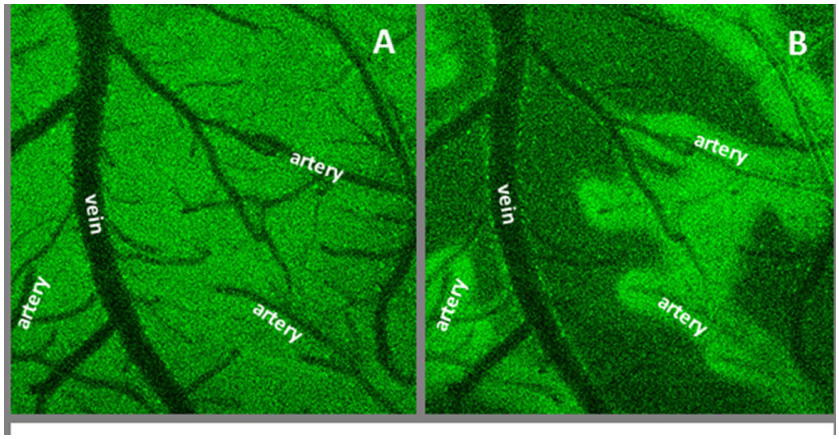

Figure 1(abstract O6) Example of an arterial halo. A) Corex of a control mouse subjected to room air and imaged fro FAD. B) Same mouse subjected to 10% inspired oxygen

controls at 37°C = 58.1% ± 3.4), but was non-significantly higher in hypothermic endotoxic mice (69.2% ± 12.5).
Conclusions: Systemic administration of LPS increases the vulnerability of cortical mitochondrial function to reductions in inspired oxygen, despite maintenance of arterial oxygen saturation. This vulnerability was reversed by spontaneous hypothermia.
Grant acknowledgment: UK MS Society, National MS Society, MRC, UCL Grand Challenge Studentship.

O7

0034. Changes of TLR2, TLR4, MYD88 MRNA expressions on peripheral blood mononuclear cell in severe sepsis patients during treatment with thymosin α1

Z Tang*, J Wu, J Chen, B Ouyang, M Chen, X Guan
The First Affiliated Hospital of Sun Yat-sen University, Department of Surgical Intensive Care Unit, Guangzhou, China
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**O7

Introduction: Severe sepsis is associated with a high mortality rate despite implementation of guidelinerecommendations. Thymosin alpha 1 (Ta1) has been used to treat severe sepsis as a promising beneficial immunomodulatory drug, but its mechanism remains unclear.
Objectives: To examine the TLR2, TLR4 and MyD88 mRNA expressions on human peripheral blood mononuclear cells during treatment.
Methods: A prospective randomized control trial was designed. Fifty-four patients with severe sepsis were enrolled and randomized into thymosin group (26 cases, treated with thymosin alpha 1) and control group (28 cases). TLR2, TLR4 and MyD88 mRNA were tested by RT-PCR on the day of enrollment, day 3 and day 7 after treatment in both groups, 28 day mortality in these patients was analyzed.
Results: There was no statistical significance between 28 day mortality rate in the control group and that of thymosin group(35.7% vs 23.1%, P=0.310). TLR2, TLR4 and MyD88 mRNA expressions in the thymosin group were respectively increased on enrollment day, day 3 and day 7, however, this increasing trend was not found in the control group. On day 3, TLR2 and TLR4 mRNA expressions in the thymosin group were higher than those in the control group (2.31±0.79 vs 1.83±0.51, 7.31±0.79 vs 6.55±0.92, P<0.05). On day 7, TLR2, TLR4 and MyD88 mRNA expressions in the thymosin group were higher than those in the control group (2.75±1.17 vs 1.63±0.36; 7.75±1.03 vs 6.39±0.72; 4.26±0.77 vs 3.77 ±0.68, P<0.05).
Conclusions: Thymosin alpha 1 may increase TLR2,TLR4 and MyD88 mRNA expressions in severe sepsis patients.

References

1. Hotchkiss RS, Karl IE: **The pathophysiology and treatment of sepsis[J].** *N Engl J Med* 2003, **348(2)**:138-150.
2. Garaci E: **Thymosin alpha1: a historical overview[J].** *Ann N Y Acad Sci* 2007, **1112**:14-20.
3. Romani L, Bistoni F, Gaziano R, *et al*: **Thymosin alpha 1 activates dendritic cells for antifungal Th1 resistance through toll-like receptor signaling[J].** *Blood* 2004, **103(11)**:4232-4239.
4. Peck-Palmer OM, Unsinger J, Chang KC, *et al*: **Deletion of MyD88 markedly attenuates sepsis-induced T and B lymphocyte apoptosis but worsens survival[J].** *J Leukoc Biol* 2008, **83(4)**:1009-1018.
5. De Luca A, Montagnoli C, Zelante T, *et al*: **Functional yet balanced reactivity to Candida albicans requires TRIF, MyD88, and IDO-dependent inhibition of Rorc[J].** *J Immunol* 2007, **179(9)**:5999-6008.

O8

0035. Mitochondrial uncoupling contributes to fever in sepsis

E Greco*, N Arulkumaran, A Dyson, M Singer
Bloomsbury Institute of Intensive Care Medicine, University College London, London, UK
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**O8

Introduction: The major sources of body heat production are muscle activity, chemical reactions involving ATP synthesis and usage, coupled (oxidative phosphorylation) and uncoupled (proton leak) mitochondrial respiration. The cause of fever associated with sepsis has not been

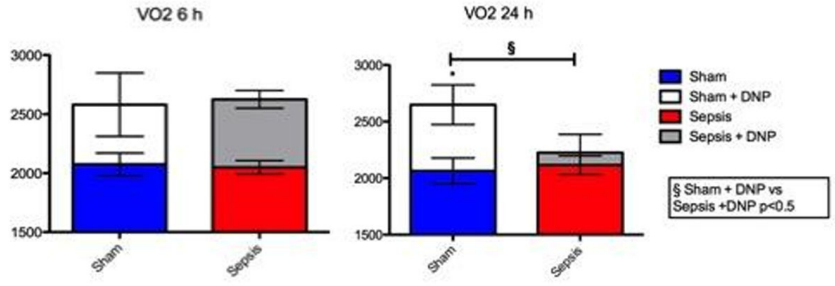

Figure 1(abstract O8)

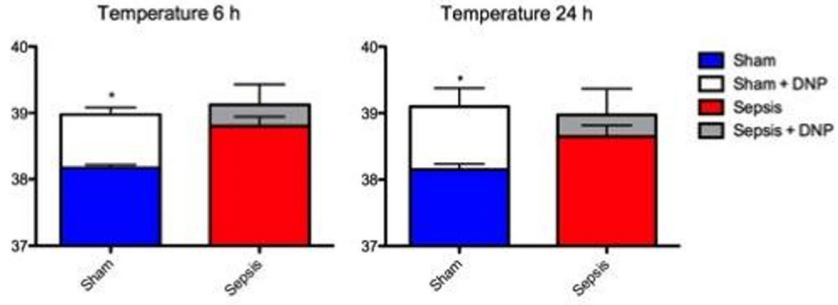

Figure 2(abstract O8)

elucidated, particularly in sedated, ventilated patients who neither perform much voluntary skeletal muscle activity nor shiver.

Objectives: To determine whether the excess heat production in febrile septic rats is mediated by mitochondrial uncoupling, and related to an increase in global oxygen consumption previously cannulated (tunneled carotid and jugular lines), male Wistar rats (approx. 300g body weight) were placed in metabolic cages to measure VO_2. Core temperature was measured intermittently with a rectal probe. Twenty-four hours later, sepsis was induced by i.p. injection of faecal slurry. Sham animals received i.p. saline. Intravenous fluid resuscitation (10 ml/kg/h crystalloid) was started 2 h later. At 6 and 24 hours, animals were randomized to receive an infusion of either the mitochondrial uncoupler dinitrophenol (DNP) (30 mg/Kg) or n-saline over 1 hour. Wilcoxon Rank Sum test was used to compare groups and two-way ANOVA was used to compare change of continuous variables from baseline between groups, with $p< 0.05$ considered significant.

Results: Sham animals were euthermic at 6 and 24h, and their VO_2 was similar to baseline. Infusion of DNP produced similar rises in both temperature and VO_2 ($p< 0.05$) at both timepoints (Figure 1). By contrast, the septic animals were febrile at 6h and 24h ($p< 0.05$ vs baseline) and DNP only induced a small, non-significant rise in temperature to the same level as seen in shams (Figure 2). Whereas 6h and 24h VO_2 values were similar to shams, the effect of DNP on VO_2 was similar to sham animals at 6h but significantly reduced at the 24h timepoint (Figure 1).

Conclusions: In febrile septic animals, mitochondrial uncoupling with DNP only produced a small rise in temperature at 6h and 24h, and a subnormal VO_2 response at 24h. This implies that increased mitochondrial uncoupling was already active in septic rats and this may explain their fever. The proportion of VO_2 directed towards ATP-coupled respiration also appears to be reduced.

Reference

1. Rolfe DF, Brown GC: *Physiol Rev* 1997, **77**:731-58.

NEUROMONITORING

O9

0036. Confocal imaging of impaired mitochondrial function in the cerebral cortex of rats during haemorrhagic shock *in vivo*

KK Ida[1,2*], LMS Malbouisson[1], DA Otsuki[1], KI Chisholm[2], A Dyson[3], M Singer[3], MR Duchen[4], KJ Smith[2]

[1]University of São Paulo, Medical School, Anaesthesiology LIM-8, São Paulo, Brazil; [2]University College London, Department of Neuroinflammation, Institute of Neurology, London, UK; [3]University College London, Bloomsbury Institute of Intensive Care Medicine, London, UK; [4]University College London, Department of Cell and Developmental Biology, London, UK

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**O9

Introduction: Haemorrhagic shock (HS)-induced hypotension impairs cerebral perfusion pressure and decreases oxygen supply to the brain (1), but the consequences for brain mitochondrial function are poorly understood.

Objectives: To investigate the effects of HS on mitochondrial function in the cerebral cortex of rats *in vivo*.

Methods: A flap of skull was removed and cerebral cortex exposed in isoflurane-anaesthetised rats undergoing HS (target mean arterial pressure (MAP) of 40 mmHg for 30 minutes) (n=7) compared with sham (n=6) controls. Mitochondrial function was assessed by observing mitochondrial membrane potential (using tetramethyl rhodamine methyl ester (TMRM); ex: 543 nm; em: 585 nm), and endogenous flavin adenine dinucleotide (FAD) autofluorescence (ex: 488 nm; em: 505-570 nm) by confocal imaging. The TMRM and FAD signals were registered before induction of HS (baseline), at MAP of 40 mmHg (shock), and at 30 (T30) and 60 (T60) minutes after shock.

Results: There was a loss of mitochondrial membrane potential (decreased TMRM signal; $p < 0.001$) and reduction of the FAD/$FADH_2$ pair (seen as decreased FAD fluorescence; $p < 0.01$) at both T30 and T60 post-shock, except for a striking protection observed as a 'halo' extending ~30-40

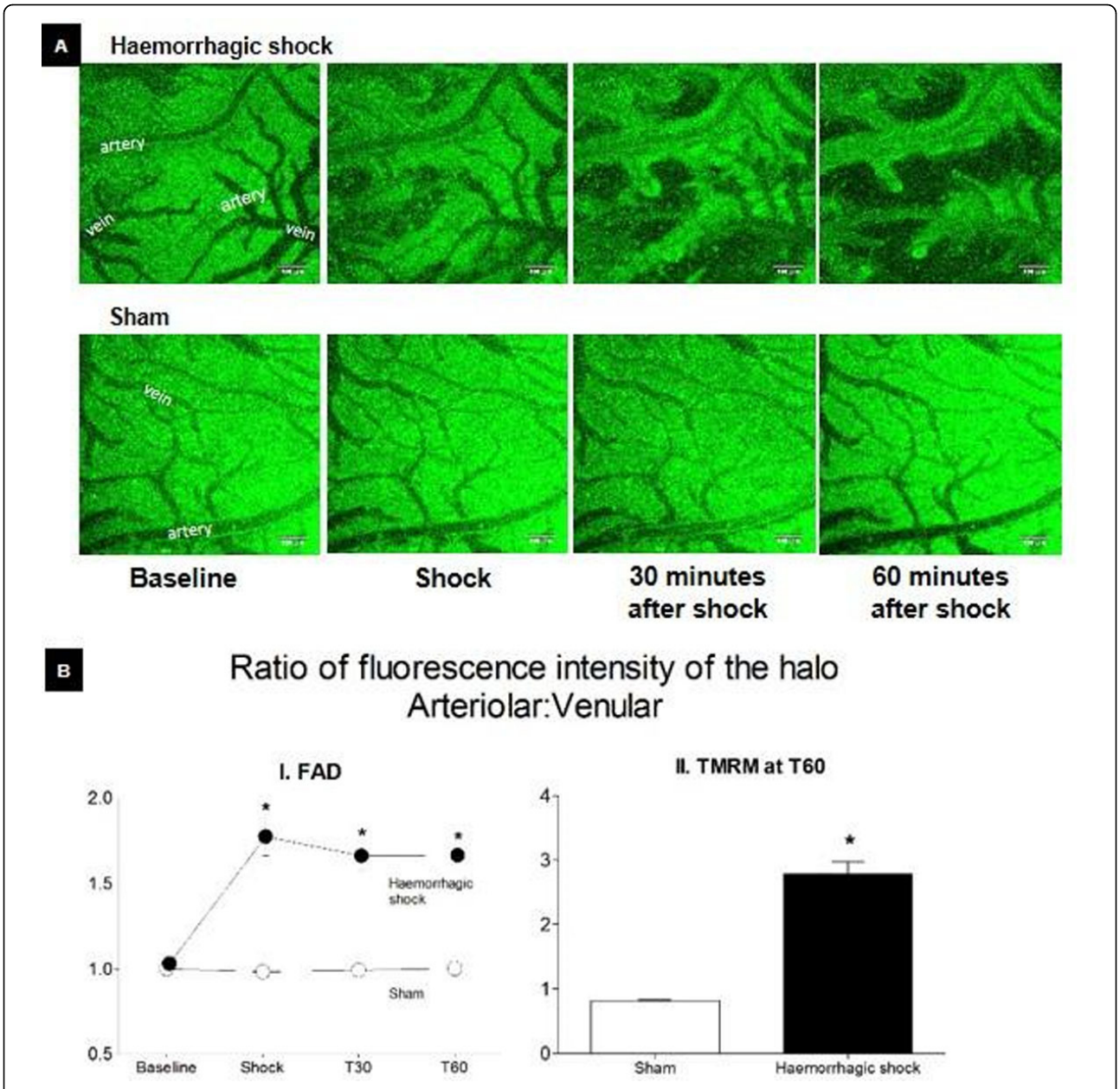

Figure 1(abstract O9) (A) Images of the somatosensory cortex at baseline, haemorrhagic shock, and at 30 and 60 minutes after shock. Green mitochondrial FAD autofluorescence indicates an oxygenated environment, and in shick this is only maintained within ~30-40 μm of arteries. (B) Ratio of the FAD (I) and TMRM (II) fluorescence intensity of the halo extending 30-40 μm, from arterioles/arteries to venules/veins in rats undergoing haemorrhagic shock. *Within a time-point, the value is significantly different from sham (p <0.01).

mm from arterioles (Figure 1). No loss of mitochondrial function was observed in sham controls (MAP >90 mmHg in all animals at both T30 and T60).

Conclusions: HS-induced hypotension causes a marked loss of mitochondrial membrane potential and FAD fluorescence in cerebral cortex, except in regions in immediate proximity to arterioles. This study reveals a profound spatial vulnerability of cortical tissue to reduced blood flow.

Grant acknowledgment: Programa Ciência sem Fronteiras 10464-4-12, UK MS Society, MRC.

Reference

1. Cavus E, Meybohm P, Doerges V, *et al*: **Cerebral effects of three resuscitation protocols in uncontrolled haemorrhagic shock: a randomised controlled experimental study.** *Resuscitation* 2009, **80**:567-72.

CARING FOR THE CRITICALLY ILL BURN PATIENT

O10

0041. The effect of low-dose hydrocortisone after severe burn injury: a microarray longitudinal study

J Textoris[1,2*], J Plassais[1], M-A Cazalis[1], F Venet[1,3], T Rimmele[2], G Monneret[3], A Pachot[1], S Tissot[2]

[1]BioMérieux, Joint Research Unit Sepsis, Lyon, France; [2]Hospices Civils de Lyon, Burn ICU, Lyon, France; [3]Hospices Civils de Lyon, Immunology Lab, Lyon, France

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**O10

Introduction: The systemic effect of inflammatory mediators release after severe burn is a severe cardiovascular dysfunction called burn shock. Patients need vasopressor infusion to maintain adequate delivery of oxygen but it could have deleterious effects on skin perfusion and worsen the burn depth. This shock could result from the interplay of the initial hypovolemia and the release of multiple inflammatory mediators. It has been shown that a low-dose of hydrocortisone could reduce the shock duration but the mechanisms involved remain unclear. In this study, we investigated the systemic genomic response after severe burn injuries and determine whether patterns of gene expression could be associated with low-dose of glucocorticoids.
Objectives: In this study, we investigated the systemic genomic response after severe burn injuries and determine whether patterns of gene expression could be associated with low-dose of glucocorticoids.
Methods: Thirty burn patients with over 30% of total body surface area were enrolled into a randomized double-blind clinical study. 15 patients were treated with low-dose of hydrocortisone and 15 patients were treated with placebo. Whole blood samples were collected after shock onset (S1) before any treatment, one day after treatment beginning (S2) and 120h and 168h after the burn injury (S3/S4). Moreover blood samples of 13 healthy volunteers were collected. Pangenomic expression was evaluated with Affymetrix HG-U133plus 2.0 microarrays. Moderated t-tests and F-test were used to compare burn patients to controls and then gene expression profiles between the 2 groups (B-H correction, $p < 0.05$).
Results: Severe burn injury induced the deregulation of a considerable number of genes (n>2200 at S1) in comparison with controls with an increased number of deregulated genes over time. Within burn patients, more than 300 genes were deregulated by hydrocortisone over time. The treatment had a rapid effect on gene expression, 339 and 627 genes were differentially expressed at S2 and S3 respectively. However the number of these genes decreased drastically at S4 (only 24 genes significant). The genes identified at S2 were mostly related to the decrease of growth, development and quantity of leukocytes but these biological processes were not found significant at S3 indicating that the action of glucocorticoid in the response to burn injury is short-lived and time dependent.
Conclusions: This study is an informative overview of the genomic responses after burn injuries. More importantly it is the first study providing information about mechanism involved in glucocorticoid's reduced shock duration after burn.

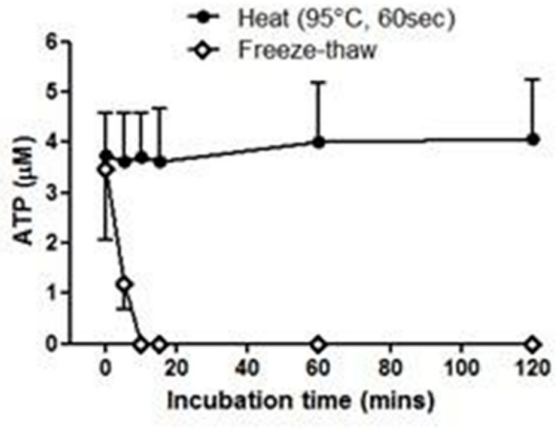

Figure 1(abstract O11) *n=4-7*

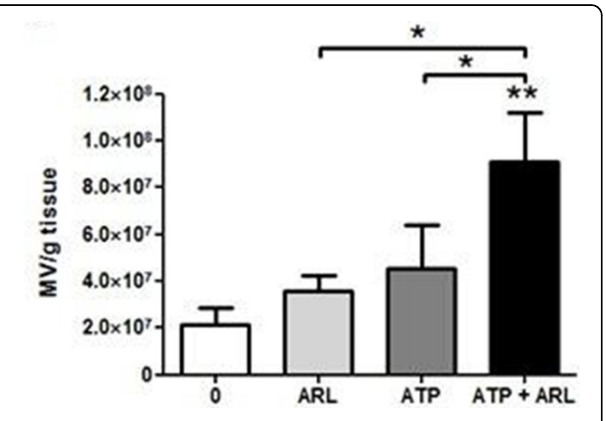

Figure 2(abstract O11) *n=4-7, *p<0.05, **p<0.01, ***p<0.001*

O11

0042. Burn injury stabilises extracellular atp and induces microvesicle production in skin

U Katbeh*, M Takata, KP O'Dea
Imperial College London, Section of Anaesthetics, Pain Medicine and Intensive Care, London, UK
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**O11

Introduction: Microvesicles (MVs) are subcellular membrane-enclosed particles released from activated or dying cells with multiple functions, including induction of inflammation. A role for MVs has been indicated in sepsis- and trauma-related systemic inflammatory response syndrome (SIRS), but their production and function in severe burns injury is unknown. Extracellular ATP released from mechanically injured cells is an important stimulus for MV production [1], but this response is usually limited spatially as well as temporally by the rapid breakdown of extracellular ATP by ectoATPases. Due to the known sensitivity of ectoATPases to thermal inactivation [2], we hypothesised that ATP released during thermal injury would be stable and capable of inducing MVs from viable responder cells within the burn tissue microenvironment.
Objectives: To investigate the release of ATP from thermally-injured cells and its potential role in inducing MV release from burn-injured tissue.
Methods: In vitro cultured human keratinocytes were injured thermally by incubation at 95°C for up to 60 seconds, or mechanically by one freeze-thaw cycle. ATP release was quantified using a fluorimetric assay. An ex vivo

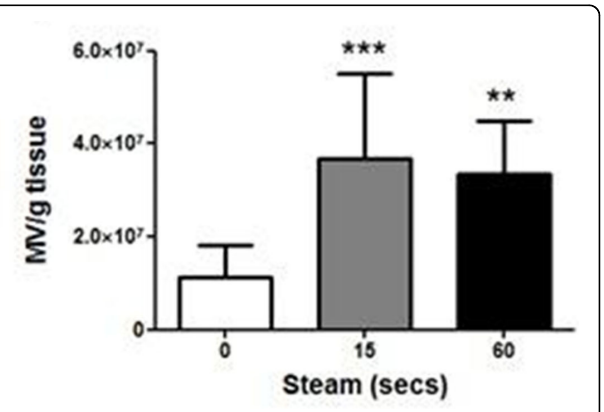

Figure 3(abstract O11) *n=4-7, *p<0.05, **p<0.01, ***p<0.001*

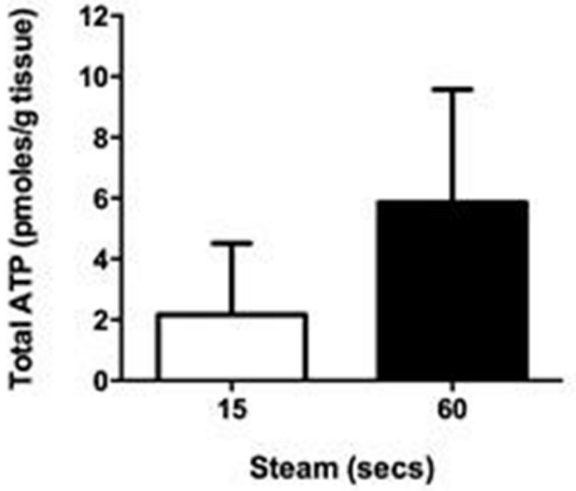

Figure 4(abstract O11) *n=4-7, *p<0.05, **p<0.01, ***p<0.001*

mouse skin explant model was developed in which skin pieces were exposed to steam for 15 or 60 secs, or to ATP (6mM) sub-dermally, and then incubated in medium for 2 hours at 37°C. Released MVs were analysed by flow cytometry. Skin oedema was assessed by weight gain during the incubation period.

Results: ATP was released by thermal injury to keratinocytes and remained stable for up to 3 hours at 37°C (Fig. 1). In contrast, ATP released by freeze-thawing decayed rapidly. Incubation of skin explants with ATP produced significant MV release (Fig. 2), but this effect required the presence of an ectoATPase inhibitor (ARL 67156) presumably due to prevention of the normal extracellular ATP degradation process. Exposure of skin to steam for 15 or 60 secs produced significant increases in MV release into the medium (Fig. 3), while ATP was detectable in medium from steam-exposed but not untreated skin (Fig. 4). Skin weight significantly increased with 15 (17.8±4.3%, $p< 0.05$, n=6) and 60 secs (40.0±18.2%, $p< 0.001$, n=6) steam exposure confirming that both treatments produced a burn injury.

Conclusions: These results demonstrate that burn injury can elicit MV production and that ATP release from injured cells could play a central role in this process. The stabilisation of extracellular ATP by burn injury would produce a unique pathophysiological environment for MV production, which, if released systemically, would play an important and more significant role in promoting systemic inflammation following severe burn injury as compared to other SIRS aetiologies.

Grant acknowledgment: Funded by the Chelsea and Westminster Health Charity.

References

1. Bianco F, *et al*: *J Immunol* 2005, **174**:7268-7277.
2. Despa F, *et al*: *Burns* 2005, **31**:568-577.

RESPIRATORY ASSESSMENT & MONITORING

O12

0449. Surface EMG of extra-diaphragmatic muscles can assess muscle unloading during neurally adjusted ventilatory support

P Somhorst[1,2*], M van Mourik[1,2], AA Koopman[1,2], D Gommers[1]
[1]Erasmus MC, Adult Intensive Care, Rotterdam, Netherlands; [2]University of Twente, Institute of Technical Medicine, Enschede, Netherlands
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**O12

Introduction: Neurally adjusted ventilatory assist (NAVA) is used to adapt mechanical ventilation to patient demand while unloading respiratory muscles. Titration methods are focused on sustained unloading of the diaphragm while maintaining a stable tidal volume [1]. It is hypothesized that the activity of accessory respiratory muscles can supply extra information about muscle unloading and patient comfort during NAVA ventilation.

Objectives: To assess the extra-diaphragmatic muscle activity (EDMA) during 100%, 50% and 150% of titrated NAVA level.

Methods: EDMA was measured in ventilated patients with mild ARDS. EDMA was defined as the amplitude of the combined surface EMG at the scalene and sternomastoid muscle (Dipha16, InBiolab, Groningen, The Netherlands). A baseline NAVA level (NAVA100) was titrated using the diaphragm activity (EAdi) response to changing NAVA levels, according to Brander et al. [1]. Patients were ventilated with NAVA100, NAVA50 (50% of NAVA100) and NAVA150 (150% NAVA100) for a period of 15 minutes.

Results: Twenty-one patients were included. In six patients EDMA was absent during NAVA100, so NAVA titration was sufficient to unload accessory respiratory muscles. Fifteen patients (71%) showed EDMA during NAVA100. In seven patients (33%) EDMA increased at NAVA50 and decreased during NAVA150. In one patient, EDMA decreased at NAVA150. One patient only showed EDMA during NAVA50. In three patients EDMA decreased at NAVA50 or increased at NAVA150. Nine patients (43%) showed no change in EDMA after a change of NAVA level.

Conclusions: Measurement of extra-diaphragmatic muscle activity using surface EMG during NAVA ventilation might be helpful in titration of ventilatory assist level in order to optimize patient´s comfort.

Reference

1. Brander L, Leong-Poi H, Beck J, Brunet F, Hutchison SJ, *et al*: Titra- tion and implementation of neurally adjusted ventilatory assist in critically ill patients. *Chest* 2009, **135(3)**:695-703.

NEW FINDINGS IN CARDIOVASCULAR DYNAMICS

O13

0450. Systemic dysregulation of the angiopoietin-1/2 system in adults undergoing cardiopulmonary bypass (CBP)

E Charbonney[1*], E Wilcox[2], Y Shan[2], P Perez d'Empaire[2], A Dugal[3], M Glogauer[2], GD Rubenfeld[2], S Sutherland[2], C Lilles[4], C Dos Santos[2]
[1]Université de Montréal, Montréal, Canada; [2]University of Toronto, Toronto, Canada; [3]Cleveland Clinic Foundation, Cleveland, USA; [4]University of Washington, Seattle, USA
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**O13

Introduction: Systemic capillary leak syndrome after cardiopulmonary bypass (CBP) is a well-known phenomenon, accompanied by interstitial fluid accumulation and inflammation, and can lead to end-organ failure or increased hospital length of stay (LOS). The pathophysiology responsible for capillary leak involves activation of inflammation, complement, and coagulation. The endothelium plays a central role in the activation of coagulation, and activated/dysregulated endothelium contributes to microvascular leak and enhanced adhesion of leukocytes. However, the characterization and measurement of endothelial dysregulation is not well established. Angiopoietin-1 (Ang-1) and -2 (Ang-2) are molecules implicated in angiogenesis and regulation of endothelial permeability. Their differential changes of expression could be a surrogate to quantify endothelial dysregulation after CBP.

Objectives: To characterize changes (delta) of Ang-1 and Ang-2 over time after elective CBP surgery in adults, and investigate conditions associated with measured level changes.

Methods: Patients > 18 years, undergoing elective cardiac surgery using CBP, were enrolled. Baseline blood samples were obtained before surgery (T0), at admission to the ICU (T1) and the day following admission to the ICU (T2). Plasma Ang-1/-2 were measured using an ELISA and cytokines were measured using ELISA-based multiplex technology®.

Results: Forty-one adult patients were enrolled consecutively, after obtaining consent.

Serum Ang-2 and Ang-2/Ang-1 ratio increased significantly over time (all $p < 0.0001$), while Ang-1 decreased ($p = 0.012$). When compared to baseline, Ang-2 and Ang-2/Ang-1 ratio were significantly higher at both post-CBP time (T1 vs T0 and T2 vs T0; all $p < 0.0001$). IL-1β, IL-6, MCP-1, IL-10, IL-12

and IL-1RA rose significantly at T1. No meaningful correlation was found between changes in Ang-1/2 and cytokines.
A positive correlation was found between delta creatinine T0-T1 and delta T0-T1 for the ratio Ang-2/Ang1 ($r = 0.38$; $p = 0.027$). The hospital LOS correlated highly with delta T0-T2 for Ang-2 ($r = 0.590$; $p < 0.0001$) in patients with LOS ≤ 14 days (90%). The predictors of delta Ang-2 in multivariate analysis were female gender, ACE inhibitor use, blood transfusion and clamping time.
Conclusions: CBP was associated with increases in serum Ang-2 and Ang-2/Ang-1 ratio, indicating endothelial activation and dysfunction post-CBP. Ang-1/2 dysregulation correlated with hospital length of stay and delta creatinine. Factors predicting endothelial dysregulation need to be further investigated.

References

1. Giuliano JS, Lahni PM, Bigham MT, Manning PB, Nelson DP, Wong HR, Wheeler DS: **Plasma Angiopoietin-2 Levels Increase in Children Following Cardiopulmonary Bypass.** *Intensive Care Medicine* **34(10)**:1851-1857.
2. Clajus C, Lukasz A, David S, Hertel B, Lichtinghagen R, Parikh SM, Simon A, Ismail I, Haller H, Kümpers P: **Angiopoietin-2 is a potential mediator of endothelial barrier dysfunction following cardiopulmonary bypass.** *Cytokine* **60(2)**:352-359.

RESPIRATORY DYSFUNCTION IN SEPSIS

O14

0468. Cerebral effects of lateral trendelenburg vs semirecumbent position in an experimental model of ventilator-associated pneumonia

J López-Aguilar[1,2*], G Li Bassi[2,3], ME Quílez[1], JD Martí[3], M Rigol[3], O Tavares-Ranzani[3], E Aguilera[3], I Ferrer[4,5], L Blanch[1,2,6], A Torres[2,3]
[1]Fundació Parc Taulí, Sabadell, Spain; [2]CIBERES, Madrid, Spain; [3]Instituto del Torax, Hospital Clinic, IDIBAPS, UB, Barcelona, Spain; [4]Idibell, Hospital Universitario de Bellvitge, L'Hospitalet de Llobregat, Spain; [5]CIBERNED, L'Hospitalet de Llobregat, Spain; [6]Corporació Sanitária i Universitária Parc Taulì-UAB, Sabadell, Spain

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**O14

Introduction: Lateral-Trendelenburg position has been postulated as a promising intervention to prevent ventilator-associated pneumonia (VAP) because it improves mucus clearance and avoids pulmonary aspiration in intubated patients [1]. Mechanical ventilation (MV) per se can modify brain status [2,3] but the effects of position during MV are unknown.
Objectives: To investigate the preventive effects of lateral Trendelenburg (TL) vs semirecumbent (SR) position in the development of VAP and its effects in the brain in an experimental model in pigs.
Methods: We have studied 17 Large White-Landrace pigs (30±2 kg) anesthetized, intubated and MV during 72 h in volume control. Animals were randomized in 3 groups:
1) SR: SR position, PEEP 0cmH2O, inspiratory-expiratory ratio (I:E) 0.33,
2) SR-inv: SR position, PEEP 5 cmH2O, I:E 0.7, and
3) TL: TL position, PEEP 0cmH2O, and I:E 0.33.
All the animals were oropharyngeal instilled (10ml) with *P aeruginosa* (10^7-10^8 cfu/ml). Mean arterial pressure (MAP) was monitorized and final bacterial pulmonary colonization, and cerebral status, consisting in a macroscopical evaluation of haemorrhage and of apoptosis indicatiors (caspase and TUNEL) in dentate gyrus in the hippocampal formation, were evaluated.
Results: Bacterial Colonization (0.22 vs 2.27 and 2.31 log cfu/gr, $p < 0.05$) and VAP (0 vs 67 and 86 %, $p < 0.05$) were drastically reduced in TL position as compared to SR and SR-inv respectively. MAP was lower in SR and SR-inv compared to TL position (80 and 73 vs 90 mmHg, $p < 0.05$). At the brain level, pigs in TL position presented high score of petequial hemorrhage (2.6 vs 1 and 1.5, $p < 0.05$), and higher levels of immunopositive cells to caspase (6.3 vs 2.5 and 1.7, $p< 0.05$) and TUNEL (5.17 vs 1 and 2.72, $p< 0.05$) in the dentate gyrus in the hippocampus, both indicators of apoptosis, in comparison with groups SR and SR-inv respectively.
Conclusions: In this pig model of MV, TL position prevents pulmonary colonization and VAP development, but enhances cerebral hemorrhage, and increased apoptosis in the hippocampus. These alterations in the brain could be related with the increase in MAP observed in TL position. More studies to evaluate risks and benefits of TL position are needed.
Grant acknowledgment: Fundació Parc Taulí; MICIN PS09/01249; *ESICM* (*2009* Alain Harf Award on Applied Respiratory Physiology); AGAUR GRC 532.

References

1. Li Bassi G, Torres A: **Ventilator-associated pneumonia: role of positioning.** *Curr Opin Crit Care* 2011, **17**:57-63.
2. Quílez ME, López-Aguilar J, Blanch L: **Organ crosstalk during acute lung injury, acute respiratory distress syndrome, and mechanical ventilation.** *Curr Opin Crit Care* 2012, **18**:23-8.
3. Quilez ME, Fuster G, Villar J, Flores C, Martí-Sistac O, Blanch L, López-Aguilar J: **Injurious mechanical ventilation affects neuronal activation in ventilated rats.** *Crit Care* 2011, **15**:R124.

NUTRITION: THE BEST NEW MIXTURE

O15

0473. Necrosis and apoptosis in liver, spleen, pancreas, kidney and intestinal tissue induced by intra-abdominal hypertension in a porcine model. Second part of an experimental study

JA Buensuseso Alfaro[1*], M Poblano Morales[1], MA Moreno Eutimio[2], J Mendoza Escorza[1], S Zamora Gómez[1], G Magdaleno Lara[1], FJ Tendillo Cortijo[3], M Lomelí Terán[4], JJ Martínez Mazariegos[5], E Deloya Tomas[6], L Torres López[1], T Mondragon Labelle[1]
[1]Hospital Juarez of Mexico, Intensive Care Unit, Mexico, Mexico; [2]Hospital Juarez of Mexico, Learning and Investigation Center, Mexico, Mexico; [3]Sanity Investigation Institute Puerta de Hierro, Medical-Surgery Investigation Unit, Madrid, Spain; [4]Hospital Tec 100, Intensive Care Unit, Santiago de Queretaro, Mexico; [5]Specialty Hospital Better Life ISSTECH, Intensive Care Unit, Tuxtla Gutierrez, Mexico; [6]General Hospital San Juan del Rio, Intensive Care Unit, Santiago de Queretaro, Mexico

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**O15

Introduction: Intra-abdominal hypertension (IAH) and abdominal compartment syndrome (ACS) are increasingly recognized as severe complications of critical illness.
Objectives: Characterize the apoptosis and necrosis in the renal, intestinal, splenic, pancreatic and hepatic tissue in an experimental porcine model after six hours of IAH.
Methods: Four York-Landrace mixed breed piglets with a mean body weight of 30 kg were obtained from Center for Teaching, Research and Extension in Swine Production, Faculty of Veterinary Medicine, National Autonomous Mexican University.
Biopsies were taken from liver and kidney by laparoscopy prior to induction of IAP, the intra-abdominal pressure was increased with an intra-peritoneal catheter that was placed in the peritoneal cavity at the level of the umbilicus; and then a saline 0.9% solution was infused to increase intra-abdominal pressure. The IAP was increased up to 20 mmHg, renal an liver biopsies were performed by exploratory laparotomy after the increase of IAP to 20 mmHg and 6 hours after sustained elevated IAP.
The biopsy was cut in two pieces and these were immediately placed into a disposable disaggregator Medicon with 50 μm separator mesh plus 1 mL of ice-cold PBS and processed for 50 s in the Medimachine System.
The cell suspension were stained with FitC-conjugated mAb specific for CDXX, PE-conjugated mAb specific for CDXX. Briefly, 1x106 were stained with the fluorochrome-conjugated mAb specific for cell surface antigen markers for 20 min in the dark at 4°C. After incubation, the cells were resuspended in 200 μL PBS for subsequent flow cytometric analysis using a Accuri C6 flow cytometerand then were analysed using FlowJo software V10.
Results: After 6 hours of sustained IAH there was no difference in the percentage of cells in early apoptosis in the spleen ($p=0.19$) and intestinal tissue ($p=0.24$). There was difference in the liver ($p=0.01$) and pancreas ($p=0.02$).
There was difference in pancreas ($p=0.04$), kidney ($p=0.01$) and intestinal tissue ($p=0.02$) after 6 hours of sustained IAH in the percentage of cells in late stage apoptosis, and only the pancreatic tissue shown necrosis ($p=0.03$) (Fig. 1, Fig. 2, Fig. 3)
Conclusions: Apoptosis and necrosis presents in pancreas above all, but also in hepatic, splenic, intestinal and kidney tissues after six hours of IAH. Our data suggests that the greatest harm of sustained IAH take place in the pancreas, kidney and intestinal tissue in an early fashion and more lately in the liver.

References

1. Al Mufarrej F, *et al*: **Understanding intra-abdominal hypertension: from the bench to the bedside.** *J.Intensive Care Med* 2012, **27**:145-160.

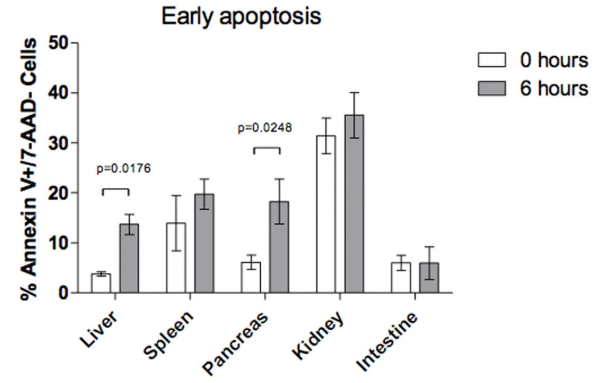

Figure 1(abstract O15)

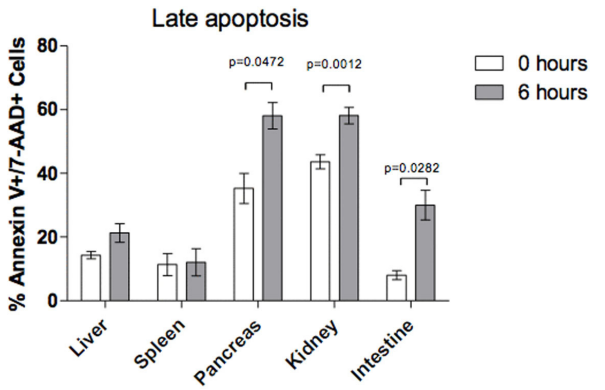

Figure 2(abstract O15)

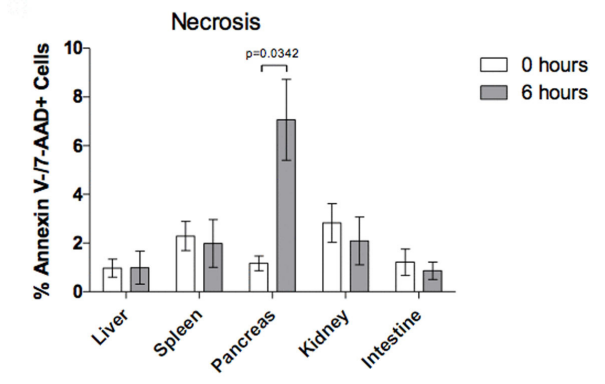

Figure 3(abstract O15)

2. Gallagher JJ: **Intra-abdominal hypertension: detecting and managing a lethal complication of critical illness.** *AACN.Adv.Crit Care* 2010, **21**:205-219.
3. Malbrain ML, *et al*: **Intra-abdominal hypertension in the critically ill: it is time to pay attention.** *Curr.Opin.Crit Care* 2005, **11**:156-171.

O16

0474. Circulating mitochondrial dna and vitamin d in critical illness

AA Litonjua[1], LE Fredenburgh[1], RM Baron[1], L Gazourian[1], AF Massaro[1], K Nakahira[2], AMK Choi[2], KB Christopher[3*]
[1]Brigham and Women's Hospital, Pulmonary and Critical Care Division, Boston, MA, USA; [2]Weill Cornell Medical Center, Department of Medicine, New York, NY, USA; [3]Brigham and Women's Hospital, Renal Division, Boston, MA, USA

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**O16

Introduction: Mitochondrial dysfunction and an impaired autophagic response is associated with mortality in experimental sepsis. Vitamin D is shown to upregulate NOD2 which is linked to autophagy. Autophagy regulates innate immunity via inhibition of mitochondrial DNA (mtDNA) release via by the inflammasome. Circulating plasma mt-DNA is a robust predictor of mortality in ICU patients.
Objectives: We hypothesized that critically ill patients with low plasma 25(OH)D would have high levels of circulating plasma mt-DNA.
Methods: We performed a prospective observational cohort of MICU patients at the Brigham and Women's Hospital from 2008-2010 [n=49]. The exposure of interest was 25(OH)D categorized *a priori* as deficiency (25(OH)D ≤ 15 ng/mL) and measured via competitive chemiluminescence immunoassay. Circulating plasma mitochondrial DNA (mt-DNA) were assessed by measuring copy number of the NADH dehydrogenase 1 gene using quantitative real-time PCR. Plasma 25(OH)D was measured from the same plasma sample as mtDNA. Adjusted associations were estimated through fitting of multivariable logistic regression models including covariate terms for potential confounders of interest. Time-to-event analysis was performed using Cox proportional hazard regression.
Results: Of the cohort patients studied, 37% were women and 84% were white. The mean age at critical care initiation was 53.5 years (SD 14.2). The mean APACHE II score was 24.5 (SD 8.3), while 100% of the cohort had SIRS, 71% had a source of infection identified and 18% had ARDS. The mean plasma 25(OH)D was 20.6 (SD 11.4) and 28.6% of the study cohort had plasma 25(OH)D ≤ 15 ng/mL. Gross unadjusted 30-day mortality was 26.5%. The mean (SD) plasma 25(OH)D was significantly higher in patients with mtDNA < 4000 copies/ml [25(OH)D 22.0 (11.2) ng/ml] relative to those with mtDNA ≥ 4000 copies/ml [25(OH)D 14.9 (10.9) ng/mL]; P < 0.001. When the cohort was analyzed with the exposure as plasma 25(OH)D and the outcome as plasma mtDNA ≥ 4000 copies/ml, we find that patients with plasma 25(OH)D < 15 ng/mL have a significantly higher odds of having plasma mtDNA ≥ 4000 copies/ml (unadjusted OR= 7.50, 95% CI 1.53-36.79; P=0.013). 25(OH)D in the cohort remains a significant predictor of plasma mtDNA ≥ 4000 copies/ml following adjustment for age, gender, race and APACHE II (adjusted OR= 13.19, 95% CI 1.69-103.34; P=0.014). Cox proportional hazard multivariable regression modeling, adjusting for *a priori* defined covariates comprising APACHE II score, age, sex, race, and plasma 25(OH)D, showed that mtDNA was predictive of all cause mortality following critical care (HR, 4.02; 95% CI, 1.14-14.20 ng/ml).
Conclusions: 25(OH)D levels are associated with circulating mtDNA levels. This study cannot determine a relationship between 25(OH)D and mtDNA beyond association but raises the question as to whether vitamin D-mediated pathways might play a role in linking autophagic and inflammasome processes.

INSIGHTS INTO PATHOGENESIS OF AKI

O17

0888. Administration of tetrahydrobiopterin (BH4) protects renal microcirculation after ischemia and reperfusion

L Rahmania*, F Su, D Orbegozo Cortés, EH Post, C Santacruz, FS Taccone, J-L Vincent, D De Backer
Erasme Hospital, Université Libre de Bruxelles, Department of Intensive Care, Brussels, Belgium

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**O17

Introduction: Abdominal aortic aneurysm surgery with supra-renal clamping is associated with potential development of renal insufficiency. Ischemia and reperfusion (I-R) produced during the procedure induces

Table 1(abstract O17) Microcirculatory parameters in the 3 groups

	Group	Baseline	1h	4h	6h	P Group*Time
PVD (vessels/mm)	SHAM	2.6 (2.5-2.7)	2.7 (2.3-2.9)	2.4 (2.2-2.7)	2.3 (2.3-2.5) c	P<0.05
	I-R	3.2 (2.9-3.7)	2.0 (1.5-2.8) c	2.1 (1.7-2.7) c	1.6 (1.3-2.3) a, c	
	I-R+BH4	3.2 (2.7-3.4)	3.0 (2.6-3.3) b	2.5 (2.0-3.1) b, c	3.1 (2.4-3.8) b	
PPV (%)	SHAM	93.7 (93.3-94.7)	87.6 (84.2-90.6) c	83.0 (80.3-90.2) c	85.5 (82.0-90.4) c	P=0.269
	I-R	97.3 (96.0-97.9)	80.1 (73.5-90.2) c	82.7 (68.5-88.7) c	79.8 (74.9-84.6) c	
	I-R+BH4	97.8 (96.8-98.3)	92.3 (89.8-93.8) b, c	85.9 (80.9-91.5) c	89.3 (86.0-99.4) c	
PPV-HI (%)	SHAM	11.9 (8.9-13.2)	18.4 (16.5-22.8) c	14.2 (11.0-15.1)	14.4 (11.3-39.0)	P<0.05
	I-R	9.6 (5.5-11.0)	43.0 (22.2-84.0) a, c	41.8 (28.2-97.7) a, c	47.3 (11.1-76.3) c	
	I-R+BH4	9.3 (7.3-12.5)	24.7 (17.1-32.8) c	42.8 (29.1-56.2) c	25.4 (2.9-40.5) c	

[values are presented as median with P25-75]

a: significant difference between groups I-R and SHAM

b: significant difference between groups I-R and BH4+I-R

c: significant difference with the baseline.

endothelial dysfunction with a decrease in tetrahydrobiopterin (BH4), a cofactor used in nitric oxide synthesis.
Objectives: To assess whether BH4 administration could prevent the injury to the renal microcirculation caused by supra-renal aortic clamping with I-R.
Methods: Nineteen adult sheep were anesthetized, mechanically ventilated and invasively monitored. Renal blood-flow was measured continuously through a left lumbotomy using a peri-vascular flow probe (Transonic, USA) and an aortic clamp was positioned above the renal arteries. After surgical preparation and stabilization, animals were randomized into 3 groups (SHAM=5, I-R=7, I-R+BH4=7). SHAM animals underwent surgical preparation but no aortic clamping was performed. The I-R groups were exposed to 1 hour of aortic ischemia. The I-R+BH4 group received 20 mg/kg of BH4 before aortic clamping. Animals were followed for a maximum of 6 hours after reperfusion. Renal microcirculation was evaluated at baseline, and 1, 4 and 6 hours after reperfusion using Sidestream Dark Field video-microscopy (Microvision Medical, Netherlands). We calculated perfused small vessel density (PVD), proportion of perfused small vessels (PPV) and heterogeneity of PPV (PPV-HI). Data were analyzed using the generalized estimating equation (GEE) and p-values less than 5% were considered statistically significant. Results are presented as median [IQRs].
Results: The systemic hemodynamics variables were preserved in all 3 groups. BH4 was associated with improved renal function, as evaluated by creatinine after 6 hours of reperfusion in the I-R+BH4 group (14 mg/L [13-17]) compared to the I-R group (16 mg/L [16-22]) (P=0.072).
Conclusions: In a sheep model of renal I-R, BH4 pre-treatment can prevent microvascular injury and dysfunction. Clinical trials are warranted to evaluate the administration of BH4 to prevent I-R-induced kidney injury.

O18

0889. Mitochondrial dysfunction contributes to sepsis-induces acute kidney injury

E Greco[1*], N Arulkumaran[1,2], M Sixma[1], H Courtneidge[3,4], M Duchen[4], F Tam[2], RJ Unwin[3], M Singer[1]
[1]Bloomsbury Institute of Intensive Care Medicine, University College London, London, UK; [2]Department of Nephrology, Imperial College, London, UK; [3]Centre for Nephrology, University College London, London, UK; [4]Department of Cell and Developmental Biology, University College London, London, UK
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**O18

Introduction: Mechanisms underlying sepsis-induced acute kidney injury remain uncertain. Mitochondrial dysfunction is a hallmark of established sepsis, and may contribute to renal dysfunction.
Objectives: To determine the effect of septic serum on renal tubular epithelial cell mitochondrial physiology.
Methods: Multiphoton confocal microscopy images were recorded over 45 minutes in intact renal sections (taken from healthy rats) incubated in either physiologic saline solution (PSS) (n=6), sham serum (n=3), septic serum (n=3), and septic serum co-incubated with 10nM of the anti-oxidant 4-OH-Tempo (n=4). Pooled septic serum was obtained from (n=5) rats 24 hrs after induction of faecal peritonitis. Sham serum was obtained from 3 healthy controls. The percentage change in NADH redox state (using NADH autofluorescence), reactive oxygen species (ROS) production (using tetramethyl rhodamine methyl ester, TMRM) and mitochondrial membrane potential (using dihydroethidium) are expressed as ratios compared to baseline values. Wilcoxon Rank Sum test was used to compare groups with p< 0.05 taken as significant.
Results: Mitochondrial dysfunction was seen in both proximal and distal tubular epithelial cells incubated in septic serum. NADH became more reduced, reactive oxygen species increased and mitochondrial membrane potential fell compared to either sham serum or PSS. The increases in NADH reduction and ROS production (but not mitochondrial membrane potential) on exposure to septic serum could be prevented by co-incubation with 4-OH-Tempo (Figure 1).
Conclusions: Circulating mediators within septic serum can induce mitochondrial dysfunction in healthy renal tubular cells. Co-incubation with an anti-oxidant offered protection, suggesting these mediators are likely to be oxygen or nitrogen reactive species.
Grant acknowledgment: NA is supported by the Wellcome Trust.

ACUTE LUNG INJURY: EXPERIMENTAL STUDIES

O19

0893. High respiratory rate favors pulmonary edema in an experimental model of acute lung injury

J Retamal[1,2*], JB Borges[1], F Suarez-Sipmann[1], A Bruhn[2], G Hedenstierna[3], A Larsson[1]
[1]Hedenstierna Laboratory, Uppsala University, Department of Surgical Sciences, Uppsala, Sweden; [2]Pontificia Universidad Católica de Chile, Facultad de Medicina, Departamento de Medicina Intensiva, Santiago, Chile; [3]Hedenstierna Laboratory, Uppsala University, Department of Medical Sciences, Clinical Physiology, Uppsala, Sweden
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**O19

Introduction: The ARDS-net protocol [1], recommends that respiratory rate (RR) could be increased at hypercapnia in order to normalize PaCO2. However, **i**n heterogeneously inflated lungs, e.g., ARDS, at every breath the local alveolar distending forces will be amplified up to 4.5 times in the interphase between collapsed and aerated areas [2]. Thus, a higher RR could exaggerate the cyclic deformations of lung parenchyma and might therefore induce further lung injury. Indeed, animal studies using simultaneous modifications of flow and tidal volume have indicated that low respiratory rates are lung protective [3]. We therefore hypothesized that

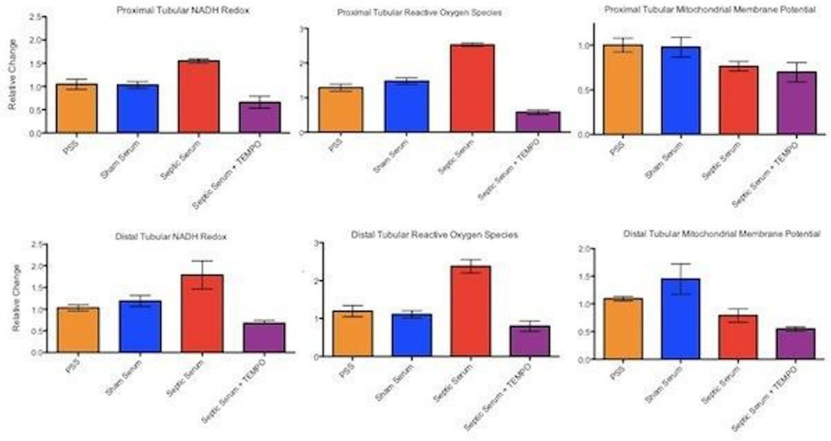

Figure 1(abstract O18)

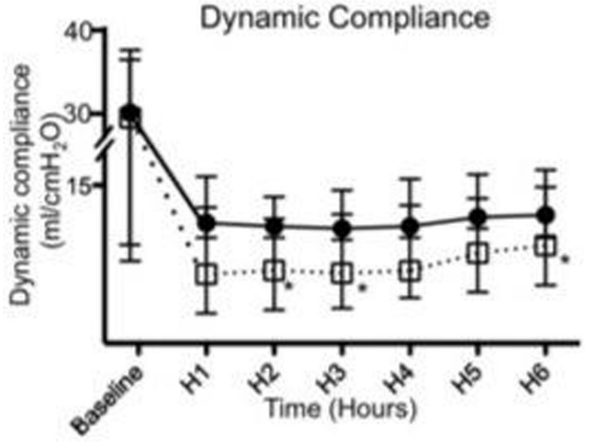

Figure 1(abstract O19) Dynamic compliance

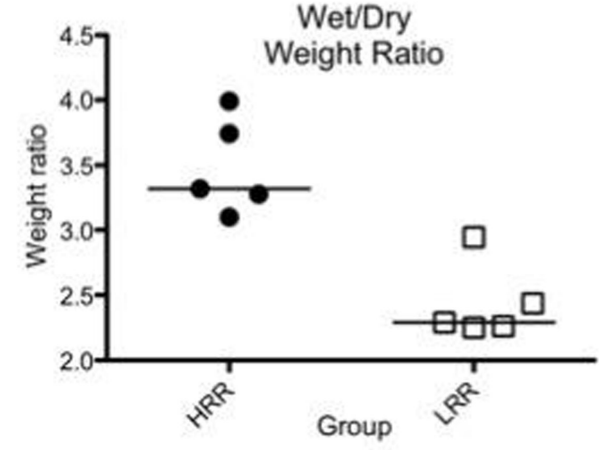

Figure 3(abstract O19) Wet/Dry weight ratio

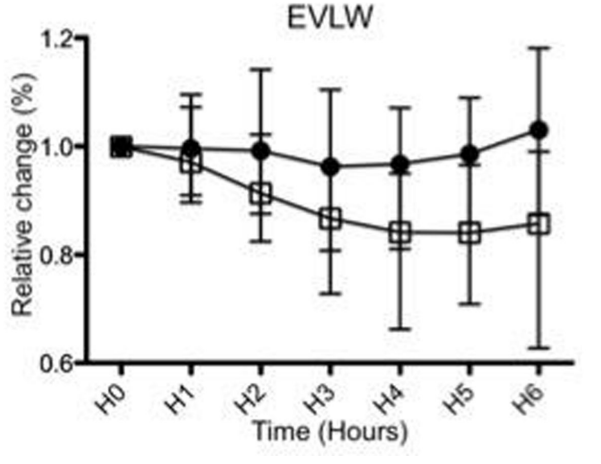

Figure 2(abstract O19) Extravascular lung water (EVLW)

an isolated increase of RR would augment the development of ventilator induced lung injury (VILI).

Objectives: To compare VILI development at two clinically relevant RR during protective mechanical ventilation setting, keeping constant flow, tidal volume (VT) and pCO2 levels.

Methods: Healthy piglets were subjected to a two-hit lung injury model (saline lavages followed by 2 hours of injurious ventilation), and then randomized into two groups: LRR 20 breaths/min (n=6), and HRR 40 breaths/min (n=6), and were mechanically ventilated during six hours according to ARDSnet protocol (VT 6 ml/kg, PEEP10 cmH2O, FiO2 0.5), keeping an inspiratory time of 0.5 sec. We used instrumental dead space to keep similar values of pCO2 in both groups. We assessed respiratory mechanics, invasive systemic and pulmonary arterial pressures, volumetric capnography and extravascular lung water (EVLW). At the end of the experiments lungs were excised and wet/dry (W/D) ratio was evaluated.

Results: Baseline data were similar between groups. No differences in oxygenation, pCO2 levels, or in systemic and pulmonary arterial pressures were observed during the protocol. We observed an increase in dynamic compliance (Fig. 1) and a decrease in EVLW (Fig. 2) over time in the LRR group ($p < 0.05$), but not in the HRR group. In addition, W/D ratio (Fig. 3) was higher in the HRR group ($p< 0.05$). Data are expressed as median and ranges.

Conclusions: In our study high respiratory rate reduced lung water clearance, which resulted in an increase of lung water content, indicating that increasing respiratory rate could augment VILI.

Grant acknowledgment: Beca Cotutela Doctoral CONICYT.

References

1. ARDSNet: **Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome.** *N Engl J Med* 2000, **342**:1301-1308.
2. Mead J: **Stress distribution in lungs: a model of pulmonary elasticity.** *J Appl Physiol* 1979, **28**:596-608.
3. Vaporidi K: **Effects of respiratory rate on ventilator-induced lung injury at a constant PaCO2 in a mouse model of normal lung.** *Crit Care Med* 2008, **36(4)**:1277-1283.

O20

0894. Time course of VILI development: a CT scan study

C Chiurazzi[1*], M Gotti[1], M Amini[1], C Rovati[1], I Algieri[1], M Brioni[1], A Cammaroto[1], C Bacile di Castiglione[1], K Nikolla[1], C Montaruli[1], S Luoni[1], B Comini[1], G Rossignoli[1], G Conte[1], T Langer[1], M Cressoni[1], L Gattinoni[1,2]
[1]Università degli Studi di Milano, Dipartimento di Fisiopatologia Medica e dei Trapianti, Milano, Italy; [2]Fondazione IRCCS Ca' Granda - Ospedale Maggiore Policlinico, Milano, Italy

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**O20

Introduction: Mechanical Ventilation at high tidal volumes and pressures induces in lung oedema which is undistinguishable from ARDS. It is possible that pleura, bronchi and vessels act as a natural stress raiser playing as local stress multipliers [1].

Objectives: To study the development of VILI with CT scan and determine where and when the first lesions appear and to follow their development in time.

Methods: Piglets were instrumented and ventilated with TV of corresponding to a strain (TV/FRC) >2.5. The whole study was performed in an animal CT scan facilities equipped as an ICU. Every three hours a CT scan was performed and data on respiratory mechanics were collected. CT scans were analysed and lesions were defined as clearly defined regions of poorly/not inflated tissue (-500/+100 HU) with a minimal diameter of 6 mm non present in the previous CT scan image. Lung weight was computed from the CT scan data.

Results: We studied 11 swine (22±5 kg), 5 healthy and 6 with basal densities which did not regain inflation after a recruitment manoeuvre. As shown in Figure 1 most the CT scan lesions were subpleural. The first CT scan lesions appeared after a median of 7 hours [4.5-12] in healthy pigs and after a median of 9 hours [6.5-11.5] in diseased ones (p=0.88 healthy vs diseased) while changes in lung mechanics developed after 20 [13 - 23] hours (p=0.04 vs time of development of CT scan lesions) and oxygenation impairment, defined as PaO2FiO2 < 200, after 25 [14 - 36] hours (p=0.003 vs time of development of CT scan lesions) while widespread lung oedema defined as presence of infiltrations in all lung fields at CT scan imaging developed after 18 [12 - 32] hours (p=0.02 vs time of development of CT scan lesions).

Conclusions: Oxygenation and respiratory mechanics impairment is evident when relevant lung oedema develops. Most of the first CT scan lesions are located in lung regions which act as stress raisers and precede alterations in gas exchange and respiratory mechanics.

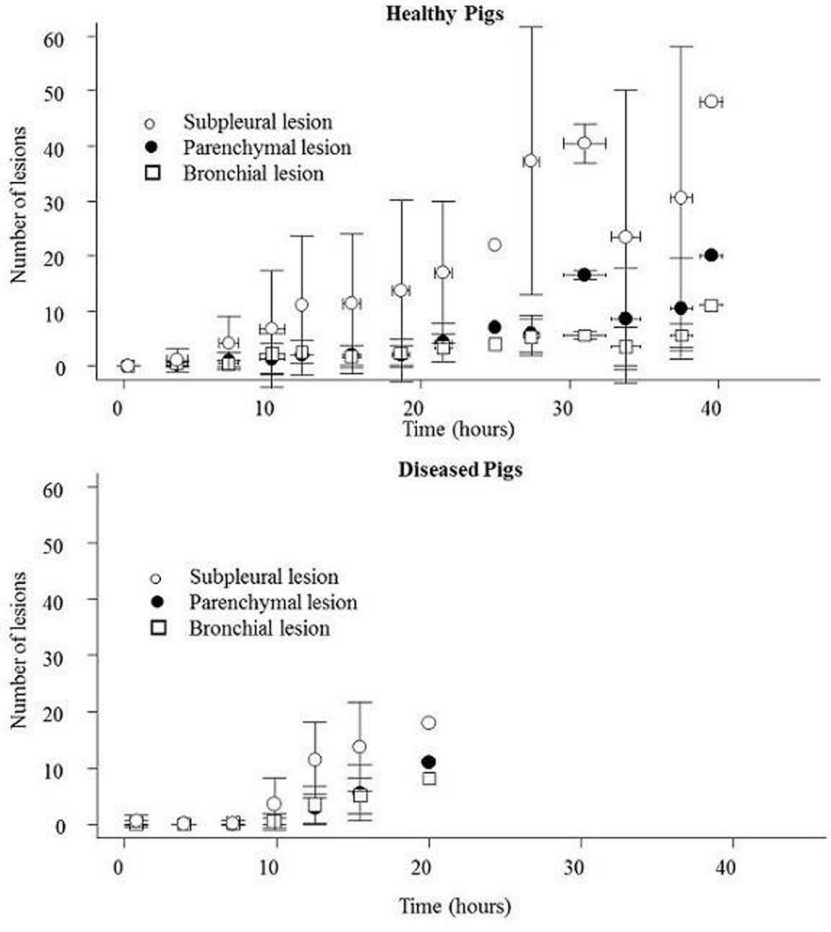

Figure 1(abstract O20) VILI Lesion Time Course.

Grant acknowledgment: ESICM Bernard Drager Award 2012 to M.C.

Reference

1. Cressoni M, Cadringher P, Chiurazzi C, Amini M, Gallazzi E, Marino A, Brioni M, Carlesso E, Chiumello D, Quintel M, Bugedo G, Gattinoni L: **Lung inhomogeneity in patients with acute respiratory distress syndrome.** *Am J Respir Crit Care Med* 2014, **189(2)**:149-158.

O21

0895. Identification and validation of a mirna as a diagnostic biomarker of diffuse alveolar damage in an animal model of acute lung injury and adult respiratory distress syndrome in mechanically ventilated patients

P Cardinal-Fernández[1,2], A Ferruelo[1,2], N Rego[3], Y Rojas[1,2], A Ballén-Barragán[1,2], R Granados[1,4], C Jaramillo[2], E Lopez-Hernández[5], L Martínez-Caro[1,2], N Nin[6,7], R Herrero[1,2], MA de la Cal[1,2], A Esteban[1,2], JA Lorente[1,2,8*]

[1]Centro de Investigación Biomédica En Red de Enfermedades RespiratoriaS (CIBERES), Getafe, Spain; [2]Hospital Universitario de Getafe, Intensive Care Service and Burn Unit, Getafe, Spain; [3]Institut Pasteur de Montevideo, Unidad de Bioinformatica, Montevideo, Uruguay; [4]Hospital Universitario de Getafe, Pathology Department, Getafe, Spain; [5]Hospital Universitario de Getafe, Getafe, Spain; [6]Hospital de Torrejon, Intensive Care Service, Madrid, Spain; [7]Hospital Español, Intensive Care Service, Montevideo, Uruguay; [8]Universidad Europea de Madrid, Madrid, Spain

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**O21

Objective: To discover a miRNA with diagnostic characteristics for diffuse alveolar damage (DAD) in an animal of acute lung injury (ALI) and in patients with the ARDS.

Methods: Male rats (325-372 gr) underwent mechanical ventilation for 2.5 h with V_T=9 ml/kg + PEEP=5 cm H_2O (low V_T, LV, n=10); or V_T=25 ml/kg + PEEP=0 cm H_2O (high V_T, HV, n=19) (11 of 19 developed DAD at histological examination). Whole miRNA expression (RNA-seq-Single Read, 72 cycles, Illumina GallxI) was analyzed in lung parenchyma. miRNA expression in LV vs. HV and in HV-DAD vs. HV-no-DAD was compared. We used a data mining strategy to prioritize the most relevant miRNA within the miRNAs differentially expressed. Prioritized miRNAs were validated in (1) serum from the same group of rats (RT-PCR); (2) human lung tissue (preserved at -80º after sampling) from autopsies of patients with ARDS (RT-PCR and *in situ* hybridization) (n=20); (3) human serum from mechanically ventilated patients obtained during the first 24 hours of ICU admission (n=66, 14 nonsurvivors). A p value < 0.05 was considered statistically significant. Results are median (IQR), and odds ratio (OR [95% confidence interval]). RT-PCR results were expressed $x10^{-4}$. Categorical and continuous variables were compared with $\chi 2$ and Mann-Whitney, respectively. Predictive multivariate logistic analysis was used to identify independent risk factors. The area under ROC curve (AUROC, mean±SEM) was used to assess the discriminatory capacity. Multiple-comparison was adjusted by FDR. The local Ethics Committee approved this study.

Results: 19 miRNAs were differentially expressed **in rat lungs** between the 3 groups, one of them (herein named miRNA A) containing the information to classify animals in the 3 groups (97% correct classification in the original dataset, and 86% in 5 fold cross-validation analysis). **In rat serum**, miRNA A expression differed in the 3 groups (p=0.045) (AUROC for the presence of DAD 0.76 [0.521-1.000], p=0.036 for the difference with the line of identity). miRNA expression **in human serum** was associated with mortality. Variables in multivariate analyses for the preediction of mortality were: SAPS II score (OR 1.05 [1.01-1.10], p=0.02), and miRNA A expression (OR 1.06 [1.01-1.12; p=0.04) (AUROC for the logistic model 0.84 [0.73-0.96]; for SAPSII 0.76 [0.60-0.92] and for miRNA A 0.76 [0.63-0.89]). miRNA A was more expressed **in lung tissue** from 39 patients with the diagnosis of ARDS than in patients without ARDS (p < 0.01). In **human lung parenchyma** miRNA A was detected (RT-PCR), localized in alveolar type II cells, macrophages and interstitium (*in situ* hybridization).

Conclusions: We have identified one miRNA associated with DAD in an animal model of ALI, that is expressed in human lung tissue, and whose expression in human serum correlates with mortality and with the diagnosis of ARDS.

Grant acknowledgment: FIS 12/02898, FIS 11/02791, FIS 12/02451, European Network (7th FP) ITN 264864.

O22

0896. Temporal changes in tidal recruitment compared to histologic outcome in experimental acute respiratory distress syndrome

J Haase[1*], S Hammermüller[1], A Beilicke[1], P Spieth[2], D Buchloh[1], S Haska[1], K Noreikat[1], T Muders[3], A Reske[1], H Wrigge[1]

[1]University of Leipzig, Anesthesiology and Intensive Care Medicine, Leipzig, Germany; [2]University of Dresden, Department of Anesthesiology and Intensive Care Medicine, Dresden, Germany; [3]University of Bonn, Department of Anesthesiology and Intensive Care Medicine, Bonn, Germany

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**O22

Introduction: Acute Respiratory Distress Syndrome (ARDS) and Ventilator-Associated Lung Injury (VALI) are characterized histologically by Diffuse Alveolar Damage (DAD)[1]. Tidal recruitment (TR) of nonaerated airspaces during mechanical ventilation (MV) causes VALI. Different MV strategies aim to reduce TR and VALI by individualized PEEP[2-4].

Objectives: For 3 MV strategies we quantified TR by computed tomography (CT) and electrical impedance tomography (EIT) as well as their association with DAD.

Methods: After approval by the animal welfare committee, pigs underwent anesthesia, tracheostomy, MV and ARDS induction by tracheal hydrochlorid acid installation. MV was randomized according to the ARDS network lower PEEP (ARDSnet, n=8), Open Lung Concept (OLC, n=8) or EIT (n=10) protocols. In ARDSnet PEEP was set according to the table. In groups OLC and RVD, recruitment maneuvers preceded decremental PEEP titration. In OLC the PEEP at which the highest individual PaO_2/FiO_2 dropped by 10% was chosen. The minimal Regional Ventilation Delay Index (RVD) determined during a low-flow maneuver defined the best PEEP step in the EIT group[4]. TR was measured every 4 hours as the difference in nonaerated tissue between expiratory and inspiratory CT. The RVD was used to set PEEP only in the EIT group, but it was measured every 4 hours to assess heterogeneous lung aeration in all groups.

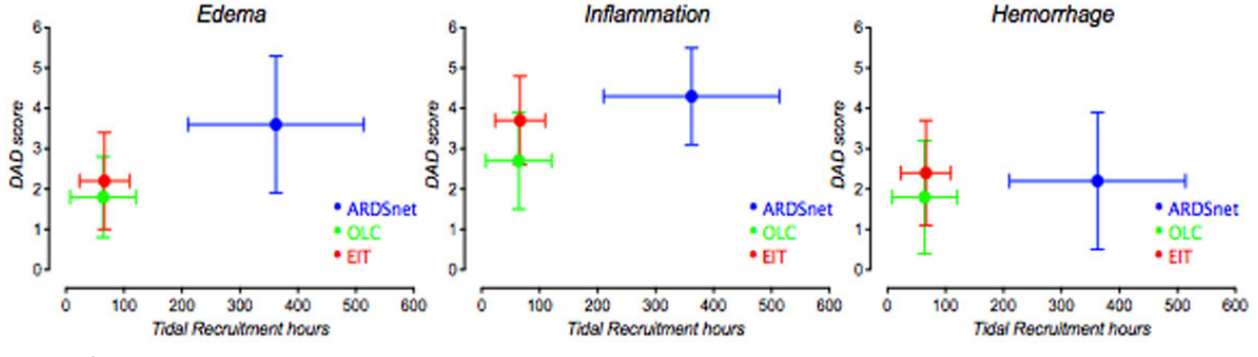

Figure 1(abstract O22)

TR- and RVD-data were multiplied by the time difference to the last measurement and cumulatively expressed as TRhours and RVDhours over 24 hours. Lung tissue samples from the left lower lobe were stained with hematoxylin/eosin. The DAD score for intraalveolar edema, inflammatory infiltration and hemorrhage was calculated as mean of 3 blinded investigators. ANOVA (Sidak's post-hoc) and linear regression were used. Results are presented as mean±SD.

Results: Figure 1 summarizes the results. TRhours were significantly higher in ARDSnet (362±152) than in OLC (70±57) and RVD (65±45). RVDhours showed the same behavior (ARDSnet 211±53, OLC 120±30, RVD 104±40) (both $P < 0.0001$). Correlation between TRhours and RVDhours was strong ($R^2=0.7$, $P < 0.0001$). The edema score of ARDSnet (3.6±1.7) significantly exceeded OLC (1.6±0.9, P=0.04), but not RVD (2.2±1.3, P=0.13). Inflammation scores were also higher in ARDSnet (4.3±1.1) than OLC (2.5±1.1, P=0.03), but not higher than RVD (3.6 ±1.1, P=0.7). OLC and RVD differed neither for edema nor for inflammation. Hemorrhage did not differ between groups (P=0.68). DAD scores did not increase significantly across quintiles of TRhours or RVDhours (all P>0.16).

Conclusions: MV according to ARDSnet is associated with more TR, greater ventilation inhomogeneity and increased histological DAD scores compared to OLC or EIT-RVD approaches. The association of TR surrogates assessed by CT or EIT with histological DAD features warrants further studies.

Grant acknowledgment: DFG (WR47/1-1,Wrigge)

References

1. Spieth P: 2007.
2. ARDSnet: 2000.
3. Schreiter D: 2004.
4. Muders T: 2012.

O23

0897. Mechanisms of pulmonary inflation during lung injury assessed by synchrotron radiation computed tomography

G Perchiazzi[1*], JB Borges[2], G Hedenstierna[2], L Porra[3], L Broche[4], M Pellegrini[1,2], A Sindaco[1], AP Tannoia[1], S Derosa[1], FF Todisco[1], T Fiore[1], A Larsson[2], S Bayat[5]
[1]Bari University, Emergency and Organ Transplant, Bari, Italy; [2]Uppsala University, Dept of Medical and Surgical Sciences, Uppsala, Sweden; [3]University of Helsinki, Dept of Physics, Helsinki, Finland; [4]European Synchrotron Radiation Facility, Grenoble, France; [5]Université de Picardie Jules Verne, Amiens, France
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**O23

Introduction: The process of lung inflation during mechanical ventilation is characterized by phenomena of alveolar recruitment and distension but regional interaction and temporal sequence are not known for the core areas of the lung. The only information available are referred to subpleural alveoli, studied by microscopy. Relevance of this issue derives from the notion that 1) radiologic studies reveal that air spaces behavior in the core regions of the parenchyma is very complex 2) unsuited patterns of mechanical ventilation (MV) can trigger a Ventilator-Induced Lung Injury (VILI). Synchrotron Radiation Computed Tomography (SRCT) yields tomographic images at resolutions higher than conventional CT.

Objectives: The aim of this study was to evaluate the behavior of air spaces using SRCT in an animal model of VILI at different Positive End Expiratory Pressure (PEEP) levels.

Methods: Six anesthetized, tracheostomized rabbits were mechanically ventilated to preserve healthy conditions (HC) of the lung (Tidal Volume=7ml/kg, PEEP=3 cmH_2O) and a sequence of SRCT exposures was performed at 40 different consecutive gravitational levels, at PEEP levels of 12, 9, 6, 3, 0 cmH_2O, during end-expiratory pauses. Then VILI was induced combining repeated lung lavages followed by injurious MV in Pressure Control mode with Peak Pressure=35 cmH_20 and ZEEP, for one hour; then the same sequence of SRCT exposures was performed. SRCT images (resolution = 45.5 μm) were transformed into binary images. From each animal, from a stack of 40 images at different gravitational levels, a Region Of Interest (ROI) of 100x200 voxels was studied by using the Image Processing Toolbox for Matlab. The increase of air spaces numerosity (NA) was considered as an index of alveolar recruitment while the increase of air spaces area (SA) and perimeter (PE) as indices of alveolar expansion. The statistical analysis was performed applying the Student's T test (a=0.05) on NA, SA and PE during HC and VILI at different PEEP levels.

Results: During healthy conditions (HC), SA and PE increased in direct proportion to PEEP up to 9 cmH_2O, then remained stable; NA increased up to PEEP=6 cmH_2O and then decreased with higher PEEP values. During VILI, SA, PE and NA increased with PEEP in a uniform manner.

Conclusions: In HC the changes of shape and size of air spaces seemed to provide a greater contribution to the increase of lung volume than recruitment mechanisms. NA tendency to decrease after a peak was interpreted as a consequence of progressive stretching of interstitial septa with creation of false connections between adjacent air spaces (false derecruitment). In VILI, alveolar recruitment during lung inflation was a continuous pattern throughout the range of PEEP levels that were studied.

Grant acknowledgment: The School of Anesthesia and Intensive Care of Medicine, Bari University; The Swedish Research Council; The Swedish Heart Lung Fund; The European Synchrotron Radiation Facility.

WHAT'S NEW IN SEPSIS AND INFECTION?

O24

0900. Effects of oxygen status on the innate immune response in humans *in vivo*

D Kiers[1,2,3*], A John[1,2], E Janssen[1,2], GJ Scheffer[2,3], H van der Hoeven[1,2], P Pickkers[1,2], M Kox[1,2,3]
[1]Radboud University Medical Center, Department of Intensive Care Medicine, Nijmegen, Netherlands; [2]Radboud Institute for Infectious Diseases, Nijmegen, Netherlands; [3]Radboud University Medical Center, Department of Anesthesiology, Nijmegen, Netherlands
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**O24

Introduction: *In vitro* and animal studies have shown that hypoxia and hyperoxia influence the innate immune response. Therefore, hypoxia and hyperoxia could be cheap, non-pharmacological, non-invasive treatment modalities to modulate inflammatory conditions. Hypoxia has shown to exert pro-inflammatory effects, supposedly mediated by the transcription factor hypoxia inducible factor 1α (HIF1α), whereas hyperoxia is related to immune suppression. However, apart from direct effects of oxygen status adjustment on the immune response, other mechanisms such as a hypoxia-induced stress response might play a role in humans *in vivo* as well. Up till now, the interplay between oxygen status adjustment and the innate immune response in humans *in vivo* has not been investigated.

Objectives: To evaluate the effects of hypoxia and hyperoxia on the systemic innate immune responseduring experimental endotoxemia in healthy volunteers.

Methods: We performed a parallel randomized controlled study in 30 healthy male volunteers. Using a non-invasive ventilation helmet, subjects were exposed to a total of 3.5 hours of either hypoxia (mixture of nitrogen and room air titrated to an arterial oxygen saturation of 80-85%, n=10), normoxia (room air, n=10), or hyperoxia (100% oxygen, n=10). Actual FiO_2 in the helmet was measured using a gas analyzer. One hour after the start of oxygen status adjustment, 2 ng/kg purified *E. Coli* endotoxinwas administered intravenously.

Results: Both hypoxia and hyperoxia were well-tolerated. An FiO_2 of 11.5±0.8% was required to induce hypoxia (SaO_2: 81.9±0.5%, PaO_2: 5.8±0.4 kPa), while hyperoxia (FiO_2: 97.9±0.2 %) resulted in a mean PaO_2 of 54.1±4.1kPa. Hypoxia attenuated the endotoxin-induced increase in plasma levels of pro-inflammatory cytokines TNFα, IL-6, and IL-8 , while potentiating the anti-inflammatory IL-10 response (Figure 1). Hyperoxia did not affect cytokine levels. Hypoxia resulted in a profound increase of plasma adrenaline levels, which were not influenced by hyperoxia (Figure 2). Furthermore, inverse correlations between adrenaline and the pro-inflammatory cytokines IL-6 and IL-8 (r=-0.48, p=0.007 and r=-0.47, p=0.004, respectively) and a positive correlation between adrenaline and IL-10 (r=0.52, p=0.004) were found. Finally, intracellular HIF-1α expression was increased in circulating neutrophils 2,5 and 6 hours after endotoxin administration, and 6 hours post-endotoxin in circulating lymphocytes. Hypoxia or hyperoxia did not affect HIF-1α expression.

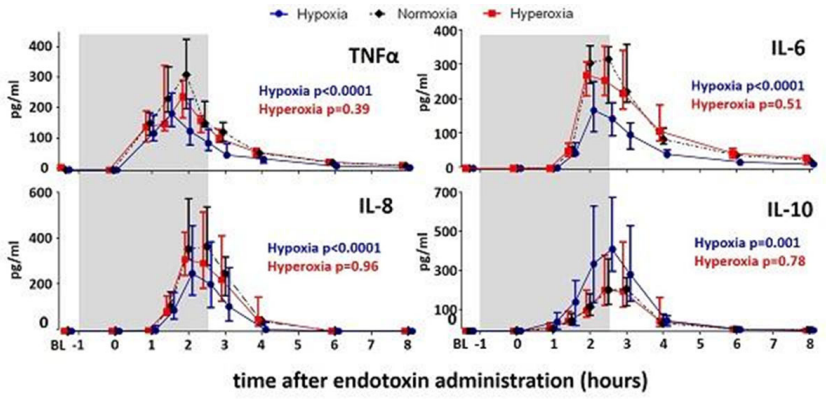

Figure 1(abstract O24) Plasma cytokine levels during hypoxic, normoxic, and hyperoxic endotoxeimia. Data are represented as median with interquartile range of 10 subjects per group. Gray box indicates period of oxygen status adjustment. P-values represent separate comparisons of the hypoxia and hyperoxia group with the normoxic group (two-way ANOVA-repeated measures [interaction term] on the log-transformed data). BL:baseline.

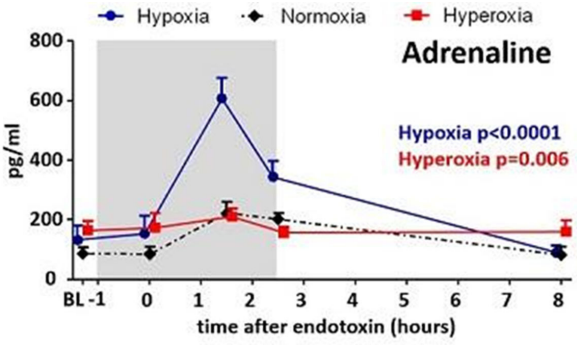

Figure 2(abstract O24) Plasma adrenaline levels during hypoxic, normoxic, and hyperoxic endotoxeimia. Data are represented as means with SEM of10 subjects per group. Gray box indicates period of oxygen status adjustment. P-values represent separate comparisons of the hypoxia and hyperoxia group with the normoxic group (two-way ANOVA-repeated measures [interaction term] on the log-transformed data). BL:baseline.

Conclusions: In contrast with *in vitro* and animal data, three-and-a-half hours of moderate hypoxia in healthy volunteers attenuates the endotoxin-induced systemic innate immune response, whereas hyperoxia exerts no immunomodulatory effects. The hypoxia-induced immunosuppression is (at least in part) mediated by an increased endogenous adrenaline response. HIF-1α expression increases in circulating leukocytes after endotoxin administration, but is not affected by oxygen status.

PREVENTING DELIRIUM IN CRITICAL ILLNESS

O25

0915. Effect of propofol combined with opioids on guinea pig's small bowel motility in vitro

M Schörghuber[1*], E Tatzl[1], P Holzer[2], W Toller[1], S Fruhwald[1]
[1]Medical University of Graz, Department of Anaesthesiology and Intensive Care Medicine, Graz, Austria; [2]Medical University of Graz, Institute of Experimental and Clinical Pharmacology, Graz, Austria
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**O25

Introduction: Critically ill patients frequently develop gastrointestinal (GI) motility disorders resulting in feeding intolerance with increased morbidity and mortality. Sedatives and opioids per se have adverse effects on GI motility and can aggravate GI motility disorders.

Objectives: The aim of this study was to evaluate the inhibitory potency of propofol alone and in combination with remifentanil or sufentanil.

Methods: Guinea pig´s small bowel segments of 8 cm length were set up in organ baths containing oxygenated Tyrode´s solution. Peristalsis was elicited by luminal perfusion (0.5 ml/min) against an aboral resistance of 400 Pascal (Pa). Perfusion of the segments resulted in an increase of the intraluminal pressure up to a pressure threshold (PT; mean ±SEM), where peristaltic contractions were triggered. The pressure was recorded at the aboral end of the segments. An increase of the PT was interpreted as an inhibition of peristalsis, while a decrease of the PT was interpreted as a stimulation of peristalsis. A PT of 400 Pa was equated with a complete block of peristalsis. In a first setting increasing concentrations of propofol (1; 3; 10; 30; 100; 300 μM) were added to the organ bath and the PT was evaluated at each concentration. These results were compared to increasing concentrations of propofol after pretreatment with sufentanil (0.1 resp.0.3 nM) or remifentanil (3 resp. 10 nM). Statistic calculations were performed using the general linearlized model for repeated measures and the two sided t-test of IBM SPSS 21.0.

Results: Basic PT without any substances added to the organ bath was 73.83 ± 5.3Pa. Propofol had a dose dependent inhibitory effect on peristalsis ($p < 0.001$, partial $\eta^2 = 0.9$; observed power= 1.00). Pretreatment with sufentanil 0.1 nM (124.2 ± 16.1Pa; p = 0.039) and 0.3 nM (156.2 ± 51.3Pa; p= 0.04) had an additional inhibitory effect at both tested concentrations (p= 0.041 resp. 0.029, figure 1). Remifentanil 3 nM (97.7 ± 8.6Pa; p = 0.49) and 10 nM (304.2 ± 49.1Pa; p = 0.005) resulted in an increase of the PT compared to basic PT with a slight aggravation of propofol's inhibitory effect (p = 0.242) at low doses and a more pronounced effect at high doses (p = 0.011, low vs. high concentration: $p < 0.001$, figure 2).

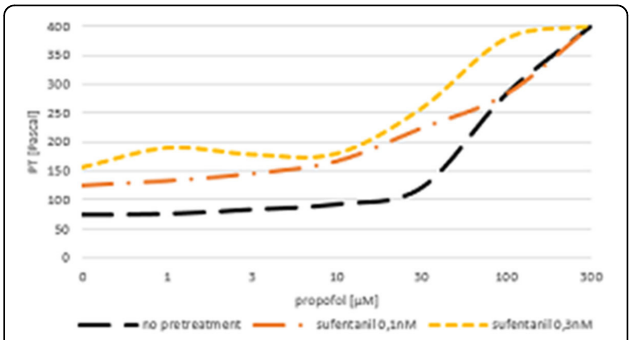

Figure 1(abstract O25) Dose-effect curve of propofol solitary and after pretreatment with sufentanil on guineapig's small bowel motility.

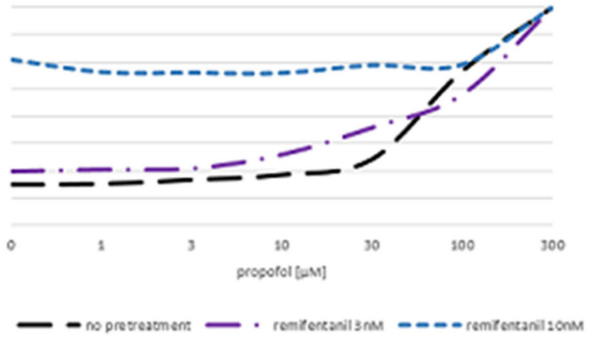

Figure 2(abstract O25) Dose-effect curve of propofol solitary and after pretreatment with remifentanil on guineapig's small bowel motility.

Conclusions: Propofol and opioids affect neuronal activity via different points of action. While propofol acts on GABA receptors, opioids bind to μ, κ and δ receptors. In this experimental setting the combination of propofol with opioids shows a more pronounced inhibition of small bowel motility than propofol alone. Propofol combined with remifentanil at a high concentration had a pronounced inhibitory effect on peristalsis, while the difference between the two tested concentrations of sufentanil was minor.

TISSUE PERFUSION IN TRAUMA

O26

0918. Grey matter perfusion is preserved during normotensive hypovolaemia, despite reductions in total cerebral blood flow: evidence of local, pressure independent, intra-cerebral vascular autoregulatory response

SC Beards[1*], JR Cain[2], LM Parkes[2], A Jackson[2]
[1]University Hospital of South Manchester, Acute Intensive Care Unit, Manchester, UK; [2]University of Manchester, Wolfson Molecular Imaging Centre, Manchester, UK
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**O26

Introduction: We have recently demonstrated, using MRI measurements, that normotensive hypovolaemia (NTH) is associated with correlated decreases in cerebral blood volume flow (CBVF) and cardiac output (CO). This is in disagreement with previous studies using trans-cranial Doppler measurements.
Methods: Eleven healthy volunteers (20-31 years; 8-male 3-female) underwent MRI studies with a lower body negative pressure (LBNP) chamber. Imaging included quantitative phase contrast angiographic (PCA) measurements of blood volume flow in the carotid and vertebral arteries and ASL of the whole brain. An ASL sequence followed by PCA acquisition was performed at rest (control) and under -20mmHg LBNP. ASL imaging used STAR labeling collected at 4 inversion times: 800ms, 1200ms, 1600ms and 2000ms. PCA acquisition was collected using 2D cine phase-contrast images. Throughout scanning the subjects pulse and blood pressure were monitored. ASL images were analysed using in-house code assuming a single blood compartment model[1]. Control and labelled images were subtracted and a two-parameter fit for bolus arrival time (BAT) and perfusion was performed on a voxel by voxel basis, producing perfusion and BAT maps. Perfusion was calculated with units ml/100ml/min. Automated tissue segmentation masks were created from aligned T1 images applied to co-registered perfusion and BAT maps for both cortical and sub-cortical grey matter structures.
Results: During -20mmHg LBNP, CBVF was reduced by 0.5 l/min, blood pressure remained constant and pulse was raised by 7 bpm. There was no difference between ASL grey matter perfusion values (mean 39.2ml/100ml/min and 42.3ml/100ml/min respectively) or sub-cortical grey matter perfusion values (31.3ml/100ml/min and 33.1ml/100ml/min) between control and -20mmHg (Fig 1A). BAT was significantly delayed ($p < 0.05$) during -20mmHg LBNP compared to control in both the cortical (782ms and 831ms) and sub-cortical grey matter (896ms and 1033ms); Fig 1B).
In the presence of constant perfusion this indicates regional vasodilatation. Comparing the sub-cortical structures, the hippocampus (19.5%) caudate (21%) and putamen (28%) showed the greatest % increase in BAT during -20mmHg LBNP.
Conclusions: In young healthy individuals NTH induces reductions in CBVF which are compensated by vasodilatation of small vessels in normal grey matter, preserving grey matter perfusion despite reductions in total cerebral blood flow. This presumably occurs at the expense of white matter blood flow although that could not be measured with the current methodology. These results suggest a local, intra-cerebral autoregulation mechanism, independent of perfusion pressure, capable of preserving grey matter blood flow in the face of decreasing minute volume cerebral blood flow.
Grant acknowledgment: This work was supported by the Wellcome Trust.
Reference
1. Parkes L, Tofts P: *Magnetic Resonance in Medicine* 2002, **48(1)**:27-41.

O27

0919. Effect of catecholamine immediately after blast lung injury caused by laser-induced shock wave in a mouse model

H Miyawaki[1], D Saitoh[2], K Hagisawa[3], M Noguchi[2], S Satoh[4], M Kinoshita[5], H Miyazaki[2], Y Satoh[6], T Sakamoto[1*]
[1]National Defense Medical College Hospital, Department of Traumatology & Critical Care Medicine, Tokorozawa, Japan; [2]National Defense Medical College, Division of Traumatology, Research Institute, Tokorozawa, Japan; [3]National Defense Medical College, Division of Physiology, Tokorozawa, Japan; [4]National Defense Medical College, Division of Biomedical Information Sciences, Tokorozawa, Japan; [5]National Defense Medical College, Department of Immunology and Microbiology, Tokorozawa, Japan; [6]National Defense Medical College, Department of Anesthesiology, Tokorozawa, Japan
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**O27

Introduction: The physical damage inflicted by blast waves is called primary blast injury, and lungs are vulnerable to blast waves [1]. Blast lung injuries (BLI) can be extremely critical during the super-acute phase, and hypotension is supposed to be the main cause of death (1) , but its etiology has not been elucidated. Recent studies have demonstrated that hypotension is mediated by the absence of vasoconstriction [2]. However, research investigated the effectiveness of catecholamine for BLI during the super-acute phase was not identified.

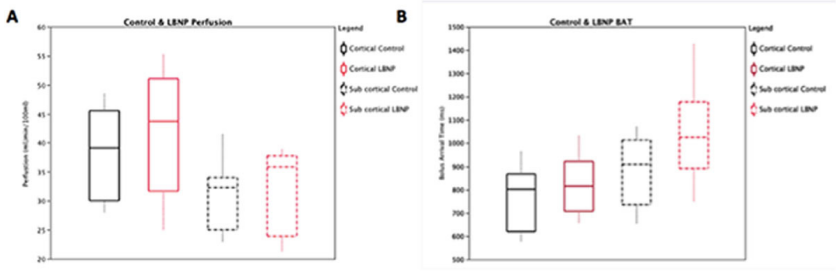

Figure 1(abstract O26)

Objectives: The present study aimed to establish a small-animal model of severe BLI using laser-induced shock wave (LISW) and to evaluate the effect of catecholamine on the super-acute phase of severe BLI.
Methods: The investigation comprised two parts. Study 1 assessed the validity of the BLI model using LISW as follows. Mice were randomly allocated to groups that received 1.2, 1.3 or 1.4 J/cm^2 LISW. Survival rates, systolic blood pressure (sBP), heart rate (HR), and peripheral oxyhemoglobin saturation (SpO_2) were monitored for up to 60 min thereafter and lung tissues were histopathologically analyzed. Study 2 evaluated the effects of catecholamines as follows. The mice were randomly assigned to groups that received 1.4 J/cm^2 LISW followed by the immediate intraperitoneal administration of dobutamine, noradrenaline or normal saline. A sham group received no LISW or drugs. Survival rates were measured for 48 h. We also measured sBP, HR, and SpO_2 before and 5 and 10 min after LISW, and left ventricular ejection fraction (EF) and systemic vascular resistance (SVR) before and 1 min after LISW.
Results: (Study 1) The triad of BLI (hypotension, bradycardia, and hypoxemia) was evident immediately after LISW. The degree of the triad and the survival rates were aggravated with increasing doses of LISW. The histopathological findings were compatible with BLI.
(Study 2) The survival rate was highest in the group that received noradrenaline, with significantly elevated SVR and decreased EF after LISW.
Conclusions: The LISW induced lung injury model seems to be useful as severe BLI in small animals without any large scaled equipment. The main cause of death during the super-acute phase of severe BLI might be hypotension due to the absence of peripheral vasoconstriction. The immediate administration of an α1-adrenergic receptor agonist such as noradrenaline right after exposure to blast waves might be an effective treatment during the super-acute phase of severe BLI.
Grant acknowledgment: This work was supported by a grant-in-aid for the Special Research Program from the National Defense Medical College (D.S.).
References
1. Guy RJ, *et al*: Physiologic responses to primary blast. *The Journal of Trauma* 1998, **45(6)**:983-987.
2. Ohnishi M, *et al*: Reflex nature of the cardiorespiratory response to primary thoracic blast injury in the anaesthetised rat. *Experimental Physiology* 2001, **86(3)**:357-364.

O28

0920. Cerebrovascular autoregulation in normotensive hypovolaemia: a study combining lower body negative pressure and MRI

SC Beards[1*], S Brodie[2], J Cain[2], LM Parkes[2], A Jackson[2]
[1]University Hospital of South Manchester, Acute Intensive Care Unit, Manchester, UK; [2]Manchester University, Wolfson Molecular Imaging Centre, Manchester, UK
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**O28

Introduction: Previous studies using transcranial Doppler sonography have suggested that cerebral circulation is preserved relative to cardiac output during normotensive hypovolaemia (NTH). We have further investigated the cerebral autoregulatory responses to hypovolaemia using using MRI measurements of total cerebral blood flow combined with graded levels of lower body negative pressure (LBNP) to induce steady state central hypovolaemia.
Methods: 11 healthy male volunteers (21-24 years) underwent MRI scanning with their legs and torso within a LBNP chamber; phase contrast angiography based flow measurements (MRI-PCA) were acquired at 10mmHg increments from baseline, to -50 mmHg . Measurements were made through the carotid and vertebral arteries to measure total cerebral volume flow (CBVF) and through the ascending and proximal descending aorta to measure cardiac output (CO).
Results: At low levels of LBNP (≤ -20mmHg) decrease in CBVF was directly proportional to the decrease in CO (Figure 1). Between LBNP of -20 mmHg and -40 mmHg there was a relative maintenance of CBVF in relation to cardiac output ($P < 0.05$) in keeping with classic, pressure mediated, cerebrovascular autoregulation. LBNP of -50mmHg produced a decrease in CBVF of 65.5% decrease ($P < 0.01$) in keeping with a 63.6% ($P < 0.01$) decrease in CO.
Conclusions: These findings demonstrate a correlation between decreases in CO and CBVF during normotensive hypovolaemia (NTH), which is in disagreement with previous studies using transcranial Doppler to measure CBVF. The findings do not support previous descriptions of relative sparing of CBVF due to compensatory reductions in cerebrovascular resistance in response to NTH [1]. This difference is likely to reflect the different methods of cerebral blood flow measurement. MRI-PCA allows direct volume flow measurements from the carotid and basilar arteries whereas TCD uses flow velocity in the middle cerebral arteries as a surrogate of volume flow. This potentially important discrepancy requires further investigation to elucidate the mechanisms controlling CBF responses to nth.

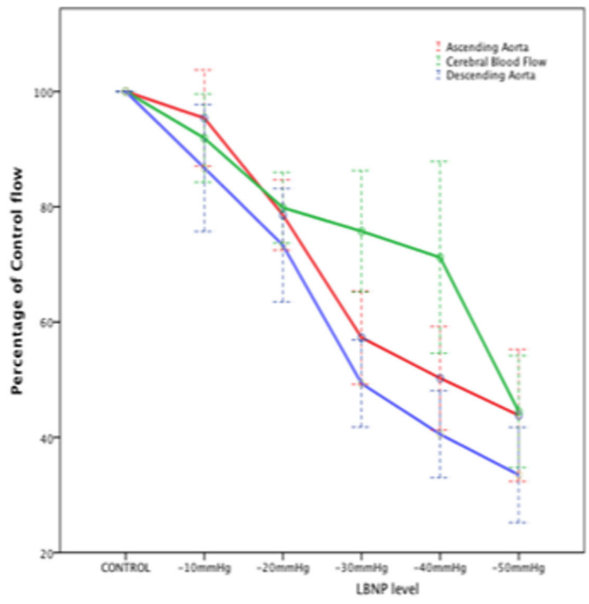

Figure 1(abstract O28)

Reference
1. Brown CM, Dütsch M, Hecht MJ, Neundörfer B, Hilz MJ: Assessment of cerebrovascular and cardiovascular responses to lower body negative pressure as a test of cerebral autoregulation. *J Neurol Sci* 2003, **208**:71-8.

O29

0921. Effect of norepinephrine on intestinal oxygenation during fluid resuscitation of hemorrhagic shock in mice

A Harrois[1,2*], N Baudry[2], E Vicaut[2], J Duranteau[1,2]
[1]Hôpital de Bicêtre, Département d'Anesthésie-Réanimation, Le Kremlin Bicêtre, France; [2]Laboratoire Microcirculation, Bioénergétique, Inflammation, Insuffisance Circulatoire Aigue, Université Paris 7/Paris 11, Paris, France
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**O29

Introduction: Hemorrhagic shock resuscitation aims at maintaining tissue perfusion while waiting for hemorrhage control. Fluid resuscitation remains the first line therapy to improve tissue perfusion but a vasoconstrictor (most often norepinephrine) may be associated to stabilize the mean arterial pressure (MAP) level and avoid hemodilution while waiting for transfusion. However, the arteriolar vasoconstriction induced by norepinephrine could have deleterious effects on tissue perfusion during hypovolemia due to hemorrhage.
Objectives: To evaluate the effects of norepinephrine administration on intestinal perfusion and oxygenation during hemorrhagic shock resuscitation in mice.
Methods: Tracheotomised and ventilated Balb/c mice were submitted to a controlled hemorrhagic shock to reach a MAP level of 40 mmHg during sixty minutes. Mice groups differed from their resuscitation between the 60th and the 120th minute: a fluid resuscitated group (FR) with NaCl 0,9% to reach a MAP level of 60 mmHg and a group resuscitated with fluid and norepinephrine (FRNE) to reach a MAP level of 60 mmHg. Mice were then retransfused with their shed blood and an equal volume of Ringer Lactate and were observed between the 120th and the 165th minute. Intestinal

microcirculation was observed by intravital microscopy. Intestinal mucosal PO_2 level (PO_{2muq}) was measured by phosphorescence quenching (OxyMicro probe) at jejunal mucosal level. A sham group (no hemorrhage, no resuscitation) was constituted. Data are presented as mean ± SEM. The group effect on microcirculatory and oxygenation parameters was analysed with ANOVA and Mann Whitney tests.

Results: Hemorrhagic shock induced an alteration of the intestinal microcirculatory perfusion with a decrease in the fraction of perfused villi and a decrease in the villous red blood cells flux. A decrease of the PO_{2muq} from 33,9 ± 3 to 8,2 ± 1 mmHg ($p < 0,01$) and from 32,9 ± 2 to 10,3 ± 2 mmHg ($p < 0,01$) was observed during hemorrhage in FR and FRNE groups respectively. During hemorrhagic shock resuscitation, MAP goal was reached in FRNE group (59 ± 1 mmHg) but not in FR group (52 ± 2 mmHg). Fluid resuscitation amount was 67 ± 14 $\mu L.g^{-1}$ and 176 ± 19 $\mu L.g^{-1}$ in FRNE and FR groups respectively ($p < 0.05$). The alteration of intestinal microcirculatory perfusion was corrected in the same proportion in FRNE and FR groups. The PO_{2muq} recovered to its basal level during resuscitation in the FR group: 30 ± 3 mmHg and in the FRNE group: 33 ± 2 mmHg.

Conclusions: In a mice model of uncontrolled hemorrhagic shock, a MAP directed resuscitation associating norepinephrine and fluid resuscitation decreased blood loss and fluid requirements compared to a MAP directed resuscitation with fluid without norepinephrine while preserving intestinal microcirculatory perfusion and oxygenation.

Grant acknowledgment: This work was supported by a grant from "La Société française d´Anesthésie-Réanimation"

POSTER PRESENTATIONS

LIVER & GUT FAILURE

P1

0078. Establishing a detailed short-term rat model of partial ischaemia/reperfusion injury

G Sabbatini*, A Dyson, M Singer
University College London, Bloomsbury Institute of Intensive Care Medicine, London, UK
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P1

Introduction: Liver ischaemia/reperfusion (I/R) injury may be observed after major hepatic surgery or resuscitation from severe trauma/haemorrhage. Well-characterised and representative animal models are needed to better understand mechanisms of injury, apply effective treatments and prevent complications.

Objectives: To establish a well-characterized rat model of partial liver I/R injury.

Methods: Under isoflurane anaesthesia, tracheotomized male Wistar rats underwent left common carotid artery and right jugular vein instrumentation for BP measurement/blood sampling and fluid infusion (10 ml/kg/hr), respectively, and bladder catheterization for urine output measurement. Via a transverse subcostal laparotomy, blood vessels to the median and left liver lobes were occluded with a surgical clamp for 60 mins. On release of the clamp, the remaining liver lobes were ligated to prevent a steal phenomenon. The animals were observed for a further 5 hours. Sham animals underwent the same procedure except for vascular occlusion. Measurements were made of haemodynamics (BP, echocardiography), blood gas analysis, and biochemical and functional (Indocyanine Green plasma disappearance rate, ICG-PDR) liver function tests. A tissue PO_2 probe (tPO_2) (Oxford Optronix, UK) was placed in contact with liver tissue to measure hepatic tissue PO_2. Tissue samples were taken to assess microscopic and ultrastructural injury both locally and remotely (data not shown).

Results: See figure 1.

Liver ischaemia resulted in an increase of markers of hepatic injury (AST) and function (ICG-PDR and Lactate). Cardiac output was maintained but arterial base excess was different between groups. tPO_2 at 5 hours post-reperfusion recovered in shams but not in I/R group.

Conclusions: This severe liver I/R model demonstrates derangement of hepatic function, biochemistry, haemodynamic and ultrastructure. It offers utility for the assessment of interventions aimed at preventing or reducing I/R injury.

References

1. Eguchi S, Kamlot A, Ljubimova J, Hewitt WR, Lebow LT, Demetriou AA, Rozga J: **Fulminant hepatic failure in rats: survival and effect on blood chemistry and liver regeneration.** *Hepatology* 1996, **24(6)**:1452-1459.
2. Merle U, Sieg O, Stremmel W, Encke J, Eisenbach C: **Sensitivity and specificity of plasma disappearance rate of indocyanine green as a prognostic indicator in acute liver failure.** *BMC Gastroenterology* 2009, **9**:91.

P2

0082. Early circulating lipid and cytokine profiles prognosticate in a rat model of faecal peritonitis

W Khaliq*, M Singer
Bloomsbury Institute of Intensive Care Medicine, University College London, London, UK
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P2

Introduction: In stress states, catecholamines induce lipolysis and insulin resistance with hyperglycaemia. Lipid profiles differ between surviving and non-surviving septic patients [1,2] but, hitherto, little attention has been paid to this finding and its significance remains unknown. We used a previously characterized 72h fluid-resuscitated rat model of faecal peritonitis where prognostication can be made with high sensitivity and specificity as early as 6h from heart rate or stroke volume [3].

Objectives: To determine the relationship between early changes in plasma cytokine and metabolic profiles, and their prognostic significance.

Methods: Under general anaesthesia male Wistar rats (325±15g) underwent tunneled insertion of carotid arterial and jugular venous lines, followed by i.p. injection of 4µl/g faecal slurry. They were then woken and attached to a swivel-tether system allowing free movement in their cage with, from 2h, fluid resuscitation (1:1 mix of 5% dextrose:Hartmann's) at 10ml/kg/h. An echocardiography-measured HR cut-off of 460 bpm was used to classify animals into predicted survivors or non-survivors. At 6h, animals were sacrificed for blood and tissue sampling. We here report plasma levels of IL-6, IL-10, and a metabolic profile using blood gas analysis, ELISA and enzymatic colorometric testing.

Results: At 6h the animals manifested only mild clinical features of illness, however significant differences were seen in IL-6 and all lipid measurements between predicted survivors and non-survivors. Glucose, lactate and IL-10 levels did not differ. Table 1

Conclusions: In this long-term rat model of faecal peritonitis, predicted non-survivors had a significantly different IL-6 and lipid profile as early as 6 hours after sepsis. IL-6 impacts on lipid metabolism [4] but the relationship in sepsis has not, to our knowledge, been previously described. The impact of early hypolipidaemia on outcome warrants further investigation.

Grant acknowledgment: UK Intensive Care Foundation and NIHR

References

1. Barlage S, *et al*: **Changes in HDL-associated apolipoproteins relate to mortality in human sepsis and correlate to monocyte and platelet activation.** *Intensive Care Med* 2009, **35**:1877-1885.
2. Cappi SB, *et al*: **Dyslipidemia: a prospective controlled randomized trial of intensive glycemic control in sepsis.** *Intensive Care Med* 2012, **38**:634-641.
3. Rudiger A, *et al*: **Early functional and transcriptomic changes in the myocardium predict outcome in a long-term rat model of sepsis.** *Clin Sci* 2013, **124**:391-401.
4. Glund S, Krook A: **.** *Acta Physiol (Oxf).* 2008, **192(1)**:37-48.

P3

0083. Hepatoprotective effects of hydrogen sulphide against acute liver failure

K Tokuda[1,2*], F Ichinose[2]
[1]Kyushu University Hospital, Intensive Care Unit, Fukuoka, Japan;
[2]Massachusetts General Hospital, Department of Anesthesia, Critical Care and Pain Medicine, Charlestown, MA, USA
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P3

Introduction: Acute liver failure is a fatal syndrome attributed to massive hepatocyte apoptosis that is resistant to conventional medical therapies. Consequently, liver transplantation is required in many cases. An experimental liver failure model induced by galactosamine (Gal) and lipopolysaccharide (LPS) mimics clinical acute liver failure. In this model, LPS

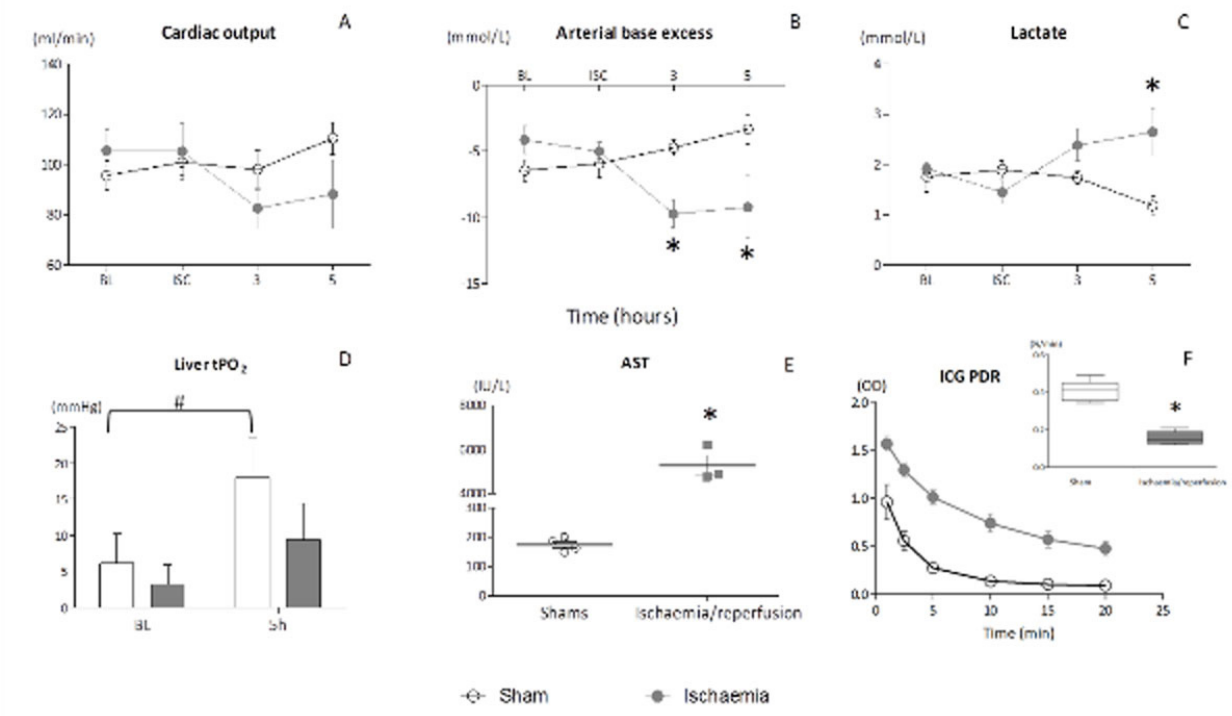

Figure 1(abstract P1) ISC= Ischaemia, BL= baseline, OD= optical density AST= aspartate aminotransferase. Data shown as mean (SEM) (A-E) or median (interquartile range) (F). Sham= 6, Ischaemia= 5/group, *p< 0.05 comparing ischaemia to sham, #p< 0.05 comparing BL to 5h. Statistics performed using t-test or two-way ANOVA and Bonferroni's test for multiple comparisons, as appropriate.

Table 1(abstract P2)

	Predicted survival (n=6)	Predicted non-survival (n=6)
IL-6 (ng/mL)	0.94 ± 0.23	3.70 ± 0.83*
IL-10 (ng/mL)	0.33 ± 0.05	0.30 ± 0.12
Glucose (mmol/L)	6.8 ± 0.7	6.9 ± 0.6
Lactate (mmol/L)	1.9 ± 0.5	1.6 ± 0.5
HDL cholesterol (mmol/L)	0.88 ± 0.04	0.73 ± 0.07*
LDL/VLDL cholesterol (mmol/L)	0.50 ± 0.03	0.39 ± 0.03*
Triglyceride (mmol/L)	1.12 ± 0.04	0.75 ± 0.08*

*[Data shown as median ± SE; * p<0.05]*

stimulates macrophages to release TNFα, which induces apoptosis in Gal-sensitized hepatocytes, causing acute liver failure. Hydrogen sulphide (H_2S), which is an endogenously produced gaseous signaling molecule, has anti-apoptotic as well as anti-inflammatory properties. Previously, we reported that H_2S attenuates liver dysfunction arising from LPS-induced systemic inflammation [1]. It has also been reported that H_2S reduces hepatic ischemia/reperfusion injury by inhibition of apoptosis in the liver [2]. However, it is still unknown whether H_2S exerts hepatoprotective effects against acute liver failure, in which both inflammatory responses and apoptosis have critical roles. Here, we examined the impact of H_2S on acute liver failure in mice induced by Gal and LPS.

Methods: Mice were challenged with saline or combination of Gal and LPS intraperitoneally, then divided into two groups: one group breathed air alone, and another breathed H_2S (80 ppm) for 6h followed by breathing air.

Results: Mice that breathed air after Gal/LPS challenge showed poor survival rate (13%) and marked increase of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in plasma. On the other hand, H_2S inhalation for 6h after challenge markedly improved survival (60%, $p < 0.05$) and suppressed Gal/LPS-induced elevation of ALT and AST levels in plasma. Inhaled H_2S suppressed TNFα in plasma at 1h after Gal/LPS challenge. Mice that breathed air after Gal/LPS challenge exhibited activation of caspase 3, 8, and 9 in the liver, whereas H_2S breathing inhibited activation of caspase 3, 8, and 9, suggesting inhaled H_2S after Gal/LPS challenge suppressed both extrinsic and intrinsic pathways of caspase-dependent apoptosis in the liver. Gal/LPS challenge increased phosphorylated STAT3 transcription factor. H_2S inhalation after Gal/LPS challenge further augmented phosphorylation of STAT3 compared to air alone. The protective effects of H_2S inhalation after Gal/LPS challenge were associated with upregulation of gene expression of

anti-inflammatory IL-10, which stimulates STAT3 phosphorylation, in the liver. These results suggest that inhaled H_2S contributes to survival of mice in acute liver failure at least in part through activation of IL-10/STAT3 pathway.

Conclusions: These results suggest that H_2S shows hepatoprotective effects against acute liver failure at least in part by inhibition of caspase activation and by augmentation of IL-10/STAT3 signaling pathway in the liver.

References

1. Tokuda K: **Inhaled hydrogen sulfide prevents endotoxin-induced systemic inflammation and improves survival by altering sulfide metabolism in mice.** *Antioxid Redox Signal* 2012, **17**:11-21.
2. Jha S: **Hydrogen sulfide attenuates hepatic ischemia-reperfusion injury: role of antioxidant and antiapoptotic signaling.** *Am J Physiol Heart Circ Physiol* 2008, **295**:H801-806.

SEPSIS FUNDAMENTALS

P4

0092. Ischemic pre/post-conditioning protects the microcirculation in experimental sepsis

D Orbegozo Cortes*, S Fuhong, C Santacruz, K Hosokawa, K Donadello, J Creteur, D De Backer, J-L Vincent
Erasme Hospital, Dept of Intensive Care, Brussels, Belgium
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P4

Introduction: Ischemic preconditioning induces complex physiological adaptations to improve the tolerance of cells and tissues to future ischemic episodes. This phenomenon has been most studied in myocardial cells but also occurs in other tissues.

Objectives: Ischemic preconditioning could be used to improve the microcirculatory response to sepsis related hypoperfusion.

Methods: ixteen adult sheep (24-34 Kg) were anesthetized (midazolam, ketamine and morphine), mechanically ventilated and invasively monitored. Abdominal sepsis was induced by injecting autologous feces into the peritoneal cavity. Animals were randomly allocated to undergo remote ischemic preconditioning followed by intermittent post-conditioning (PRECON) or not (CONTROL). Controlled ischemic episodes of the lower extremities and pelvis were obtained by inflating an intravascular balloon in the aortic bifurcation. We performed 4 cycles of 2 min ischemia (4 min apart) 1 hour before sepsis induction and, thereafter, 1 inflation every 4 hours until death. Sublingual microcirculation was evaluated using side-stream dark field (SDF) video-microscopy capturing 5 videos of 12 sec at baseline and every 6 hours thereafter for later blinded analysis. We calculated the perfused vessel density (PVD), proportion of perfused vessels (PPV), mean flow index (MFI) and heterogeneity of PPV (PPV HI). Animals were followed until death or for a maximum of 30 hours. Data are presented as median values with inter-quartile ranges. Repeated measurement data were analyzed using a Generalized Estimating Equations approach in SPSS 19.0 (IBM,USA) with a $p < 0.05$ considered as significant.

Results: See table 1.

Conclusions: Repeated ischemic pre- and post-conditioning of the pelvis and lower extremities protected the microcirculation in this sheep model of severe abdominal sepsis.

Grant acknowledgment: Institutional funds only.

P5

0093. Mitochondrial function of immune cells in severe sepsis and septic shock - a prospective observational cohort study

TM Merz*, AJ Pereira, V Jeger, JM Stephan, T Jukka, S Djafarzadeh
University Hospital Bern, Department of Intensive Care Medicine, Bern, Switzerland
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P5

Introduction: Circulating immune cells contribute to sepsis pathophysiology. Immune system activation increases cell energy requirements [1]; impaired mitochondrial function and ATP production may modulate the immune response in sepsis.

Objectives: To investigate mitochondrial enzyme activities and ATP content of monocytes, B cells and $CD4^+$ T cells in patients with severe sepsis and septic shock.

Methods: 30 patients with severe sepsis or septic shock were studied at ICU admission and after 24 and 48 hours. Immune cells were identified and isolated using an immunomagnetic positive cell isolation procedure (Dynabeads® for Human Monocytes, Human B cells, Human CD4 cells, Invitrogen Dynal AS, Oslo, Norway). Enzymatic activity of mitochondrial complexes I, IV, and ATP synthase and ATP content were measured spectrophotometrically and expressed as ratio to citrate synthase for enzymatic activities to account for mitochondrial mass and as mass per µg of cellular protein for ATP content. Maximal mitochondrial enzymatic activities and ATP in the first 48h of sepsis were compared with samples from 20 healthy volunteers (Mann Whitney test).

Results: Complex I and ATP synthase activities in sepsis were increased ($p < 0.0001$ to 0.0002; Figure 1, 2, 3). Complex IV activity was increased in monocytes (p=.0138). Complex IV activity in B and T cells was highly variable (Figures 1a-c). ATP content was increased in B cells but not in monocytes or T cell in sepsis compared to control. There were no differences between survivors and non-survivors in any of the enzymatic activities or in ATP content.

Conclusion: Mitochondrial enzyme activities of human immune cells are increased in severe sepsis and septic shock. We suggest that this helps to preserve normal or increased cell ATP content in acute inflammation.

GRANT ACKNOWLEDGEMENT: This study wass supported by Stiftung für die Forschung in Anästhesiologie und Intensivmedizin and the Gottfried und Julia Bangerter-Rhyner-Stiftung, both Bern, Switzerland.

References

1. Belikova I, *et al*: **Oxygen consumption of human peripheral blood mononuclear cells in severe human sepsis.** *Crit Care Med* 2007, **35(12)**:2702-2708.
2. Munoz C, *et al*: **Dysregulation of in vitro cytokine production by monocytes during sepsis.** *The Journal of clinical investigation* 1991, **88(5)**:1747-1754.

Table 1

		T0	T6	T12	T18	T24
PVD (vessel/mm)	**PRECON**	13.0 (12.8-15.8)	10.6 (9.8-12.1)	9.0 (8.6-11.1)	9.2 (8.5-10.1)	8.2 (7.3-9.3)*
	CONTROL	13.8 (12.5-15.7)	10.4 (9.5-11.3)	9.1 (8.6-10.8)	7.7 (6.9-9.6)	6.2 (5.0-6.7)
PPV (%)	**PRECON**	98 (97-99)	91 (89-93)*	86 (82-93)	84 (82-89)*	77 (76-83)*
	CONTROL	97 (97-98)	89 (86-90)	84 (82-87)	77 (66-84)	58 (50-65)
MFI (0-3)	**PRECON**	3.0 (2.9-3.0)	2.6 (2.5-2.7)	2.2 (2.1-2.6)	2.3 (2.1-2.6)*	2.2 (2.1-2.4)*
	CONTROL	2.9 (2.9-3.0)	2.4 (2.3-2.5)	2.2 (2.1-2.4)	1.7 (1.5-2.1)	1.2 (1.0-1.4)
PPV HI (%)	**PRECON**	3 (3-5)	9 (5-15)*	16 (11-40)	21 (18-39)	35 (25-39)*
	CONTROL	4 (2-5)	19 (13-25)	27 (23-35)	38 (21-55)	57 (44-66)

[Sublingual microcirculatory variables]

* = $p < 0.05$ compared with control group.

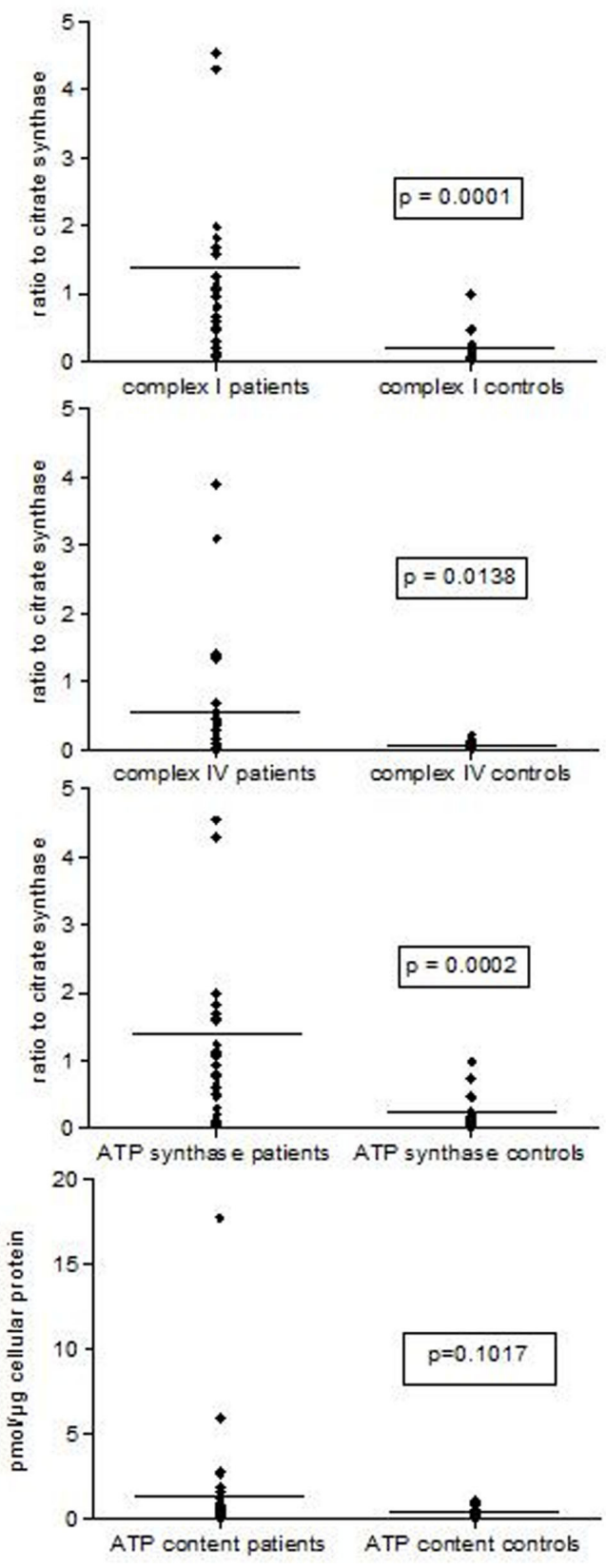

Figure 1(abstract P5) Maximal mitochondrial enzymatic activities and ATP content of human immune cells in the first 48h of sepsis : Monocytes

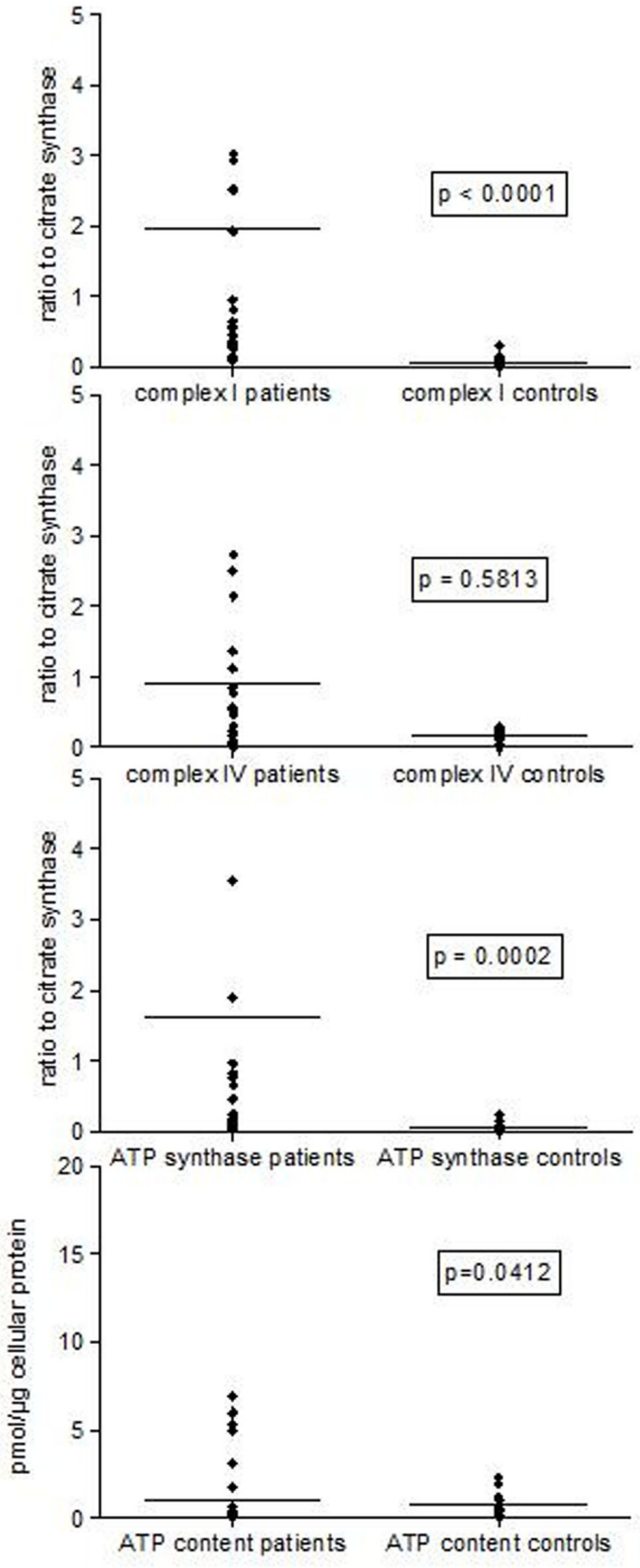

Figure 2(abstract P5) Maximal mitochondrial enzymatic activities and ATP content of human immune cells in the first 48h of sepsis: B cells

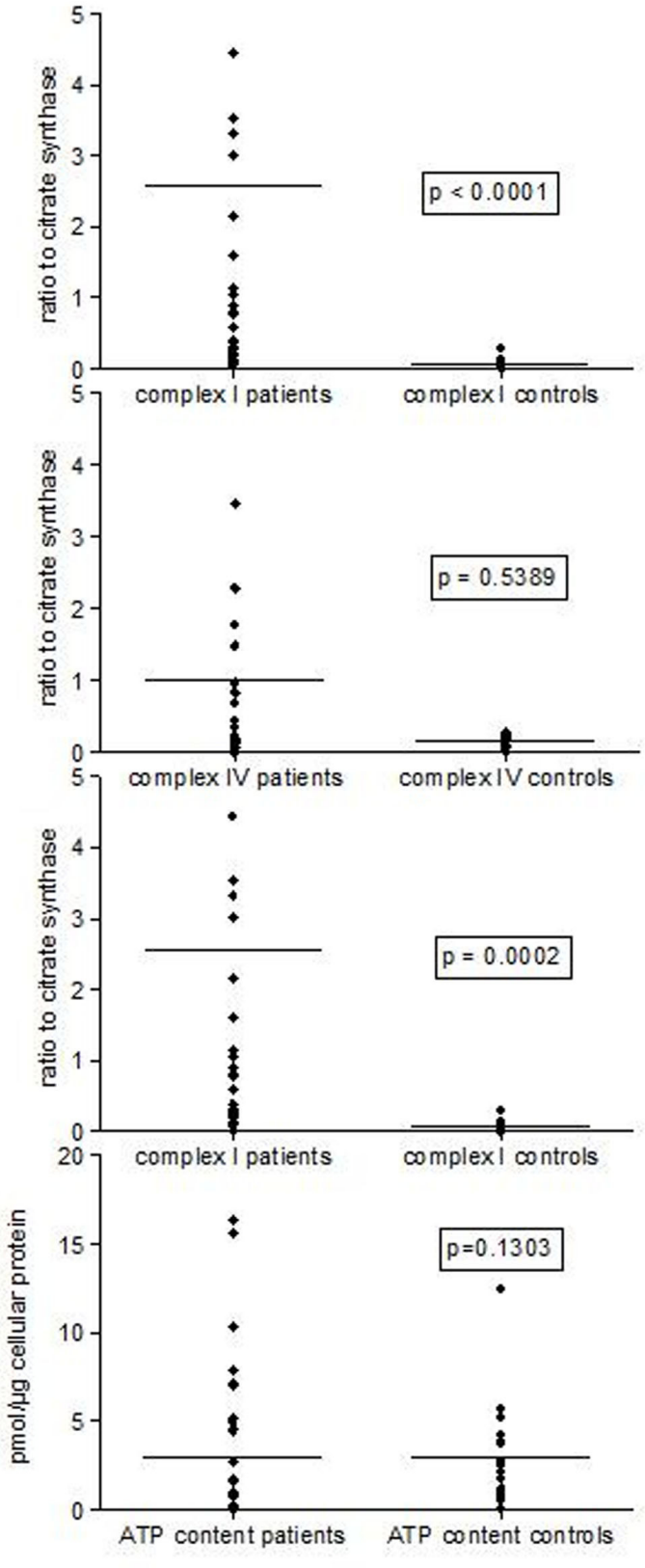

Figure 3(abstract P5) Maximal mitochondrial enzymatic activities and ATP content of human immune cells in the first 48h of sepsis: T cells

P6

0094. Monocyte tace activity profile during sepsis and systemic inflammatory response syndrome

DJ O'Callaghan[1,2], KP O'Dea[1*], M Takata[1], AC Gordon[1,2]
[1]Imperial College London, London, UK; [2]Imperial College Healthcare National Health Service Trust, London, UK
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P6

Introduction: To date, immune-modulatory treatments in sepsis have proved unsuccessful. Better understanding of inflammatory processes might help tailor such therapies more effectively and improve their efficacy. It is now recognised that some patients may require immune stimulating therapies.
Monocytes display altered behaviour in response to systemic inflammation; this has been called "deactivation" and is characterised by reduced HLA-DR expression and attenuated tumour necrosis factor-α (TNF) release in response to lipopolysaccharide (LPS) stimulus[1]. TNF converting enzyme (TACE), activated via p38-mitogen activated protein kinase (MAPK) mediated pathway[2], is essential for the shedding of TNF and TNF receptors (TNFRs)-1&2. Altered TACE behaviour may be mechanistically important in deactivation.
Objectives: To determine whether monocyte TACE activity is affected by sepsis and is reflective of illness severity.
Methods: Sixteen patients with sepsis, 15 healthy volunteers and 8 patients with non-infectious systemic inflammatory response syndrome (NI-SIRS) were recruited and data collected at baseline (D0), day 2 (D2) and day 4 (D4). Peripheral blood mononuclear cells were separated by density gradient centrifugation with HLA-DR, TACE and TNFR expression (basal and LPS-induced) determined by flow cytometry. LPS-induced TNF release was measured using ELISA. Monocytes were isolated using magnetic bead selection and TACE catalytic activity (basal and LPS-induced) was measured using fluorescence resonance energy transfer assay[3].
Results: HLA-DR expression and LPS-induced monocyte TNF release were attenuated in sepsis and NI-SIRS ($p< 0.01$), consistent with deactivation. Sepsis, but not NI-SIRS, produced elevated basal monocyte TACE activity ($p < 0.01$) while LPS-induced increases in TACE activity were attenuated. Elevated D0 basal TACE activity positively correlated with APACHE II score in sepsis ($p < 0.01$), but not NI-SIRS.
TACE expression was unaltered across all groups, but LPS-induced TNFR shedding appeared reduced by sepsis. Sepsis patients displayed an attenuated p38-MAPK response to LPS.
Conclusions: Our data indicate that sepsis induces specific changes in monocyte TACE catalytic activity profiles that may reflect illness severity, whilst such changes were not seen in NI-SIRS. These changes in TACE activity attenuated LPS-induced release of TNFR and TNF from monocytes, and appear to be mediated through altered upstream p38-MAPK signalling.
Grant acknowledgment: UK Intensive Care Foundation Young Investigator Award.

References

1. Volk HD, Reinke P, Krausch D, Zuckermann H, Asadullah K, Müller JM, Döcke WD, Kox WJ: **Monocyte deactivation–rationale for a new therapeutic strategy in sepsis.** *Intensive Care Med* 1996, **22(Suppl 4)**:p. S474-p.S481.
2. Scott AJ, O'Dea KP, O'Callaghan D, Williams L, Dokpesi JO, Tatton L, Handy JM, Hogg PJ, Takata M: **Reactive oxygen species and p38 mitogen-activated protein kinase mediate tumor necrosis factor a-converting enzyme (TACE/ADAM-17) activation in primary human monocytes.** *J Biol Chem.* 2011, **286(41)**:35466-35476.
3. Alvarez-Iglesias A, Wayne G, O'Dea KP, Amour A, Takata M: **Continuous real-time measurement of tumor necrosis factor-alpha converting enzyme activity on live cells.** *Lab Invest.* 2005, **85(11)**:1440-1448.

P7

0095. Transvenous vagus nerve stimulation does not modulate the innate immune response in humans *in vivo* during experimental endotoxemia

M Kox[1,2*], LT van Eijk[1,2], T Frenzel[1,2], T Verhaak[1,2], JF Gerretsen[1,2], JG van der Hoeven[1,2], L Kornet[3], A Scheiner[4], P Pickkers[1,2]
[1]Radboud University Medical Center, Intensive Care Medicine, Nijmegen, Netherlands; [2]Radboud Institute for Infectious Diseases, Nijmegen, Netherlands; [3]Medtronic Inc., Maastricht, Netherlands; [4]Medtronic Inc., St. Paul, MN, USA
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P7

Introduction: In a variety of conditions excessive and/or persistent activation of the innate immune system has detrimental effects. In animals, electrical vagus nerve stimulation (VNS) inhibits the innate immune response in models of endotoxemia (administration of lipopolysaccharide [LPS]), sepsis, trauma, and hemorrhagic shock, via the so-called cholinergic anti-inflammatory pathway. However, human *in vivo* evidence is lacking. Up till now, VNS was possible through implantation of a cuff electrode wrapped around the nerve, which limits its use in acute inflammatory situations frequently encountered on the ICU. A novel, less invasive VNS method is transvenous VNS (tVNS).
Objective: To determine whether tVNS exerts anti-inflammatory effects during experimental human endotoxemia.
Methods: A parallel randomized double-blind sham-controlled study in healthy male volunteers was performed. Subjects were randomized to receive either tVNS (n=10) or sham tVNS (n=10).
In both groups, a stimulation catheter with multiple circular electrode pairs was inserted in the left internal jugular vein at C5-C7 spinal level to be situated adjacent to the vagal nerve. In the tVNS group, stimulation (0-10 V, 1 ms, 20 Hz) was continuously performed during 30 minutes, starting 10 minutes before intravenous administration of 2 ng/kg *E. Coli* LPS. In sham subjects, the exact same procedures were performed, but the stimulator was not switched on by an unblinded team member.
Results: In all 20 subjects placement of the stimulation electrode was successful and uneventful. Furthermore, in all subjects of the tVNS group, laryngeal vibration was confirmed by an unblinded team member, indicating stimulation of vagal fibers. LPS administration resulted in an increase in heart rate of 26±3 bpm and a decrease in mean arterial pressure of 16±2 mmHg, as well as fever (increase of 1.5±0.1 °C) and flu-like symptoms. No differences between groups were observed. Furthermore, plasma levels of inflammatory cytokines increased sharply, but responses were similar between groups (Figure 1). Likewise, cytokine production by leukocytes *ex vivo* restimulated with LPS as well as neutrophil phagocytosis capacity were unaffected by tVNS. Finally, heart rate variability analysis revealed that during (sham) stimulation, the ratio between low frequency and high frequency (HF) spectral power was significantly lower, and HF power in normalized units significantly higher in the tVNS group compared with the sham group, suggestive of increased parasympathethic activity (Figure 2, p=0.02 for both indices, unpaired Student's t-test at T=0). However, when corrected for baseline differences, this effect was no longer statistically significant (p=0.35 for both indices).
Conclusions: Thirty-minute transvenous vagus nerve stimulation is feasible and safe but does not modulate the innate immune response in humans in vivo during experimental human endotoxemia.
Grant acknowledgment: This study was sponsored by Medtronic Inc.

P8

0096. Evaluation by videomicroscopy (SDF) of the renal cortex microcirculation and convoluted tubules in acute renal failure during severe sepsis. Experimental study

AMA Liberatore[1*], JC Vieira[2], J Almeida-Filho[3], RC Tedesco[4], IHJ Koh[5]
[1]Federal University of São Paulo, Surgery, Sao Paulo, Brazil; [2]Federal University of São Paulo, Surgery, São Paulo, Brazil; [3]Federal University Foundation of Vale do São Francisco, Surgery, Petrolina, Brazil; [4]Federal University of Sao Paulo, Anatomia, Sao Paulo, Brazil; [5]Federal University of Sao Paulo, Surgery, Sao Paulo, Brazil
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P8

Introduction: The microcirculatory dysfunction as the triggering event of organ dysfunction in sepsis is a universal concept[1-3],but the microcirculatory dysfunction and its relationship with the deterioration of adjacent tissue is still unsolved, thus, the macrocirculation parameters guide therapeutic decisions although the impairment of microcirculation precedes the macrocirculation dysfunction.
Objectives: Investigate the dynamic relationship between microcirculatory injury and adjacent tissue in tubular kidney failure during severe sepsis by SDF.
Methods: Wistar rats underwent severe sepsis (iv. *E. coli* $2x10^9$ CFU, DL_{70-80} in 26 hours[3]) and under general anesthesia the dynamics of microcirculatory dysfunction of the renal cortical area was monitored by SDF[4] at T0,T30 min and T1-T6 hours and the tissue injury by histology (T0,T2h,T6h).

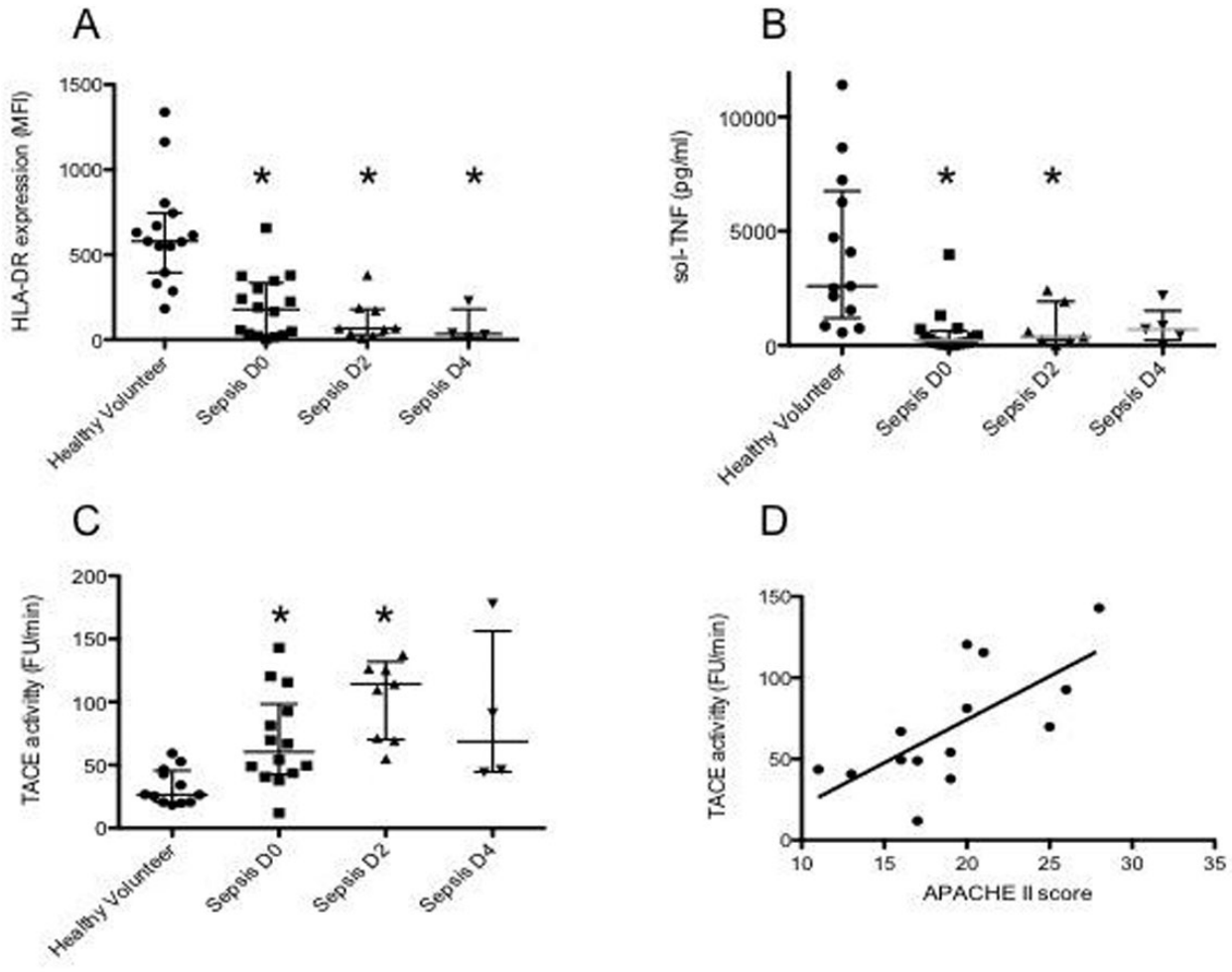

Figure 1(abstract P6)

Results: T0 and T30min SDF allowed identification of convoluted tubules with lumen and peritubular microvessels without alterations. The architectural feature of the kidney was of convoluted tubules surrounded by peritubular microvessels forming a homogeneous aspect that merges the sequence of a microvessel followed by a convoluted tubule to the fullest extent. From T1h, the outlining of tubules became blurred by their enlargement and the onset of the compression of tubular lumen and peritubular microvessels, suggesting an obstructive phenomenon by cellular edema. This focal occurrence in T1h became increasingly widespread at T6 changing the homogeneous organization of the cortex architecture.These findings suggested that the process involved in the genesis of renal failure in sepsis could be due to the cyclical repetition of the event: peritubular microcirculatory dysfunction-cytopathic hypoxia of the tubular wall epithelia-edema of the tubular cells-compression of both peritubular microvessel and tubular lumen-exacerbation of microvessel and nephron dysfunction. This hypothesis raised on visual analysis of SDF images could be confirmed by histology which showed a progressive swelling of the epithelial cells of convoluted tubules and reduction of tubular lumen and peritubular vessels with the progression of sepsis. In addition, epithelial cells showed membrane injury, pyknosis and necrosis.
Conclusions: The genesis of acute renal failure in severe sepsis appears to depend on the repetitive cycle of peritubular microcirculatory dysfunction and subsequent tubular injury that exacerbates the progression of the renal injury, thus suggesting the conjoined

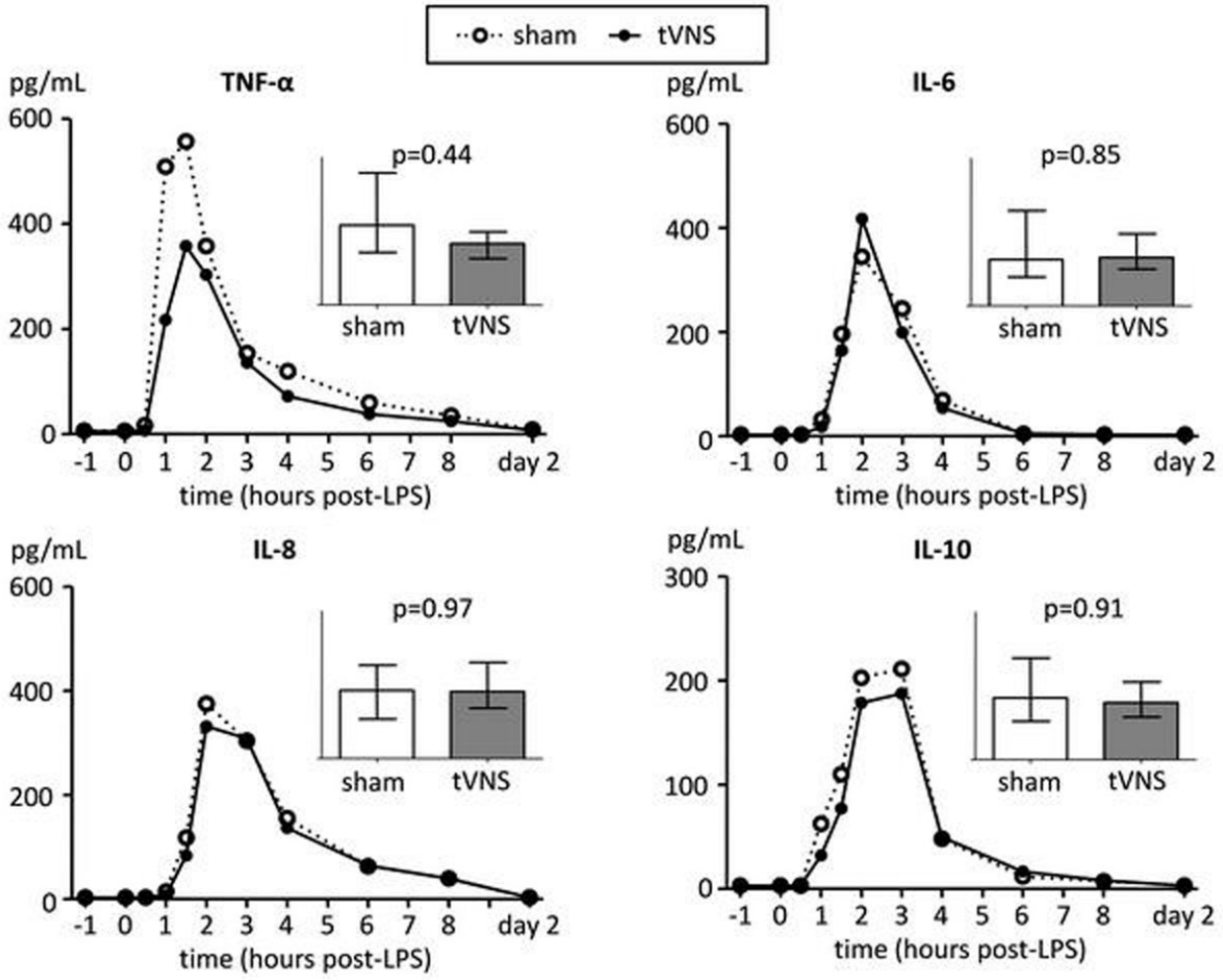

Figure 1(abstract P7) Plasma concentrations of inflammatory cytokines during endotoxemia. Data are expressed as medians od 10 subjects per group. Inserted bar graphs depict median interquartile range of area under curve (AUC) of cytokines. P values calculated using Mann-Whitney U-tests.

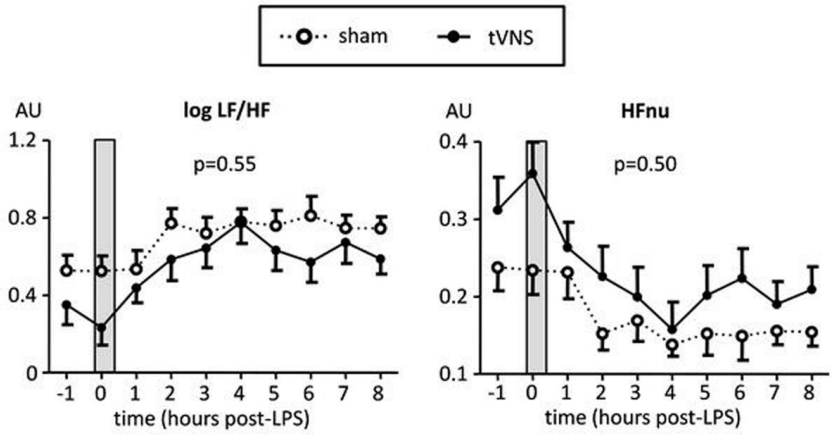

Figure 2(abstract P7) Heart rate variability parameters during endotoxemia. Left panel: ratio between low frequency and high frequency power (LF/HF). Right panel: high frequency power in normalized units (HFnu). Data are expressed as mea SEM of 10 subjects per group. LH/HF was log-transformed to attain normal distribution. Gray box indicates period in which the (sham) stimulation took place. P-values indicate differences between groups over time calculated using repeated measures two-way analysis of variance (ANOVA, interaction term). AU: arbitrary units.

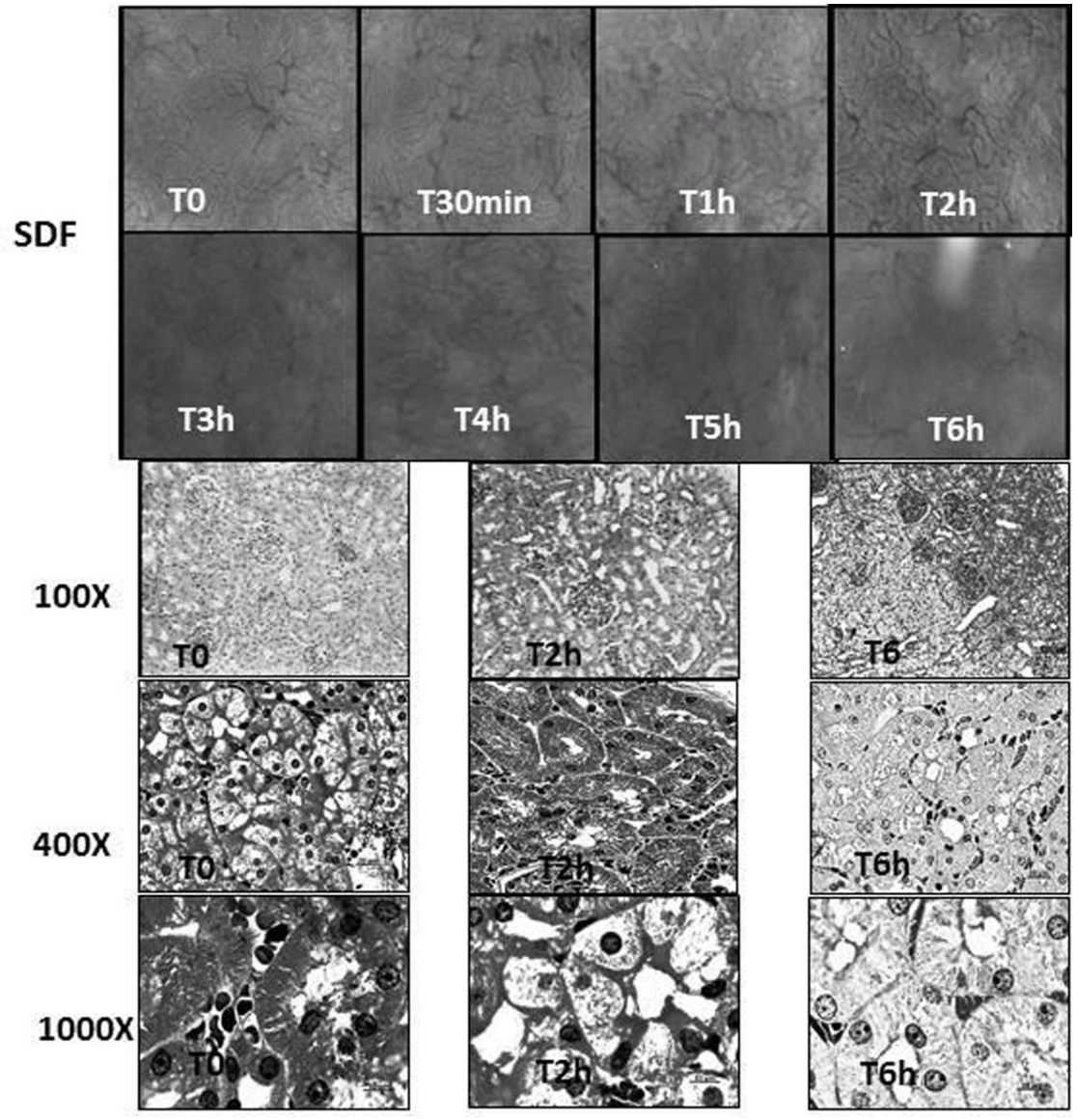

Figure 1(abstract P8) Images of Kidney by SDF and Histology

participation of microvessels and their adjacent cells in the genesis of the solid organ dysfunction.

Grant acknowledgment: FAPESP 2011/20401-4.

References

1. Sakr Y, *et al*: *Crit Care Med* 2004, **32**:1825-1831.
2. *Ince C. Crit Care* 2005, **9**:S13-S19.
3. Koh Ivan HJ, *et al*: *Shock* 2010, **34(7 Suppl 1)**:27-33.
4. De Backer D, *et al*: *Intensive Care Med,* 2010, **36**:1813-1825.

P9

0097. Myeloid-derived suppressor cells attenuate inflammation and improve the survival of microbial sepsis

L Tong, GX Hu*, JC Cai
The 1st Affiliated Hospital, Sun Yat-Sen University, Surgical Intensive Care Unit, Guangzhou, China

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P9

Introduction: Myeloid-derived suppressor cells (MDSCs) are heterogeneous population of immature myeloid cells with suppressive activity, containing precursor of granulocytes, macrophages, and dendritic cells [1-3]. They expand in inflammatory conditions and regulate immune responses. However, the role that MDSCs played in other chronic or acute inflammatory conditions like sepsis is not well understood.

Objectives: To find the role of MDSCs in sepsis and their relationship with the inflammatory cytokines.

Methods: The model of caecal ligation and puncture (CLP) was used to induce polymicrobial sepsis in adult male BALB/c mice. Mice were killed under anesthesia, then blood, peritoneal lavage fluid, and organs were harvested. After the spleen MDSCs were isolated, 30 BALB/c mice were randomized divided into three groups which were CLP+MDSCs (day3),CLP +MDSCs (day 10), CLP+saline whereas MDSCs were adoptive transferred into these mice respectively. And the MDSCs accumulation in spleen were observed at day1,3,6,10.

Results: MDSCs progressively accumulated in spleens during sepsis in survived mice. They were increasing from ~8% at day 1 to ~50% at day 10.

Adoptive transfer of late MDSCs into naïve mice after CLP could increase the level of IL-10, decrease the level of INF-γ, and improve the survival rate.
Conclusions: MDSCs could influence the inflammation response after CLP. Contrasting with the deleterious role of MDSCs in tumor associated diseases, the protective role of these cells was appeared during the early period of polymicrobial sepsis. This may provide a new direction for the treatment of sepsis.
Grant acknowledgement: We thank to the staff members of the department of SICU of the First Affiliated Hospital, Sun Yat-sen University.
References
1. Gabrilovich DI, Bronte V, Chen SH, Colombo MP, Ochoa A, Ostrand-Rosenberg S, Schreiber H: **The terminology issue for myeloid-derived suppressor cells.** *Cancer Res* 2007, **67(1)**:425, author reply 426.
2. Poschke I, Kiessling R: **On the armament and appearances of human myeloid-derived suppressor cells.** *Clin Immunol* 2012, **144(3)**:250-268.
3. Gabrilovich DI, Nagaraj S: **Myeloid-derived suppressor cells as regulators of the immune system.** *Nature Reviews Immunology* 2009, **9(3)**:162-174.

P10

0099. Nitrite reductase activity during sepsis

V Simon[1,2*], A Dyson[1], M Minnion[3], M Feelisch[3], M Singer[1]
[1]University College London Hospital NHS Foundation Trust, Bloomsbury Institute of Intensive Care Medicine, London, UK; [2]University Hospital Aachen, Intensive Care Medicine, Aachen, Germany; [3]University of Southampton, Faculty of Medicine, Southampton, UK
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P10

Introduction: Nitric oxide (NO) excess is considered to be the main cause of hypotension in sepsis. Research has mainly focussed upon NO production by isoforms of NO synthase (NOS). However, alternative pathways may make an important contribution, including nitrite (NO_2^-) reduction by the reductase activity possessed by numerous heme- and pterin-based enzymes. The temporal contribution of these pathways to NO production in sepsis is currently unclear.
Objectives: To investigate changes in nitrite reductase activity during sepsis.
Methods: Male Wistar rats (approx 300g wt) with tunnelled right jugular venous and left common carotid arterial lines *in situ* received i.p. injection of either faecal slurry (septic) or n-saline (sham). Fluid (1:1 mixture of 5% glucose/Hartmann`s; 10 ml/kg/h) was started 2h later. At either 6h or 24h, rats were anaesthetized and tracheotomized. After stabilization, measurements were made of haemodynamics (blood pressure, echocardiography) and methaemoglobinaemia (oxidation of Hb to the Fe^{3+} form induced by NO or nitrite) before and after a 25 ml/kg bolus fluid challenge (BL, baseline) to ensure adequate LV filling. Animals then received (A) a single dose of sodium nitrite ($NaNO_2$, 15mg/kg i.v.) to stimulate nitrite reductase activity, (B) a combination of $NaNO_2$ and the NO-scavenger cPTIO (3.4mg/kg i.v.) or (C) a combination of $NaNO_2$ and the non-specific NOS-inhibitor SEITU (1mg/kg i.v.). Recordings were made over the next 2-4h prior to sacrifice.
Results: At 6h, there was no significant difference between groups (data not shown). However, at 24h, septic animals showed a greater fall in BP following $NaNO_2$ with a longer time to recovery, signifying increased nitrite reductase activity (Fig 1A). After scavenging of free NO (1B) and NOS inhibition (1C), both groups showed an initial rise in BP, followed by a significant fall with $NaNO_2$. However, on removal of NO there was no prolonged BP recovery time in the septic animals. Baseline methaemoglobinaemia was low in both control and septic animals at 24h and increased markedly with $NaNO_2$, again with a longer recovery time in septic animals. This rise was blunted by pre-treatment with the NO scavenger, c-PTIO, but not by SEITU though SEITU did prevent the delayed normalization in metHb levels .
Conclusions: In this rat model, increased nitrite reductase activity was apparent during established (24h) sepsis, but not at an early (6h) timepoint. The clinical relevance of this finding needs to be elucidated.
Grant acknowledgment: Internal lab funds and salary support from University Hospital Aachen.

P11

0100. Apelin is cardioprotective and life-saving over dobutamine in a murine model of endotoxin-induced myocardial dysfunction

O Lesur[1*], F Chagnon[2], A Murza[3], P Sarret[4], E Marsault[3], D Salvail[5]
[1]University de Sherbrooke, ICU / Medicine / CHUS, Sherbrooke, Canada; [2]ICU/Medicine/CRCHUS, Sherbrooke, Canada; [3]IPS/ U de Sherbrooke, Pharmacology, Sherbrooke, Canada; [4]IPS/ U de Sherbrooke, Physiologie, Sherbrooke, Canada; [5]IPS Therapeutique Inc, Sherbrooke, Canada
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P11

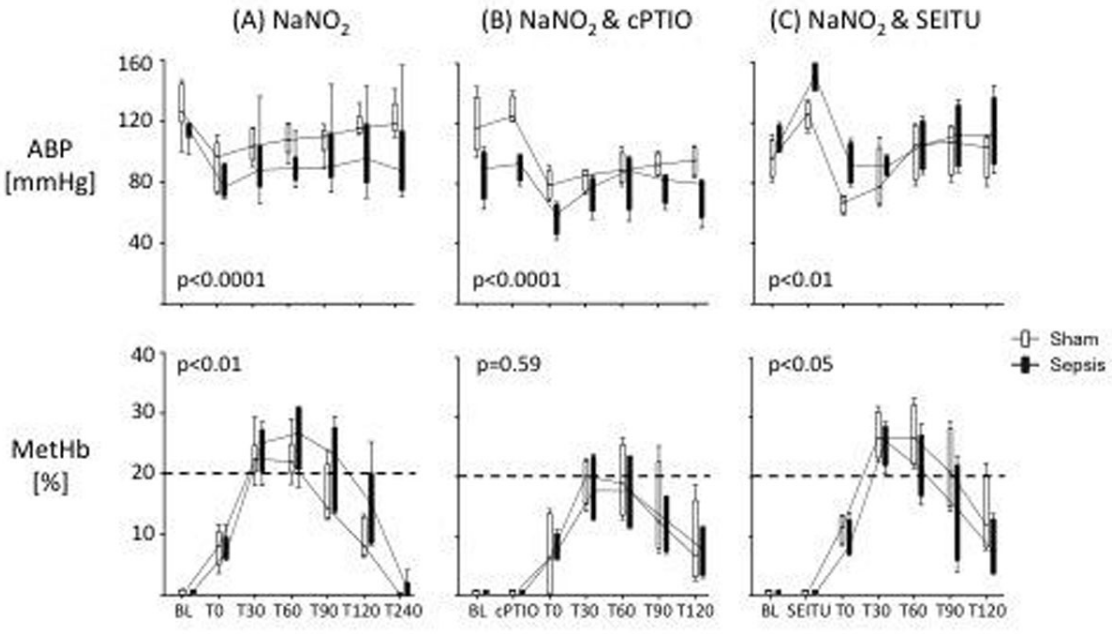

Figure 1(abstract P10) Effects of $NaNO_2$, cPTIO and SEITU on arterial blood pressure (ABP) and methaemoglobin levels (MetHb) in sham and septic animals at 24h. BL, baseline; T0, after drug administration (2-way ANOVA + Bonferroni test, *p< 0.05).

Introduction: Dobutamine (DOB) is the actual recommended b-adrenergic inotropic drug to support sepsis-induced myocardial dysfunction when cardiac output index is still low after preload correction. In this context, DOB cardiovascular response predicts outcome in septic shock. Alternative supportive and safer therapies are however mandatory because:
1) only 35-45% of septic patients do respond to DOB, and
2) numerous side-effects of DOB can be observed, including potential harmful impact on cardiomyocyte function. Apelin (APLN) is a powerful inotrope, is widely expressed by the cardiovascular system with its receptor APJ-R, and should be considered as an alternative noncatecholaminergic support.
Objectives: Perform a comparative evaluation of APLN-13 (APLN active peptide) vs DOB in terms of hemodynamic efficacy, cardioprotection and outcome in a model of "sepsis-induced cardiac dysfunction".
Methods: A rat model of LPS-induced myocardial dysfunction (*E. Coli* 055: B5, 10-12mg/kg intraperitoneal).
Interventions: APLN-13 vs DOB (0.23 vs 7.5µg/kg/min i.v continuous infusion respectively, as determined by preliminary experiments with or without "in parallel" fluid resuscitation (saline, 2mL/kg/hr).
Time-course: 0-6-18h.
Outcomes: *in vivo*: echocardiography, final hemodynamics; urine output, weight and plasma volume variation, survival study, *ex vivo*: Langendorff dP/dt, *in vitro*: APJ-R and beta1-adrenergic receptor (AR) myocardial expressions, Pi3K/Akt/GSK3/mTOR activation-expression profiles, cTnI (troponin, myocardial injury) and cleaved caspase-3 (apoptosis).
Results: Both drugs restored LPS-induced fall of left ventricular ejection fraction (LVEF) with dominant chronotropic impact and mean arterial pressure (MAP) restoration for DOB, and lower peripheral vascular resistances (PVR) for APLN. APJ-R but not beta1 AR myocardial expressions were upregulated by LPS challenge ($p< 0.05$). The deepness the induced LVEF drop, the higher the dP/dt response to APLN but not to DOB ($p < 0.05$). In 18h LPS-challenged hearts, Langendorff assays peak dP/dt responses were 3nM and 100nM for APLN and DOB, respectively ($p < 0.05$). Combining fluid resuscitation with APLN infusion declined urine output (UO) with plasma volume (PV) expansion, whereas DOB induced less PV expansion but more UO ($p < 0.05$). Survival proportions were clearly distinctive ($p < 0.05$).
APLN further dampened down LPS-induced overphosphorylation/inhibition of Pi3K/Akt/mTOR/GSK3 and reduced injury/apoptosis (i.e. cTNI and cleaved caspase-3 expressions).
Conclusions: APLN potentially offers distinctive mechanisms of hemodynamics, cardioprotective effects, and survival benefits, over DOB. Chemical optimization of APLN-13 with more extensive preclinical data, would pave the way for first phase clinical trials.
Grant acknowledgment: Canadian Heart & Stroke Foundation.

P12

0101. Early and severe impairment of lactate clearance in endotoxic shock is not related to liver hypoperfusion: preliminary report

P Tapia[1], D Soto[1], A Bruhn[1], T Regueira[1], N Jarufe[2], L Alegria[1], JP Bachler[2], F Leon[2], C Vicuña[1], C Luengo[3], G Ospina-Tascón[4], J Bakker[5], G Hernandez[1*]
[1]Pontificia Universidad Católica de Chile, Facultad de Medicina, Departamento de Medicina Intensiva, Santiago, Chile; [2]Pontificia Universidad Católica de Chile, Facultad de Medicina, Departamento de Cirugía Digestiva, Santiago, Chile; [3]Universidad de Chile, Hospital Clínico, Unidad de Pacientes Críticos, Santiago, Chile; [4]Fundación Valle del Lili, Intensive Care Unit, Cali, Colombia; [5]Erasmus MC University Medical Centre, Department of Intensive Care Adults, Rotterdam, Netherlands
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P12

Introduction: Although the prognostic value of persistent hyperlactatemia in septic shock is unequivocal, its physiological determinants are controversial. In particular, the role of impaired hepatic clearance has been considered as relevant only in severe shock with liver ischemia or advanced cirrhosis. However, very few studies have addressed this subject.
Objectives: To determine the evolution of lactate clearance [1] in an endotoxic sheep model.
Methods: This study is part of a major project exploring the influence of adrenergic stimulation and blockade over the determinants of lactate production and utilization in septic shock. Eight anesthetized sheep subjected to a multimodal hemodynamic/perfusion assessment including pulmonary artery, hepatic and portal vein catheterizations, portal/hepatic artery flow, gut tonometry, sublingual microcirculation, muscle microdialysis and hepatic mitochondrial high-resolution respirometry, were randomized to LPS or sham. LPS sheep received 5 mcg/kg bolus (E coli O127:B8®) and then 4 mcg •kg-1•hr-1 for the rest of the experiment [2]. After 1h they were volume resuscitated. Sampling and exogenous lactate clearances were performed at 4 points (fig 1).
Results: LPS sheep presented an early hyperlactatemic hyperdynamic septic shock and an increased muscle lactate production compared to sham (MAP 63 vs 87 mmHg; MPAP 20 vs 10 mmHg; CO 3.7 vs 2.5 l/min; arterial lactate 6.1 vs 2.6 mmol/l). Total liver flow (865 vs 692 ml/min), proportion of liver

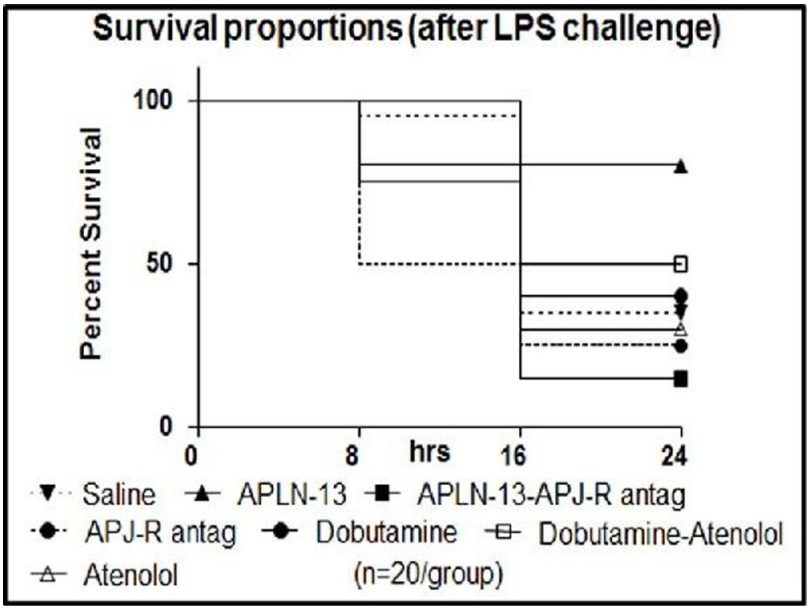

Figure 1(abstract P11) APLN survival

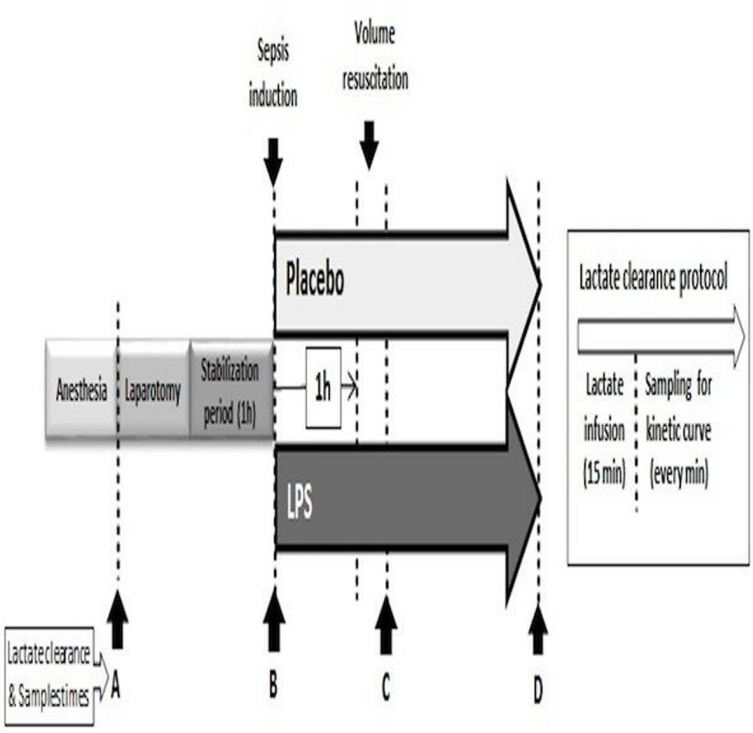

Figure 1(abstract P12)

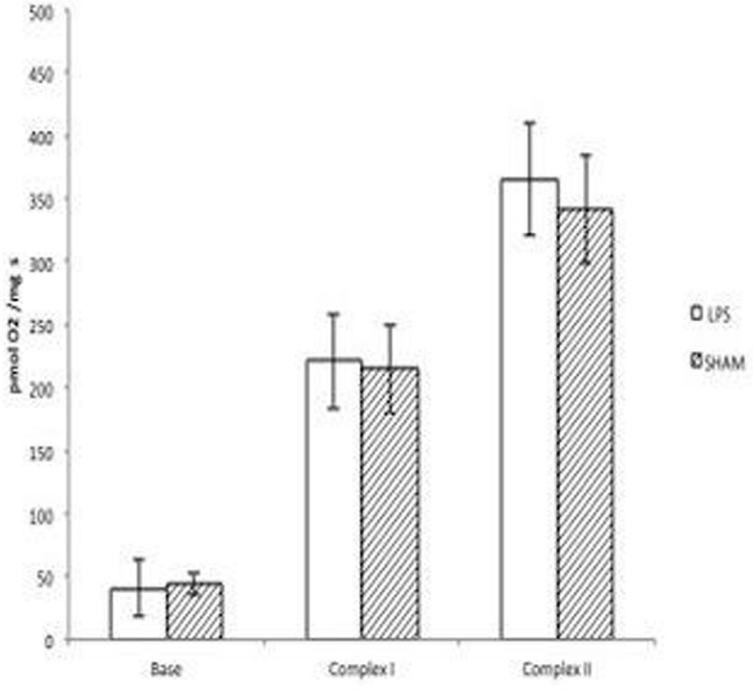

Figure 2(abstract P12)

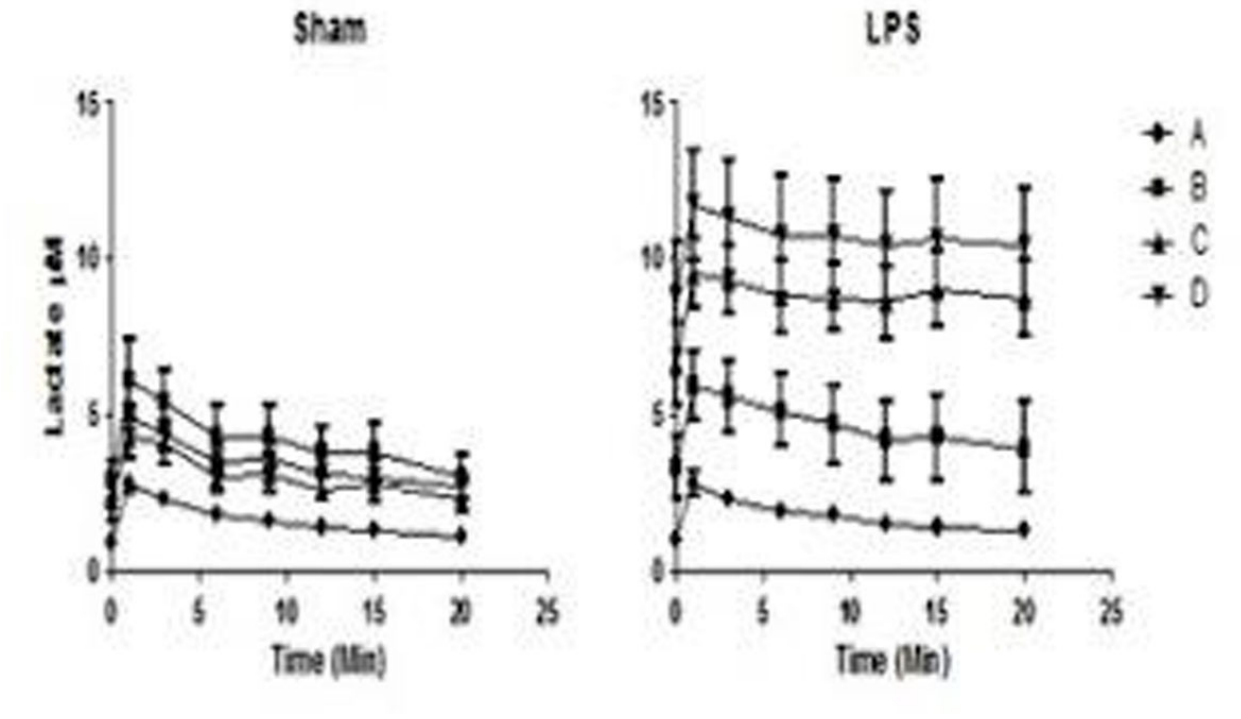

Figure 3(abstract P12)

flow/CO, O_2 liver extraction, liver enzymes, and mitochondrial function were comparable between LPS and placebo (fig 2).
However, LPS sheep presented an early severe and persistent decrease in lactate clearance (C: 575 vs 1188 and D: 907 vs 1410 ml/kg/h), fig 3.
Conclusions: Hyperdynamic endotoxic shock induces an early, severe and persistent impaired lactate clearance that is not related to liver hypoerfusion, O_2 extraction or mitochondrial function.
Grant acknowledgment: FONDECYT 1130200, Chile.
References
1. Levraut J, *et al*: *Am J Respir Crit Care Mer* 1998, **157**:1021-1026.
2. Dubin A, *et al*: *Crit Care Med* 2008, **36**:535-542.

P13

0102. *Ex vivo* effects of hyperglycemia on phenotype and production of reactive oxygen species by the nadph oxidase of human immune cells in acute inflammatory response

B Soyer*, V Faivre, C Damoisel, A-C Lukaszewicz, D Payen
Hôpital Lariboisiere - APHP, Département d'Anesthésie Réanimation, Paris, France
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P13

Introduction: Glucose is a major energy substrate for polymorphonuclears (PMNs) and monocytes (Monos). In acute stress, glucose was shown to be pro-inflammatory favoring the production of reactive oxygen species (ROS).
Objectives: 1- Demonstrate an increase in ROS production during moderate hyperglycemia by increased production of the NADPH oxidase through the pentose shunt pathway (PSP), the major source of NADPH.
2- Evaluate the phenotypic consequences on PMNs, Monos, and Lymphocytes (Lymphos).
Methods: Whole blood from healthy volunteers (CCPSL UNT -No 13/SL/015) was incubated 90 minutes either in normoglycemia (NG) or hyperglycemia (HG, 12.5 mM glucose), in the presence of deoxyglucose (DOG, 12.5 mM) blocking glycolysis, or inhibitors of the PSP (Epi Androsterone & 6 Aminonicotinamide (EPI, 6AN)). For the all conditions: evaluation of overall ROS production (luminometry[1]: area under the curve (AUC) & slope of activation (Slope)) in resting cell condition & after stimulation by PMA-Ionomycin. Following phenotypic parameters were evaluated: HLA-DR expression on Monos (overall population, and subtypes CD16- and CD16+) and on B cells; expression of CD11b on Monos and PMNs; CTLA4 on T4/T8 Lymphos, and CD62L (marker of cell activation) on Monos, PMNs and Lymphos. Statistics: non-parametric tests.
Results: 1-ROS: In resting conditions: AUC of luminometry (increased production) (n = 19) and Slope (system reactivity) were higher in HG than in NG ($p < 0.05$), a difference that disappeared after PMA-Ionomycin stimulation. The inhibitors (DOG, EPI + 6AN) reduced the AUC and the Slope in NG and HG ($p < 0.05$ and $p < 0.05$).
2-Cell phenotype: HG did not change the cell expression of markers for Monos (total, CD16- and CD16+), PMNs, T4 and T8 Lymphos. Only in B cell HG increased HLA-DR expression ($p < 0.05$). The DOG condition decreased expression of HLA-DR, CD11b and CD62L on Monos and CD62L on PMNs ($p < 0.05$), with no effect on Lymphos.
Conclusions: HG amplifies ROS production and NADPH oxidase reactivity via PSP pathway and NADPH synthesis, especially after stimulation in Monos. Absence of any impact of acute glycemic variation and of blocking glucose metabolism on T4, T8 Lymphos phenotype, strongly suggests that Lymphos do not use glucose as a major energetic substrate[2]. Except for B lympho, Mono and PMNs did not change their studied phenotype, with *ex vivo* non-significant effect on T Lymphos. Acute HG as glucose metabolic inhibitors changed ROS production but not innate immune cell phenotype.
References
1. Lukaszewicz A-C, Gontier G, Faivre V, Ouanounou I, Payen D: **Elevated production of radical oxygen species by polymorphonuclear neutrophils in cerebrospinal fluid infection.** *Ann. Intensive Care* 2012, **2**:10.
2. Pearce EL, Pearce EJ: **Metabolic pathways in immune cell activation and quiescence.** *Immunity* 2013, **38**:633-643.

P14

0104. Modulatory effects of heat shock with or without glutamine compared to LPS on peripheral blood mononuclear cells heat-shock-protein 90α expression in severe sepsis and trauma

E Briassouli[1*], M Tzanoudaki[2], G Daikos[1], K Vardas[3], M Kanariou[2], C Routsi[3], S Nanas[3], G Briassoulis[4]

[1]1st Department of Propaedeutic InternalMedicine, University of Athens, Athens, Greece; [2]Department of Immunology - Histocompatibility, Specialized Center & Referral Center for Primary Immunodeficiencies - Paediatric Immunology, "Aghia Sophia" Children's Hospital, Athens, Greece; [3]First Critical Care Department, University of Athens, Evangelismos Hospital, Athens, Greece; [4]PICU, University of Crete, University Hospital, Heraklion, Greece

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P14

Introduction: Inflammatory stimuli cause posttranslational modifications of inducible 90α-kDa-heat-shock-protein (HSP90α) that are Hsp90-inhibitor sensitive and may be important to the pro-inflammatory actions of Hsp90α.
Objectives: We investigated the heat-shock (HS) and lipopolysaccharide (LPS)-stress response effect on HSP90α in cultured peripheral blood mononuclear cells (PBMCs) from patients with severe sepsis (SS) or trauma-related systemic inflammatory response syndrome (SIRS) compared to healthy-controls (H) and any possible modulating Glutamine (Gln)-effect.
Methods: PBMCs of 16/H, 11/SS, and 7/SIRS were incubated with 1μg/ml LPS or 43°HS vs.no stimulation for 4h. In each group 3 experiments involved L-Ala-Gln10mM incubation 1h before (Gln-b) or after (Gln-a) induction, or no glutmanine (1088 measurements). Intracellular Mean Fluorescence Intensity (MFI) levels of monocytes (mHSP90α) or lymphocytes (lHSP90α) determined using Flow Cytometry.
Results: Baseline mHSP90α was higher in SIRS (187±30 vs. 112±10, $p < 0.01$) and lHSP90α in SS (91±19 vs. 47±3, $p< 0.001$) compared to H. LPS induced H-mHSP90α (141±12 vs. 112±10, $p< 0.001$) and HS H-lHSP90α (66±7 vs. 47±3, $p < 0.0001$). Neither LPS nor HS exhibit any significant effect in SIRS- or SS-mHSP90α or lHSP90α. Glutamine given before LPS suppressed SS-lHSP90α (Gln-b 61±5 vs. 91±19, $p < 0.004$). Similarly, when glutamine was given before or after HS suppressed SS-lHSP90α (Gln-a 73±5 vs. 91±19, $p < 0.001$; Gln-b 78±4 vs. 91±19, $p < 0.05$), respectively.
Conclusions: PBMCs express higher baseline mHSP90α in SIRS and lHSP90α in SS, not further induced by LPS or HS, contrasting their induction effects in H. Gln pre-treatment may attenuate the LPS or HS-induced lHSP90α in SS.
Grant acknowledgment: This research has been co-financed by the European Union (European Social Fund (ESF)) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF)-Research Funding Program: THALES.

WEANING & ASSISTED VENTILATION

P15

0108. Patients' response to changes in ventilator support. A modelling approach

S Larraza[1*], N Dey[2], DS Karbing[1], M Nygaard[2], R Winding[2], SE Rees[1]

[1]Aalborg University, Department Health Science and Technology, Aalborg, Denmark; [2]Regions Hospital Herning, Department of Anaesthesia and Intensive Care, Herning, Denmark

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P15

Introduction: Understanding patients' response to changes in support ventilation is difficult. Reducing support may result in different patterns of response including changes in depth and frequency of ventilation, possible fatigue and changes in metabolism due to increased respiratory work.
Objectives: Present and evaluate a mathematical model approach describing patients' response to changes in ventilator support.
Methods: A set of mathematical models including pulmonary gas exchange, acid-base chemistry of blood and cerebrospinal fluid, blood oxygenation, O_2 and CO_2 transport and storage, ventilation, muscle function and chemoreflex respiratory drive were combined. The set of models was used to simulate changes in support during volume and pressure support ventilation. The model set was evaluated in 12 and 5 patients on volume and pressure support respectively. Ethical approval was obtained from the committee of Mid-Jutland, Denmark. Patient or relatives and general practitioners consent was obtained.
For each patient, measurement of pulmonary gas exchange was followed by a series of up to 5 step modifications in the level of ventilator support, with these steps being 50 ml, for volume support, or 2 cm H_2O for pressure support. Each level was maintained for 15 minutes, and arterial blood gases measured. Airway pressure, flow, CO_2 and O_2 were measured continuously throughout the protocol.
The model set was tuned to the data of each patient at baseline conditions. Simulations of patient response at each level of ventilator support were then performed. Agreement between model simulated values and measurements of arterial pH, end tidal CO_2 ($FeCO_2$) and respiratory frequency (f) are reported as Bland-Altman bias and limits of agreement.
Results: Volume support levels ranged from 0.26-0.65 l and pressure support from 0-14 cmH_2O. The resulting values of respiratory frequency ranged from 10-42 min^{-1}, pHa from 7.29-7.50 and $FeCO_2$ from 2.2-7.7 %. The bias and limit of agreement between model predicted and measured values across all patients and at all levels of volume and pressure support were: f 0.0±1.3 min^{-1}, pHa -0.001±0.03 and $FeCO_2$-0.00±0.002 %.
Discussion: The model describes accurately patient response to changes in ventilator support, identifying the individual patient's chemoreceptor drive and situations where the patient could not respond adequately, resulting in changes in pH. The model description may help to describe patients' response to changes in ventilator support and hence optimize mechanical ventilation for the specific patient.

References

1. Georgopoulos D, Roussos C: *Eur Respir J* 1996, **9**:2151-2160.
2. Duffin JJ: *Appl Physiol* 2005, **99**:2255-2265.
3. Gilstrap D, MacIntyre N: *Am J Respir Crit Care Med* 2013, **188**:1058-1068.

NON INVASIVE VENTILATION

P16

0232. Evaluation of HFNC'S wash out effect; a comparison of open- and closed-mouth models

N Nakamura[*], M Kurota, T Watanabe, Y Onodera, H Suzuki, M Nakane, K Kawamae

Yamagata Unversity Faculty of Medicine, Anesthesiology and Intensive Care Medicine, Yamagata, Japan

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P16

Introduction: Although clinical studies of the high-flow nasal cannula (HFNC) and its effect on positive end-expiratory pressure (PEEP) have been performed, the mechanism of the washout effect and its relation with HFNC flow have not been well evaluated. Therefore, we made a respiratory model that can exhale with controllable end-tidal PCO_2 ($P_{ET}CO_2$) to evaluate the washout effect of HFNC.Objective. To evaluate the quantitative results of HFNC's washout effect comparing open- and closed-mouth models.
Methods: Optiflow™ (Fisher and Paykel Healthcare, Auckland, NZ) was used as the HFNC system. The artificial respiratory model consisted of a lung model (the Dual Adult Training and Test Lung, Michigan Instruments Inc., Grand Rapids, MI, USA) and a ventilator (Puritan Bennett™ 840, Covidien, Dublin, Ireland). The HFNC and the respiratory model were connected by the airway model (Endotracheal Intubation Training Model LM-059, Koken Co., Ltd., Tokyo, Japan). Respiratory settings were as follows: respiratory rate, 16 breaths/min; inspiratory time, 1 second; and tidal volume (V_T), 300, 500, or 800 mL. CO_2 was infused into a distal site of the lung model to maintain $P_{ET}CO_2$, measured just below the glottis, at 40 mmHg at each V_T setting without HFNC. HFNC flow was changed from 10-60 L/min in each V_T setting, and the change of $P_{ET}CO_2$ was measured in the open- and closed-mouth models.
Results: With any V_T setting in the open-mouth model, $P_{ET}CO_2$ quickly decreased to 20-25 mmHg as HFNC started at 10 L/min. Thereafter, $P_{ET}CO_2$ did not change with an increasing HFNC flow (Figure: solid lines). With the closed-mouth model, $P_{ET}CO2$ gradually decreased as the HFNC flow was increased. The V_T settings of 300 and 500 mL had the same trends and reached the bottom level of 22 mmHg with HFNC flow over 50 L/min. The V_T setting of 800 mL had a smaller decrease in $P_{ET}CO_2$ to 28 mmHg (Figure 1: dotted lines).

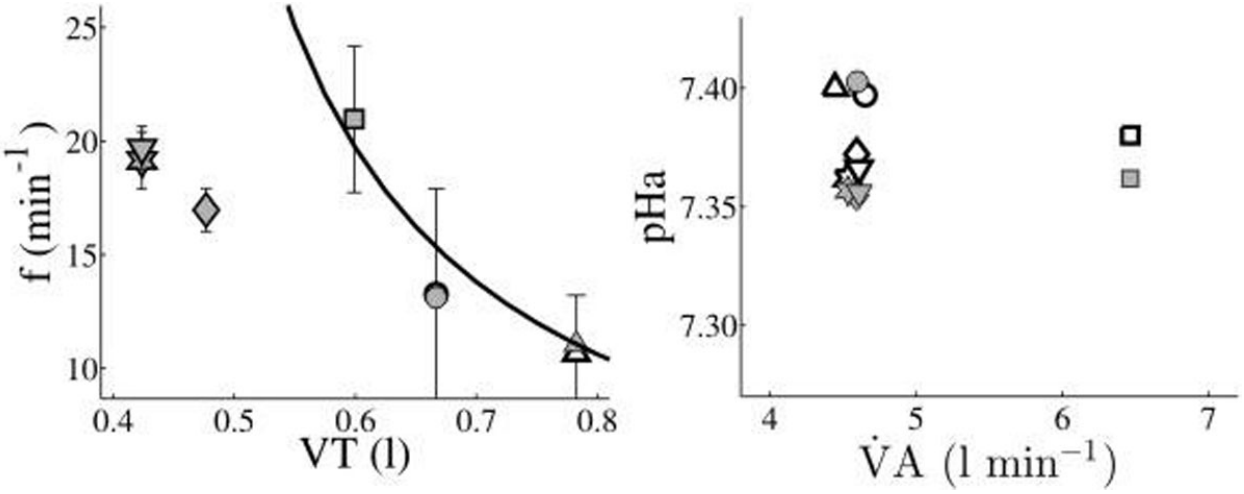

Figure 1(abstract P15) Patient response to changes in PS ventilation. Symbols represent each level of PS. Blank symbols represent measurements. Filled symbols represent model simulated values.

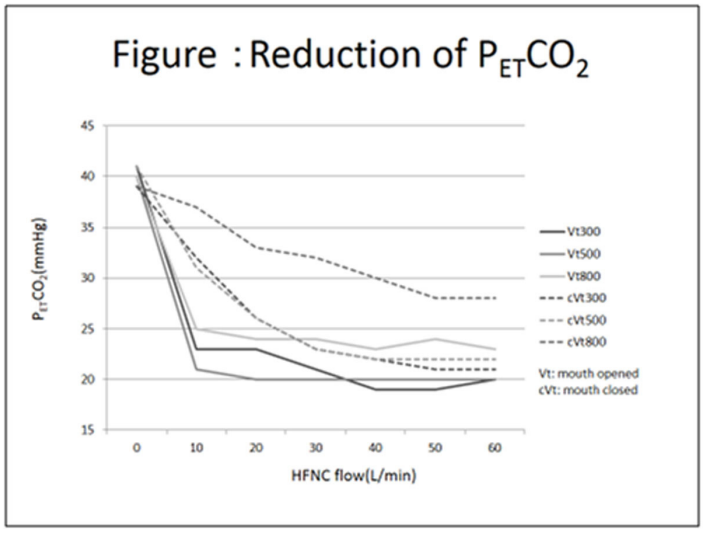

Figure 1(abstract P16) Reduction of $P_{ET}CO_2$

Discussion: Generation of PEEP by HFNC needs high flow as 35 L/min to generate PEEP of 3 cmH_2O[1]. In this study, it was demonstrated that HFNC's washout of the dead space is effective with relatively low flow as low as 10 L/min in open-mouth model. HFNC flow of 10 L/min can deliver gas of 166 mL/min, and this amount of gas delivery was thought to be enough to wash out the dead space during the exhalation time. The effect was weaker in the closed-mouth model, but by increasing the HFNC flow produced an adequate effect. In this closed-mouth model, more gas leaked from the nostril instead of the mouth, and therefore, less gas washed out the dead space, which caused a need for more HFNC flow to lower the $P_{ET}CO_2$.
Conclusions: We concluded that the washout effect depends on HFNC flow especially with closed-mouth breathing while it may reach maximum with a relatively low flow of 10 L/min with open-mouth breathing.
Grant acknowledgment: No conflict of interest.
Reference

1. Parke R: **Nasal high-flow therapy delivers low level positive airway pressure.** *Br J Anaesth* 2009, **103**:886-890.

SEPSIS BIOMARKERS

P17

0295. Induction and repression effects of heat shock (HS) and LPS and modulatory effects of glutamine on blood mononuclear cells -hsprotein-72 from icu patients with severe sepsis, trauma and healthy controls

E Briassouli[1*], M Tzanoudaki[2], G Daikos[1], K Vardas[3], M Kanariou[2], C Routsi[3], S Nanas[3], G Briassoulis[4]
[1]1st Department of Propaedeutic InternalMedicine, University of Athens, Athens, Greece; [2]Department of Immunology - Histocompatibility, Specialized Center & Referral Center for Primary Immunodeficiencies - Paediatric Immunology, "Aghia Sophia" Children's Hospital, Athens, Greece; [3]First Critical Care Department, University of Athens, Evangelismos Hospital, Athens, Greece; [4]PICU, University of Crete, University Hospital, Heraklion, Greece
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P17

Introduction: In severe sepsis (SS) or trauma-related systemic inflammatory response syndrome (SIRS), induction of heat-shock-protein-72 (HSP72) may protect cells from stress.
Objectives: We compared the heat-shock (HS) with the lipopolysaccharide (LPS) induction/repression effect on HSP72 of peripheral blood mononuclear cells (PBMCs) in SS or SIRS patients and healthy-controls (H) and investigated any possible modulating glutamine (Gln) effect.
Methods: PBMCs from 16/H, 11/SS, and 7/SIRS were incubated with 1μg/ml LPS or 43° HS vs. no stimulation for 4h. In each group 3 experiments involved L-Ala-Gln10mM incubation 1h before (Gln-b) or after (Gln-a) induction, or no glutamine (1088 measurements). Intracellular Mean Fluorescence Intensity (MFI) levels of monocytes (mHSP72) or lymphocytes (lHSP72) were determined using Flow Cytometry.
Results: In H-PBMCs, LPS did not affect mHSP72 (79±10 MFI vs. 78±13) or lHSP72 (7±1.7 vs. 7±2). HS induced mHSP72 (454±60, $p < 0.0001$) and lHSP72 (41±7, $p< 0.0001$) with or without Gln ($p< 0.0001$). Basal mHSP72 was higher in SIRS compared to H (144±25 vs. 78±10, $p < 0.03$). A HS-induction effect on SIRS-mHSP72 (394±108, $p < 0.04$) and lHSP72 (37±5, $p < 0.02$) was further enhanced by Gln-b (495±114, $p < 0.01$ and 58±14, $p< 0.04$). LPS suppressed SIRS-mHSP72 (120±54 vs. 144±25, $p < 0.02$) especially in the Gln-b group (107±19, $p < 0.02$). Basal Gln-b mHSP72 in SS was higher compared to H (112±16 vs. 69±10, $p < 0.03$). In SS-PBMCs HS, but not LPS, induced mHSP72 (492±56 vs. 108±19, $p < 0.003$). LPS repressed the SS-lHSP72 (10±2 vs. 17±2, $p < 0.007$) an effect attenuated by Gln-b (13±5).
Conclusions: Heat shock greatly induces mHSP72 and lHSP72 of ICU patients' PBMCs. LPS may repress lHSP72 in septic or trauma patients. Glutamine pre-treatment may either enhance HS-induction or LPS-repression on mHSP72 or attenuate LPS-repression on lHSP72 in SS and SIRS groups.
Grant acknowledgment: This research has been co-financed by the European Union (European Social Fund (ESF)) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF)-Research Funding Program: THALES.

MULTI-DRUG RESISTANT BACTERIA

P18

0329. Beneficial effects of azithromycin combined with ceftazidime without activity against P. aeruginosa in a murine sepsis model of peritonitis by Pseudomonas aeruginosa

ME Pachón-Ibañez[1], A Díaz-Martín[2*], J Dominguez-Herrera[1], G Labrador[1], Y Smani[1], J Pachón[1], J Garnacho-Montero[2]
[1]UCEIMP/IBIS-University Hospital Virgen del Rocío, University of Seville, Seville, Spain; [2]University Hospital Virgen del Rocio, Intensive Care Unit, Seville, Spain
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P18

Introduction: Despite a proper management, the mortality of patients with severe sepsis and septic shock is very high. During sepsis, an uncontrolled cascade of inflammatory mediators is triggered, leading to tissue damage that can cause death. Macrolides, apart from their antibiotic properties, are capable of modulating inflammatory response. Macrolides inhibit the production and secretion of pro-inflammatory cytokines (IL-1, IL-6, IL-8 and TNF-α) levels and increase anti-inflammatory cytokines such as IL-10.
Objectives: To demonstrate whether the use of azithromycin (AZM) in a murine sepsis model of *P. aeruginosa* increases ceftazidime (CFZ) efficacy.
Methods: MIC/MBC and bactericidal activity (time-killed curves using 1MIC) were performed using four clinical isolates of *P. aeruginosa*. The bactericidal activity on Pa4, chosen for the *in vivo* study, was carried out too at the maximum plasma concentration (Cmax) in mice. Pharmacokinetic/pharmacodynamic (PK/PD) parameters of CFZ and AZM were calculated (HPLC-MS/MS). The activity of each antimicrobial agent and its combination was assessed in a murine peritonitis model (n=15), using the minimum lethal dose (MLD) of Pa4 as inoculum. Treatment were: 100 mg/kg/ip/12h for CFZ and 30 mg/kg/ip/24h for AZM, during 72 hours; a group of 15 untreated mice were used as control (CON). CFU/g spleen, mortality and blood cultures were compared.
Results: The MIC/MBC (mg/L) ranges of CFZ were 2-4/2-32 and for AZM 64/128->128. In time-kill curves no bactericidal activity was observed with any of the strains at concentration of 1MIC. At concentration of Cmax, CFZ +AZM reached bactericidal activity, but not synergy was found. PK/PD results are shown in table 1. In the animal model (table 2), CFZ and CFZ+AZM were better than CON and AZM, and the combination better than the CFZ and AZM ($p< 0.001$, ANOVA, post hoc tests) respect to the CFU/g spleen and the blood cultures. No differences were found in the mortality rates (chi-square test). In the survival analysis, CFZ+AZM delayed the mortality compared with the other groups ($p< 0.001$, Kaplan-Meier).

Table 1(abstract P18) PK/PD results

		CFZ	AZM
PK	**Cmax (μg/mL)**	107.14	4.57
PK	**Tmax (h)**	1.08	2.14
PK	**AUC0-∞ (μg*h/L)**	126.83	3.81
PD	**Cmax/MIC**	107.14	0.07
PD	**T1/2/MIC**	1.08	0.02
PD	**AUC0-∞/MIC**	126.83	1.98

Table 2(abstract P18) In vivo studies

	CON	CFZ	AZM	CFZ +AZM
Log UFC/g Spleen (Mean ± SD)	8.40 ± 0.32	6.57 ± 0.61	7.58 ± 0.31	4.63 ± 0.77
Positive blood culture (%)	100	47	100	0
Exitus (%)	100	100	100	100

Conclusions: The combination of AZM with CFZ increases bacterial clearance from spleen and blood and delays the time to mortality in a murine sepsis model by *P. aeruginosa*.
Grant acknowledgment: This project (PI10/01563) was funded by the "Fondo de Investigación Sanitaria", Instituto de Salud Carlos III.

CARDIAC ARREST: CPR & BEYOND

P19

0353. Do the noble gases helium and argon exert neuroprotective effects in a rodent cardiac arrest model?

P Zuercher[1*], D Springe[1], A Putzu[1], D Grandgirard[2], S Leib[3], SM Jakob[1], J Takala[1], M Haenggi[1]
[1]University of Bern - Inselspital, Dept. of Intensive Care Medicine, Bern, Switzerland; [2]University of Bern, Institute for Infectious Diseases, Neuroinfection Laboratory, Bern, Switzerland; [3]Federal Office for Civil Protection, Biology Division, Spiez Laboratory, Spiez, Switzerland
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P19

Introduction: The noble gas xenon exerts neuroprotective effects after various insults, but availability of xenon is limited. Helium and argon are readily available noble gases, but the results of studies using helium as neuroprotective measure are mixed, and the effects of argon are less well studied. In a recent cardiac arrest (CA) study, 70% argon (in O2) administered for 1 hour post CA improved outcome [1].
Objectives: We examined the effect of prolonged administration of both gases as neuroprotective agents in a rat CA model.
Methods: 8-min asystolic cardiac arrest (intravenous KCl in esmolol) was induced in 48 male Wistar rats. 9 rats died during the operation/CA procedure, the remaining 39 were randomized into 3 groups: helium (h) (CA, 24 hours in a chamber with helium/O2 mixture of 50:50, starting 15 min after ROSC, n= 10), argon (a) (CA, 24 h in argon/O2 50:50, n=10) and control (c) (CA, 24 hours in O2/N2 50:50, n=11), 8 animals with surgery only served as sham animals (not randomized). 2 rats in the c group died after 7 h and 4.5 days. All surviving animals were euthanized at day 5.
The rats were assessed preoperatively and then daily until day 5 by a behavior score for rats, a Neuro-Deficit score and a Tape-Removal-Test. On day 0, 4 and 5 locomotor activity was recorded in an Open Field Test.

Table 1(abstract P19)

	helium/O2 n=10	argon/O2 n=10	air/O2 n=9	sham n=8	Sig./ Post hoc p<0.05
CV - pyknotic cells in CA1 [%]	49 [IQR 21 - 66]	52 [36- 86]	82 [50 - 92]	0 [0 - 1]	p<0.01 / sham vs all
CA1 cell layer (normalized: surface/length) [mm2/mm]	0.074 [0.060 - 0.084]	0.076 [0.059- 0.096]	0.067 [0.046- 0.089]	0.087 [0.081- 0.100]	p<0.01 / sham vs all
Fluoro-Jade [numbers of stained cells per mm]	148 [140 - 166]	154 [124 - 199]	145 [138 - 182]	0 [0 - 0]	p<0.01 / sham vs all

The animals were then euthanized for harvest of brain for histology in the hippocampus cornus ammonis segment CA1, assessed with cresyl violet (CV) and Fluro-Jade (FJ) staining.

Results: : CV staining demonstrated an absence of pyknotic cells in the sham group, compared to (h), (a) and (c), but the differences between the resuscitated rats were not significant (see table). This resulted in a non-significant decrease of the CA1 cell layer in the resuscitated animals. FJ staining demonstrated no cell damage in the sham group, but significant injury in the (h), (a) and (c) groups. Again, there was no significant difference between the resuscitated animals. No difference could be found in the neuropsychological and functional tests within the CA groups.

Conclusions: In our 8 minutes cardiac arrest model with mild neuro-behavioral damage neither the noble gas helium nor argon administered as a gas mix with 50% oxygen had a significant positive clinical effect. Post hypoxic-ischemic cell injury in the hippocampal CA1 segment did not differ between the helium, argon and control groups. Whether the neuroprotective effect of helium and argon is dose dependent remains open.

Grant acknowledgment: Supported by the departmental funds

Reference

1. Brucken A, Cizen A, Fera C, Meinhardt A, Weis J, Nolte K, Rossaint R, Pufe T, Marx G, Fries M: **Argon reduces neurohistopathological damage and preserves functional recovery after cardiac arrest in rats.** *Br J Anaesth* 2013, **110(Suppl 1)**:i106-112, DOI 10.1093/bja/aes509.

CARDIAC ARREST: CPR & BEYOND

P20

0354. Effects of sodium nitroprusside in addition to therapeutic hypothermia after experimental cardiac arrest

K Donadello[1*], FS Taccone[1], F Su[1], K Hosokawa[1], L Gottin[2], J Creteur[1], D De Backer[1], J-L Vincent[1]

[1]Erasme University Hospital, Intensive Care Department, Brussels, Belgium; [2]University of Verone, School of Medicine, Policlinico G.B. Rossi, Intensive Care Department, Verone, Italy

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P20

Introduction: Sodium nitroprusside (SNP) has been shown to provide additional protective effects when combined with therapeutic hypothermia (TH) in some experimental models of cardiac arrest (CA) [1,2,3].

Objectives: To determine whether the addition of SNP to TH has beneficial effects on the brain in a porcine model of CA.

Methods: We studied 8 anesthetized, invasively monitored and mechanically ventilated domestic pigs, randomized into two groups (n=4): TH without or with SNP. After 3 min of untreated ventricular fibrillation, cardiopulmonary resuscitation (CPR) was started in all animals and continued until return of spontaneous circulation (ROSC); defibrillation was performed 3 minutes after the start of CPR. Hypothermia (34±1°C) was induced at the start of CPR using a rapid IV infusion of 30 mL/kg cold saline for 60 min, trans-nasal evaporative cooling (Rhinochill, Benechill Inc, USA) and surface cooling with ice packs. Cooling was maintained for 6 hours, followed by controlled slow rewarming to baseline temperature with blankets. SNP+TH animals received 3 bolus injections of 1 mg of SNP after 2, 7 and 12 minutes of CPR. Brain temperature was measured with intraparenchymal probes (Licox CC1.SB, Integra, NeuroSciences Ltd., Hampshire, UK), blood brain flow by laser Doppler (blood laser Doppler [BLD], MNP100XP, Oxyflow, Oxford Optronix, Oxford, UK) and the lactate-pyruvate ratio (LPR) was measured hourly by microdialysis (CMA20, CMA, Sweden). After left craniectomy, the microvascular network of the frontal cortex was evaluated using sidestream dark-field videomicroscopy (Microscan, MicroVision Medical, Netherlands) at baseline (T0), 1 hour after cooling induction (T1), at the end of hypothermia (T2) and after rewarming (T3). The mean flow index (MFI) and the proportion of perfused cerebral small vessels (PPV) were calculated using standard formulas.

Results: Time to return of spontaneous circulation was similar in both groups (8 [7-23] min for TH alone and 9 [6-22] min for TH+SNP). Despite its known vasodilatory effects, there were no significant differences in measured hemodynamic parameters between the groups throughout the study period. Microvascular perfusion was significantly reduced after CA in both groups, but to a lesser extent in the TH-SNP than in the TH group. The LPR was lower in the TH-SNP than in the TH group (Result Table 1).

Conclusions: In this model, the cerebral microcirculation was significantly altered after CA; addition of SNP to TH attenuated the microvascular alterations and had a protective effect on brain metabolism.

Grant acknowledgment: Fonds Erasme. Bourse de Recherche 2013-2014.

References

1. Donadello K, *et al*: *CCM* 2012, **40(12 suppl)**:545.
2. Schultz J, *et al*: *Resuscitation* 2012, **83**:374-377.
3. Yannopoulos D, *et al*: *Resuscitation* 2013, **84(8)**:1143-9.

Table 1(abstract P20)

Time	T0	T0	T1	T1	T2	T2	T3	T3
Study Group	TH	TH-SNP	TH	TH-SNP	TH	TH-SNP	TH	TH-SNP
Heart rate, bpm	73.7 ± 9.3	73.5 ± 11.5	86.5 ± 7.6	89.5 ± 7.4	55.8 ± 11.8	55.5 ± 6.4	83.3 ± 9.8	80.6 ± 4.3
Mean arterial pressure, mmHg	113.3 ± 8.4	112.0 ± 5.4	103.3 ± 8.6	94.5 ± 8.4	86.8 ± 18.1	79.0 ± 9.4	82.8 ± 10.3	90.3 ± 3.9
PPV, %	86.8 ± 3.4	86.5 ± 2.7	39.5 ± 12.6$	45.3 ± 6.3$	39.6 ± 14.2$	49.8 ± 13.2$	56.3 ± 6.8$	65.5 ± 6.7$
MFI	2.8 ± 0.1	2.8 ± 0.1	1.9 ± 0.1$	2.0 ± 0.1$	1.7 ± 0.1$	1.9 ± 0.2$	2.3 ± 0.2$	2.7 ± 0.1*$
BLD (%/baseline)	100	100	42.1 ± 1.9$	51.3 ± 3.5$	52.8 ± 2.9$	53.6 ± 2.5$	90.7 ± 0.6$	92.5 ± 2.1$
LPR	12.5 ± 1.8	13.9 ± 4.1	16.3 ± 4.1	19.7 ± 1.7	31.7 ± 5.4$	24.3 ± 3.3$	61.1 ± 13.8$	40.5 ± 1.3*$

(*= p< 0.05 versus TH-SNP;$ = p< 0.05 versus T0).

P21

0355. Optimal chest compression technique for pediatric arrest victims

MJ Kim, YS Park*
Yonsei University College of Medicine, Department of Emergency Medicine, Seoul, Korea, Republic of
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P21

Introduction: In pediatric cardiopulmonary resuscitation (CPR), high-quality chest compressions (CCs) are essential to successful resuscitation, although most pediatric arrests are caused by respiratory arrest. Therefore, current guidelines emphasize deeper and faster CCs, with complete chest recoil between contractions.[1, 2] These guidelines further indicate that rescuers may use a one-hand or two-hand technique for pediatric compressions, thereby allowing rescuers to adapt the technique to the victim's size and rescuer's strength. However, no previous studies have examined the quality of CCs, including the use of deeper and faster CCs with complete chest recoil emphasized in the guidelines. Additionally, no study has assessed compression quality of the one-hand technique comparing the right and left hands.
Objectives: The aim of this study was to assess the quality of CCs performed by inexperienced rescuers using three different techniques: two-hand (2H), right one-hand (R1), and left one-hand (L1).
Methods: We performed a prospective, randomized, crossover study in a simulated 6-year-old pediatric manikin model. Each participant performed 2-minute continuous CCs, using three different techniques (2H, R1, and L1). CC quality data, including compression rate, compression depth, and leaning, were collected and analyzed. To examine trends in CC performance over time, each 2-minute period was divided into six consecutive 20-second epochs.
Results: The 36 participants completed 108 two-minute trials, consisting of a total of 25,030 CCs. The mean compression rates (95% confidence interval [CI]) were as follows: 2H1, 116.8 compressions/minute (111.7-121.9); L1, 115.0 compressions/minute (109.9-120.1); and R1, 115.5 compressions/minute (110.4-120.6) ($p=0.565$). The mean compression depth for 2H was 38.7 mm (37.1-40.2), which was higher than for L1 (36.3 mm [34.8-37.9]) or R1 (35.4 mm [33.9-37.0]) ($p< 0.001$). The leaning rate was higher with 2H than L1 or R1 ($p=0.021$). Chest compression depth per 20-second epoch declined over time, regardless of the technique ($p< 0.001$). The pattern of compression depth change over time was similar for all techniques ($p>0.999$).
Conclusions: For pediatric cardiopulmonary resuscitation by inexperienced rescuers, the two hand CC technique has the advantage of producing deeper compressions than the one-hand technique, but it is accompanied by more frequent leaning. For the one-hand techniques, the right and left hand produced CCs of similar quality.
Grant acknowledgment: None.
References

1. Biarent D, Bingham R, Eich C, *et al*: **European Resuscitation Council guidelines for resuscitation 2010 section 6. paediatric life support.** *Resuscitation* 2010, **81**:1364-88.
2. Berg MD, Schexnayder SM, Chameides L, *et al*: **Part 13: Pediatric basic life support: 2010 American Heart Association guidelines for cardiopulmonary resuscitation and emergency cardiovascular care.** *Circulation* 2010, **18 Suppl 3**:S862-75.

P22

0356. Effect of the neuroprotective p53-inhibitor pifithrin-μ in a rodent cardiac arrest model

D Springe[1*], A Putzu[1], P Zuercher[1], D Grandgirard[2], S Leib[3], SM Jakob[1], J Takala[1], M Haenggi[1]
[1]University of Bern - Inselspital, Dept. of Intensive Care Medicine, Bern, Switzerland; [2]University of Bern, Institute for Infectious Diseases, Neuroinfection Laboratory, Bern, Switzerland; [3]Federal Office for Civil Protection, Biology Division, Spiez Laboratory, Spiez, Switzerland
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P22

Introduction: Experimental studies have shown that pifithrin-μ has neuroprotective properties by inhibiting the tumor suppressor p53, which is a key regulator of apoptotic cell death [1]. So far no animal experiments with pifithrin-μ have been carried out in the field of cardiac arrest (CA).

Table 1(abstract P22) Histology (day 5)

	pifithrin (n=11)	control (n=11)	p
Number of pyknotic cells in CA1 [%; Mean ± SD]	25 ± 17	38 ± 21	0.11
CA1 cell layer - normalized surface/length [mm2/mm; Median (IQR)]	.060 (.054 - .075)	.053 (.052-.062)	0.10
Fluoro Jade [numbers of degenerating cells per mm; Mean ± SD]	94 ± 47	128 ± 37	0.08

Objectives: We hypothesized that treatment with pifithrin-μ inhibits delayed neurodegeneration in our 8 min CA model.
Methods: 8-min asystolic CA (intravenous KCl in esmolol) was induced in 80 male Wistar rats. 5 rats died during the operation/CA procedure, the remaining 75 were randomized into 2 main groups: control (c) (8-min CA, 10ml/kg solvent (4% dimethylsulfoxamide/phosphate buffered saline) ip after ROSC) and pifithrin (p) (8-min CA, 8mg/kg pifithrin-μ in 10ml/kg solvent ip). 1 animal (p) died after 48 hours. 12 c + 11 p animals were euthanized on day 1, 11 c + 11 p on day 5 and 12 c + 11 p after day 10. 6 Sham operated animals were euthanized on day 1 (n=3) and after day 10 (n=3), but not included into statistics.The rats were assessed daily from preoperative to day 5 and again on day 10 by a behavior score for rats, a neuro-deficit score and a tape-removal-test. On day 0, 4 and 5 the locomotor activity was recorded in an open field test and after day 10 the remaining rats were tested for learning capacities in the water maze experiment. Harvesting of brain for histology of the hippocampus cornus ammonis segment CA1, assessed with cresyl violet (CV) and Fluro-Jade (FJ) staining, was performed on day 1 and day 5.
Results: After a single dose of 8 mg/kg pifithrin-μ or solvent virtually no apoptosis could be detected after 24 hours in each group. On day 5, we found a trend towards a decreased number of pyknotic cells (CV staining), a tendency towards a preserved hippocampal cell layer and a trend towards less neuronal degeneration (FJ staining) in the (p) compared with the (c) group (see table 1).
No difference between the (p) and the (c) group was found in the behavior score, the neuro-deficit score and the tape-removal-test. The open field and the water maze test did not reveal a difference between the groups either.
Conclusions: In this 8-min CA model with only mild neurobehavioral damage pifithrin- μ does not bring any clinical benefit despite a trend towards less histological damage. Further studies with longer cardiac arrest times (more severe neuronal damage), different dosages and application routes of pifithrin- μ are planned.
Grant acknowledgment: Supported by the departmental funds
Reference

1. Nijboer C, Heijnen C, van der Kooij M, Zijlstra J, van Velthoven C, Culmsee C, van Bel F, Hagberg H, Kavelaars A: **Targeting the p53 Pathway to Protect the Neonatal Ischemic Brain.** *Ann Neuro* 2011, **1(70)**:255-264.

RISK ASSESSMENT & BIOMARKERS FOR AKI

P23

0371. Temporal changes in systemic and renal inflammation and histology in a 72-hour rat model of faecal peritonitis

N Arulkumaran[1,2,3*], M Sixma[1], E Ceravola[4], R Unwin[2], FW Tam[3], M Singer[1]
[1]University College London, Bloomsbury Institute of Intensive Care Medicine, London, UK; [2]University College London, Centre for Nephrology, London, UK; [3]Imperial College London, Department of Nephrology, London, UK; [4]Major Hospital Niguarda Ca Granda, Intensive Care Medicine, Milan, Italy
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P23

Introduction: Mechanisms underlying sepsis-induced acute kidney injury remain uncertain with inflammation and altered haemodynamics being implicated.

Table 1(abstract P23) Cytokine values

Group	Creatinine (μmol/l)	Serum IL-1β (pg/mL)	Renal IL-1β (pg/μg protein)	Serum IL-10 (pg/mL)	Renal IL-10 (pg/μg protein)	Serum IL-6 (pg/mL)
Naïve	23±2.6	1.0±0.0	135±36	24±30	207±80	0.8±0.4
6hr Sepsis	26±6	1428±1006	267±46	3390±1759	220±56	36347±27646
12hr Sepsis	21±2.5	1289±296	260±57	2939±1236	89±58	15217±13285
24hr Sepsis	30±5	1434±1601	398±87	4716±2682	180±44	8835±16422
48hr Sepsis	31±4	1008±887	318±140	3699±3745	238±121	144±172
72hr Sepsis	27±5	256±294	157±66	1410±1670	168±37	163±333

Objectives: To determine temporal changes in systemic and renal inflammation, and histology, in a 72 hour fluid-resuscitated model of sepsis.
Methods: Tunnelled central venous (for fluid administration) and arterial (for BP monitoring/blood sampling) lines were inserted into male Wistar rats under isoflurane anaesthesia. Sepsis was induced 24h later by i.p. injection of faecal slurry. Fluid resuscitation was commenced at 2h post-slurry. Cardiac output was assessed by echocardiography. Animals (n=6-8 per group) were sacrificed at 6, 12, 24, 48, or 72h with kidneys taken for histological section and cytokine (IL-1β, IL-10 by ELISA) analysis, and blood samples for renal biochemistry, and pro- (IL-1β, IL-6), and anti-inflammatory cytokine (IL-10) analysis by ELISA. Renal histology was assessed by light microscopy for features of tubular injury (dilated tubules, tubular casts, cell necrosis), and cell death using TUNEL stain. Comparison was made against sham-operated controls and naïve animals. Statistics were performed using ANOVA and post-hoc Tukey's test. Values are expressed as means±standard deviation.
Results: Sham animals had values similar to naive animals. In septic animals, serum IL-6 and IL-1β were maximal at 6h; this corresponded with the highest core temperature and tachycardia, and the largest drop in stroke volume. By 24h, serum IL-6 and IL-1β fell, whereas IL-10 levels peaked; this corresponded to recovery of cardiac function and normalization of core temperature. Renal inflammation (peak renal IL-1β and lowest IL-10) was maximal at 24h when serum creatinine also peaked. By 72h, serum creatinine and IL-1β approached baseline values while renal IL-10 reached its zenith. (Table 1) Acute tubular injury was mild and sporadic, with minimal renal tubular cell death.
Conclusions: Temporal changes in renal inflammation and dysfunction lag behind changes in systemic inflammation and cardiac dysfunction. Renal dysfunction, as measured by serum creatinine, corresponds to renal inflammation. Cell death does is not a predominant feature, and does not account for the renal dysfunction seen in this sepsis model.
Grant acknowledgment: NA is supported by the Wellcome Trust.

CARDIAC CRISIS

P24

0391. Comparison of the histopathologic effects on the lungs of two external chest compression devices (lucas versus autopulse) in a swine model of ventricular fibrillation

C Pantazopoulos[1*], I Floros[1], A Mega[1], C Rigas[1], I Pavleas[1], P Vernikos[1], N Archontoulis[1], D Xanthis[1], N Iacovidou[2], T Xanthos[3]
[1]General Hospital of Athens Laiko, Intensive Care Unit, Athens, Greece; [2]University of Athens, Medical School, Neonatology, Athens, Greece; [3]University of Athens, Medical School, M.Sc. Programme in Cardiorespiratory Resuscitation, Athens, Greece
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P24

Introduction: Given the difficulty of performing efficient CPR compressions, technology has turned to automaticity. LUCAS device has a pneumatically driven piston to compress the heart and uses active decompression suction on the upstroke. AUTOPULSE is a load distributing band compressor, that is mechanically actuated and battery driven. It provides both direct compression and semi-circumferential thoracic compression.
Objectives: Lung injury may occur during cardiorespiratory resuscitation with external chest compression devices. Aim of this study is to compare 2 different external chest compression devices (LUCAS and AUTOPULSE) regarding differences in lung injury that they may cause.
Methods: Forty (40) pigs were randomly allocated into 2 groups. Group L (LUCAS), n=20 and Group A (AUTOPULSE), n=20. After anesthesia, ventricular fibrillation was induced. Five minutes post-cardiac arrest without treatment, resuscitation was initiated. After resuscitation, lung biopsy via a mini-thoracotomy was obtained (right lung lower lobe).
Results: Histopathology findings revealed a heterogeneous interstitial infiltrate and vascular congestion in all samples studied. There was no statistically significant difference between the two groups. (P>0.05)
Conclusions: LUCAS and AUTOPULSE devices present no histopathological differences concerning lung injury after cardiorespiratory resuscitation.
References

1. Axelsson C, *et al*: Clinical consequences of the introduction of mechanical chest compression in the EMS system for treatment of out-of-hospital cardiac arrest-a pilot study. *Resuscitation* 2006, **71(1)**:47-55.
2. Ong ME, *et al*: Use of an automated, load-distributing band chest compression device for out-of-hospital cardiac arrest resuscitation. *JAMA* 2006, **295(22)**:2629-37.

VENTILATORY MODES

P25

0427. Respiratory effects of noisy ventilation depend on the etiology of acute respiratory distress syndrome

L Moraes[1*], C Samary[1], RS Santos[1], DS Ornellas[1], CL Santos[1], NS Felix[1], R Huhle[2], P Pelosi[3], M Gama de Abreu[2], PL Silva[1], PRM Rocco[1]
[1]Laboratory of Pulmonary Investigation, Carlos Chagas Filho Biophysics Institute, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; [2]Department of Anesthesiology and Intensive Care Therapy, University Hospital Carl Gustav Carus, Dresden, Germany; [3]IRCCS AOU San Martino-IST, Department of Surgical Sciences and Integrated Diagnostics, University of Genoa, Genoa, Italy
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P25

Introduction: Healthy biological systems are characterized by intrinsic variability of their function, including the respiratory system. However, during pathological conditions, as observed in acute respiratory distress syndrome (ARDS), this biological variability can be lost, and the institution of variable ventilation (noisy, VV) may lead to morphofunctional improvement compared with conventional ventilation (CV). However, it is not known if this beneficial effect is dependent on the etiology of ARDS. In pulmonary ARDS (ARDSp) there is a predominance of lung tissue consolidation, whereas extrapulmonary ARDS (ARDSexp) is associated with alveolar collapse.

Objectives: The aim of the present study was to compare variable ventilation with conventionalventilation in experimental ARDSp and ARDSexp.

Methods: In twenty-four Wistar rats (365±55g), ARDSp and ARDSexp were induced by lipopolysaccharide (LPS) administered either intratracheally (200 μg) or intraperitoneally (1,000 μg), respectively. After 24h, animals were mechanically ventilated with: tidal volume (V_T)=6ml/kg, respiratory rate (RR)=80bpm, positive end-expiratory pressure (PEEP) =0 cmH_2O, and fraction of inspired oxygen (FiO_2)=0.4. Baseline data were collected to evaluate if ARDSp and ARDSexp animals presented similar degree of lung damage. Rats were then randomly assigned to be mechanically ventilated with VV or CV. VV was applied on a breath-to-breath basis as sequence of randomly generated V_T values (n = 600; mean V_T = 6 ml/kg), with 30% of coefficient of variation. After randomization, all animals were ventilatedfor 1h, and lungs were removed for histology.

Results: Variable ventilation led to decreasedrespiratory system and transpulmonary pressures in ARDSp ($p< 0.05$), but not in ARDSexp. Furthermore, in ARDSp, the increment of lung resistance along 1h was minimized in VV compared to CV (7% *vs*. 31%, respectively). Oxygenation increased in VV and CV regardless of ARDS etiology. Nevertheless, animals that underwent VV presented a higher percentage of increase in arterial oxygen partial pressure compared to those that underwent CV (ARDSp, 50% *vs*. 26%; ARDSexp, 100% *vs*. 53%, respectively). In ARDSp, but not in ARDSexp, there was a decrease in collapsed areas in VV compared to CV ($p< 0.001$).

Conclusions: In the present model of ARDSp and ARDSexp, oxygenation improved independent of ARDS etiology, however, respiratory system and transpulmonary pressures as well as collapsed areas reduced only in ARDSp. Therefore, the morphofunctional improvement in animals ventilated with VV is dependent on ARDS etiology, and this achievement could be related to better recruitment.

Grant acknowledgment: PRONEX-FAPERJ, FAPERJ, CNPq, CAPES.

Reference

1. Gama de Abreu M, Spieth P, Pelosi P, *et al*: Noisy pressure support ventilation: a pilot study on a new assisted ventilation mode in experimental lung injury. *Crit Care Med* 2008, **36**:818-827.

P26

0434. Simulation of late inspiratory rise in airway pressure during pressure support ventilation

C-H Yu[1*], P-L Su[2], W-C Lin[2], C-W Chen[2]
[1]National Cheng Kung University Hospital Dou-Liou Branch, Department of Internal Medicine, Yun-Lin, Taiwan, Province of China; [2]National Cheng Kung University Hospital, Department of Internal Medicine, Tainan, Taiwan, Province of China

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P26

Introduction: Late inspiratory rise in airway pressure (LIRAP, ΔPaw/Δt, Fig. 1) caused by inspiratory muscle relaxation or expiratory muscle contraction is frequently seen during pressure support ventilation [1,2], although the factors that modulate LIRAP are unknown.

Objectives: The aim of current study was to determine the factors related to LIRAP during PSV using a simulation lung model. Influence of inspiratory muscle relaxation was assessed under various combinations of lung models and ventilator settings. Influence of expiratory muscle contraction was assessed through its intervention at various time points during the inspiratory phase.

Methods: We investigated the effects of respiratory mechanics (normal, obstructive, restrictive, or mixed), inspiratory effort (-2, -8, or -15 cmH_2O), flow cycling-off criteria (5-40% peak inspiratory flow), and duration of inspiratory muscle relaxation (0.18-0.3 s) on LIRAP during pressure support ventilation using a lung simulator (ASL 5000) and four types of ventilators. A user-defined breath was used to simulate expiratory muscle contraction. Seven breaths were recorded for each scenario.

Results: LIRAP occurred within all lung models when inspiratory effort was medium to high and duration of inspiratory muscle relaxation was short. The normal lung model was associated with the fastest LIRAP (maximum ranged from 24.8 to 46.1 cmH_2O/s in four types of ventilators), whereas the obstructive lung model was associated with the slowest LIRAP (maximum from 11.1 to 25.1 cmH_2O/s, $p < 0.0001$ between different ventilators and different lung models). Unless lung mechanics were normal, LIRAP was unlikely to occur when inspiratory effort was low. Different ventilators were also associated with differences in LIRAP speed ($p < 0.0001$). Lowest ΔPaw/Δt was recorded in PB-840 ventilator and highest ΔPaw/Δt in GE Carestation ventilator in all lung models. Except for within the restrictive lung model, changes in flow-cycling level did not abolish LIRAP if inspiratory effort was medium to high. Increased duration of inspiratory relaxation also led to the elimination of LIRAP. Simulation of expiratory muscle contraction revealed that LIRAP occurred only when expiratory muscle contraction occurred sometime after the beginning of inspiration.

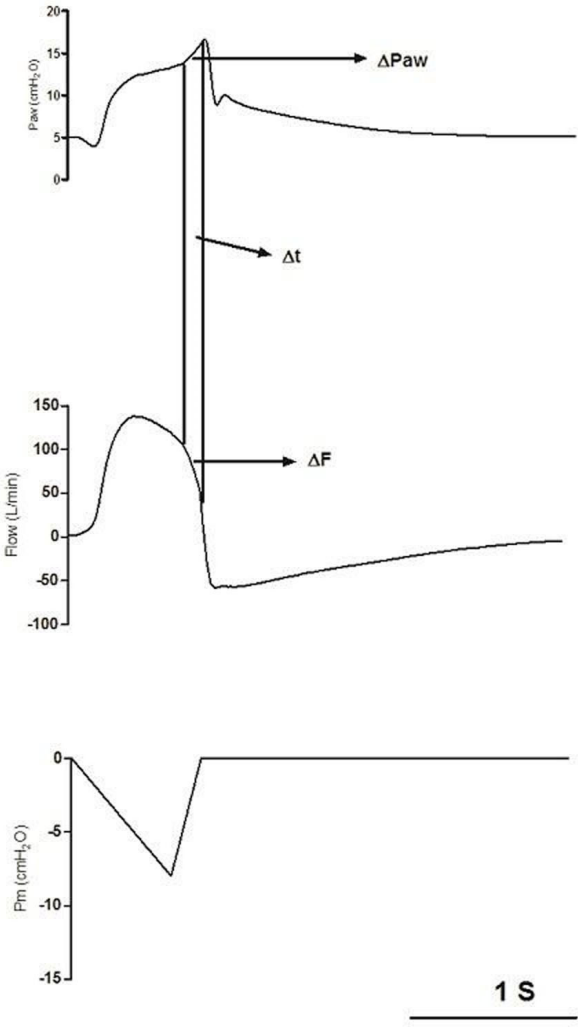

Figure 1(abstract P26)

Conclusions: Our simulation study reveals that both respiratory resistance and compliance may affect LIRAP. Except under restrictive lung conditions, LIRAP is unlikely to be abolished by simply lowering flow-cycling criteria when inspiratory effort is strong and relaxation time is rapid.

References

1. Younes M: Why does airway pressure rise sometimes near the end of inflation during pressure support? *Intensive Care Med* 2008, **34**:1-3.
2. Prinianakis G, Plataki M, Kondili E, Klimathianaki M, Vaporidi K, Georgopoulos D: Effects of relaxation of inspiratory muscleson ventilator pressure during pressure support. *Intensive Care Med* 2008, **34**:70-4.

P27

0435. Pressure-support ventilation compared to pressure-controlled ventilation in experimental emphysema

GA Padilha[1*], I Henriques[1], L Moraes[1], MV Oliveira[1], IP Ramos[1], PJ Miranda[1], LF Horta[1], RC Goldenberg[1], P Pelosi[2], PL Silva[1], PRM Rocco[1]
[1]Federal University of Rio de Janeiro, Carlos Chagas Filho Biophysics Institute, Rio deJaneiro, Brazil; [2]University of Genoa, Department of Surgical Sciences and Integrated Diagnostics, Genoa, Italy
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P27

Introduction: Emphysema is characterized by irreversible enlargement of the airspaces distal to the terminal bronchiole, accompanied by destruction of their walls, and impairment of cardiac function. Acute exacerbation of emphysema leads to increased morbidity, mortality and, in some cases, requirement of invasive mechanical ventilation (MV). So far, no study has compared the impact of pressure-controlled ventilation (PCV) and pressure-support ventilation (PSV) on lung mechanics and histology as well as cardiac function in experimental emphysema.
Objectives: Our aims were
1) to develop an elastase-induced emphysema model in rats that results in lung mechanical and histological impairment and *cor pulmonale*; and
2) to compare the effects of PCV and PSV on lung and heart.
Methods: Thirty-six Wistar rats were randomly divided into 2 groups. In ELA group, rats received porcine pancreatic elastase (2 IU) intratracheally, once a week, during 4 weeks, whereas SAL group was treated with saline. Lung mechanics and histology, as well as echocardiography were analyzed at 4, 6, 8, and 11 weeks. Additional thirty-two animals were treated with saline (n=16) and elastase (n=16) using the same protocol previously described and randomized according to ventilator strategy: pressure-controlled ventilation (SAL-PCV and ELA-PCV, n=8/each) and pressure-support ventilation (SAL-PSV and ELA-PSV, n=8/each). Tidal volume was kept constant (» 6-8 ml/kg) Lung mechanics and echocardiography were performed at the beginning (Initial) and 4 hours MV (Final), after which lungs were removed for morphometric analysis.
Results: Compared to SAL group, ELA animals showed increased specific elastance, functional residual capacity, mean alveolar diameter, and right ventricle area at 6 (61%, 37%, 64%, and 8%, respectively) and 8 weeks (37%, 37%, 40%, and 16%, respectively). At 11 weeks, no significant changes were observed in lung mechanics but Lm (53%) and right ventricle area (20%) remained elevated. Based on these data we opted to compare the mechanical ventilation strategies at 8 weeks. Mean airway pressure ($Pmean_{,aw}$) was lower in ELA-PSV compared to ELA-PCV at the Final ($p < 0.01$). Transpulmonary peak pressure (Ppeak,L) was more reduced in ELA-PSV in Final compared to Initial ($p < 0.05$). PCV resulted in greater areas of alveolar hyperinflation compared to PSV ($p < 0.05$). The ratio between the pulmonary artery acceleration time and the pulmonary artery ejection time (PAT/PET) increased in ELA-PSV (16%) along time, but not in ELA-PCV, suggesting reduction in pulmonary arterial hypertension.
Conclusion: In the current elastase-induced emphysema, we found that: 1) 8 weeks was the time associated to lung mechanical and histological changes and cardiac impairment that resemble human emphysema; and 2) PSV attenuated lung mechanics, hyperinflation, and cardiovascular dysfunction compared to PCV.
Supported by: CNPq, FAPERJ, CAPES, PRONEX, INCT-INOFAR

P28

0438. Effects of positive end-expiratory pressure (PEEP) on the pattern of breathing during neurally adjusted ventilatory assist. A pilot study in a mild ards porcine model

M Pellegrini[1,2*], G Perchiazzi[1], A Ronéus[2], I Andersson[3], T Fiore[1], A Larsson[2], G Hedenstierna[2]
[1]Università di Bari Aldo Moro, Dept. of Emergency and Organ Transplant, Bari, Italy; [2]Uppsala University, Dept. of Surgical Sciences, Uppsala, Sweden; [3]Kalmar Hospital, Dept. of Anesthesia and Intensive Care, Kalmar, Sweden
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P28

Introduction: Spontaneous breathing during mechanical ventilation is still controversial. It is well known that incongruous patterns of mechanical ventilation can trigger or exacerbate lung injury. NAVA is a mode of mechanical ventilation that delivers ventilatory assist in proportion to the Electrical activity of the Diaphragm (EDi), which mirrors the performance of the whole respiratory system. However during NAVA, the effects of PEEP on the EDi signal, respiratory rate and breathing heterogeneity are unknown.
Objectives: The purpose of the present pilot study was to evaluate the effects of PEEP on the ventilatory pattern during NAVA support in a porcine model of mild ARDS.
Methods: Three anesthetized, tracheostomized and spontaneous breathing piglets, underwent NAVA ventilation. Mild ARDS was achieved by repeated lung lavages and bronchial aspiration (targeting a PaO_2/FiO_2 ratio of 200). Each animal was ventilated with four different supports: Optimal NAVA ($NAVA_{OPT}$, defined by using Brander et al. method[1]), Low NAVA ($NAVA_{LOW}$ defined as $NAVA_{OPT}$ -20%), High NAVA ($NAVA_{HI}$ defined as $NAVA_{OPT}$ +20%) and unsupported breathing ($NAVA_{zero}$ defined as NAVA of 0 $cmH_2O/\mu V$). During each pattern of ventilation, a stepwise change in PEEP was applied, first incrementing PEEP from 0 to 15 cmH_2O in steps of 3 cmH_2O; then decrementing it from 15 to 0 cmH_2O with the same steps. During each step, after a steady state was reached, the main hemodynamic and respiratory parameters were recorded and a breath-by-breath analysis of flow, airway pressures and EDi waves, was performed by using custom made Matlab (The Mathworks, Natick, USA) codes. We defined as index of breathing pattern heterogeneity (BPH), the percentage of breaths whose maximum EDi (EDI_{max}) exceeds by 2 standard deviations the mean of EDI_{max} ($EDI_{max,\ mean}$); minimum EDi ($EDI_{min,\ mean}$) is the mean of lowest EDIs reached in a breath sample. Statistical analysis was performed by testing significant differences of respiratory rate (RR), $EDI_{max,\ mean}$, $EDI_{min,\ mean}$ and BPH at each applied PEEP using the Student's T test (α=0.05) at the various NAVA levels.
Results: RR was inversely related to applied PEEP. Increasing PEEP, $EDI_{max,\ mean}$ increases while $EDI_{min,\ mean}$ decreases. Breathing pattern heterogeneity did not change in relation to PEEP variation.
Conclusions: The application of PEEP during spontaneous breathing in a model of mild ARDS influenced the ventilatory pattern, i.e., the respiratory rate, the diaphragmatic tonic ($EDI_{min,\ mean}$) and the phasic contraction ($EDI_{max,\ mean}$). If these effects are valid in patients, they will be clinically important when using NAVA as a weaning mode.
Grant acknowledgment: The School of Anesthesia and Intensive Care of Medicine, Bari University; The Swedish heart and lung foundation.
Reference
1. Brander L, *et al*: *Intensive Care Med* 2009, **35**:1979-89.

THE BODY COMPOSITION & METABOLISM OF ICU PATIENTS

P29

0520. The role of mitochondrial dysfunction in the pathophysiology of icu-acquired weakness

K Jiroutkova[1*], J Ziak[1], A Krajcova[1], M Fric[2], V Dzupa[3], F Duska[1,4]
[1]Charles University, 3rd Faculty of Medicine, Laboratory of Bioenergetics, Prague, Czech Republic; [2]FNKV University Hospital, Intensive Care Unit, Prague, Czech Republic; [3]FNKV University Hospital, Dept. of Trauma and Orthopaedics, Prague, Czech Republic; [4]Nottingham University Hospitals NHS Trust, Adult Intensive Care Unit, Nottingham, UK
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P29

Introduction: Mitochondrial dysfunction (bioenergetic failure) is knownt to contribute to the development of multiorgan failure in acute sepsis. The defect is mainly at the level of respiratory complex I [1,2].
Objectives: To assess whether mitochondrial dysfunction in skeletal muscle perists until protracted critical illness and whether it contributes to the development of ICU-acquired weakness.
Methods: Muscle biopsies were obtained from critically ill patients with severe ICUAW (defined as MRC score < 24 on two separate tests) and from otherwise healthy controls undergoing elective hip replacement. 50-100mg of the native sample was homogenized by a technique which perserves mitochondria in their cytosolic context [3]. Oxygen consumption was measured by high-resolution respirometry (Oxygraph-2Km Oroboros) under two occasions: 1. homogenate enriched with substrates was sequentially treated with an ATPase inhibitor (oligomycine), an uncoupler (FCCP) and KCN. This allowed us to measure oxidative phosphorylation, respiratory chain capacity, proton leak through inner mitochondrial membrane and

Table 1(abstract P29) Activities of respiratory complexes [pmol/nkat]

Data as median (IQR)	Complex I	Complex II	Complex III	Complex IV	Leak	Resp. chain spare capacity
ICUAW (n=6)	22 (16-23)	23 (20-28)	7 (4-8)	67 (62-93)	7 (5-11)	43 (40-46)
Controls (n=5)	21 (20-25)	9 (3-19)	3 (2-4)	59 (42-109)	8 (5-10)	45 (36-73)
p [Mann Whitney	0.860	0.045	0.028	0.715	0.806	0.724

non-mitochondrial O2 consumption. 2. skeletal muscle homogenate enriched with abundant ADP was treated with sequential addition of substrates and inhibitors of complexes I, II, III, and IV, respectively. Integrity of outer membrane was assessed by measuring the response to addition of cytochrome c. Oxygen consumption rate was normalized to citrate-synthase activity and total protein content.

Results: In skeletal muscle of patients with protracted critical illness and severe ICUAW we have found the activity of complex II and III to be >200% of activity of healthy controls. Function of complexes I and IV was identical as were global mitochondrial function/integrity indices.

Upregulation of complex II may represent an adaptive phenomenon as skeletal muscle during critical illness is more reliant on fatty oxidation (which feeds electrons to respiratory chain via complex II), because of insulin resistance and impaired oxidative glucose disposal.

Conclusions: In patients with severe ICUAW we have demonstrated increased activity of respiratory chain complexes downstream to complex I and intact global mitochondrial function. This may represent an adaptation to insulin resistance.

GRANT ACKNOWLEDGEMENT: Supported by IGA NT 12319.

References

1. Carré, *et al*: *Am J Respir and Crit Care Med* 2010, **182**:745-751.
2. Brealey D, *et al*: *Lancet* 2002, **360(9328)**:219-23.
3. Pecinova A, *et al*: *Mitochondrion* 2011, **11(5)**:722-8.

P30

0521. A functional citrulline-arginine-no pathway and nos3 complex is essential to maintain microcirculatory function during endotoxemia

KAP Wijnands[1*], DM Meesters[1], B Vandendriessche[2], JJ Briedé[3], BAP Bessems[4], HMH van Eijk[4], P Brouckaert[2], WAP Buurman[4], A Cauwels[2], M Poeze[1]
[1]Maastricht University Medical Center, Department of Surgery, Maastricht, Netherlands; [2]Ghent University, Inflammation Research Center, VIB, Ghent, Belgium; [3]Maastricht University, Maastricht, Netherlands; [4]Maastricht University, Department of Surgery, Maastricht, Netherlands
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P30

Introduction: Sepsis is associated with high mortality rates as a result of the development of multiple organ failure (MOF). In MOF the competition for the amino acid arginine between the microcirculation and the inflammatory response is important. The endothelial nitric-oxide synthase (eNOS) uses arginine to produce nitric oxide (NO), which results in microcirculatory vasodilatation, whereas arginase and the inflammatory nitric-oxide synthase (iNOS) use arginine in the inflammatory response to produce acute phase proteins and to stimulate the host response. Enhanced arginine consumption combined with decreased arginine *de novo* synthesis from citrulline results in arginine deficiency for eNOS, eNOS uncoupling, and a decreased organ perfusion.

Objectives: To investigate the role of the NOS enzymes during endotoxemia and the effect of citrulline supplementation on the arginine-NO synthesis and microcirculation.

Methods: Wildtype (n= 39), deficient iNOS mice, ($iNOS^{-/-}$; n= 39), deficient eNOS mice ($eNOS^{-/-}$; n= 39) and double knock-out (KO) mice (mice deficient for iNOS and eNOS; n=39) received a continuous LPS (200μg total) infusion for 18 hrs alone or combined with citrulline (37.5mg) for the last 6 hrs. SDF-imaging was used to evaluate the microcirculation in jejunal villi and the NO production in jejunal tissue was determined by *in vivo* NO spin trapping and quantified by electron spin-resonance spectrometry. After the microcirculatory measurements or *in vivo* NO spin trapping, mice were sacrificed, blood and tissues were sampled. Amino-acid concentrations in blood and tissue were measured by High Performance Liquid Chromotography.

Results: LPS infusion significantly decreased plasma arginine availability in all mouse strains. However, in the jejunal tissue of $iNOS^{-/-}$ or $eNOS^{-/-}$ mice this was not accompanied by a decreased intracellular arginine availability. Jejunal NO production was significantly decreased in all mouse strains during endotoxemia, except for $eNOS^{-/-}$ mice. LPS infusion resulted in a significantly decrease in jejunal microcirculation in wildtype and $iNOS^{-/-}$ mice. However, mice lacking a functional eNOS enzyme, as present in $eNOS^{-/-}$ and double KO mice, perfusion in jejunal tissue did not decrease during LPS infusion. Also the beneficial effects of citrulline supplementation during LPS infusion on the microcirculation were not present in $eNOS^{-/-}$ or double KO mice, although citrulline significantly enhanced the plasma arginine availability in all mouse strains. In addition, tissue arginine availability did not increase in citrulline supplemented NOS deficient mice.

Conclusions: Citrulline improves the arginine *de novo* synthesis during sepsis, however to enhance the microcirculation during sepsis, citrulline requires a functional eNOS enzyme. Therefore, future research needs to focus both on improvement of the arginine *de novo* synthesis and maintenance of a functional eNOS enzyme during sepsis.

P31

0531. Cumulative effects of negative energy balance on myocardial deformity and diastolic function during the first week of ICU: a pilot study

A Gómez Blizniak*, DF Matallana Zapata, M Ruiz Bailén, A Morante, E Castillo-Lorente, MD Pola de Gallego, F Ruiz Ferrón, J La Rosa, AM Castillo, L Rucabado Aguilar
Complejo Hospitalario de Jaén, Critical Care Unit, Jaén, Spain
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P31

Objectives: • To evaluate whether a greater negative energy balance (NEB) accumulated during the first week of ICU correlates with worsening in longitudinal Strain (LS) and diastolic function (DF).
• To evaluate whether improvement in the nutritional status (NS) correlates with improvement in LS and DF.

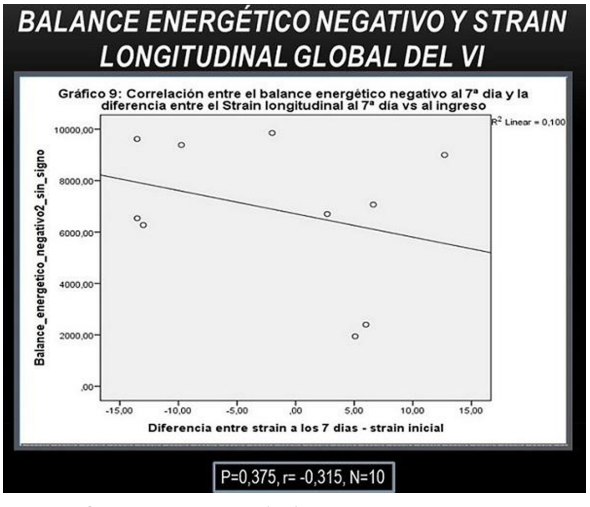

Figure 1(abstract P31) Longitudinal strain AND BEN

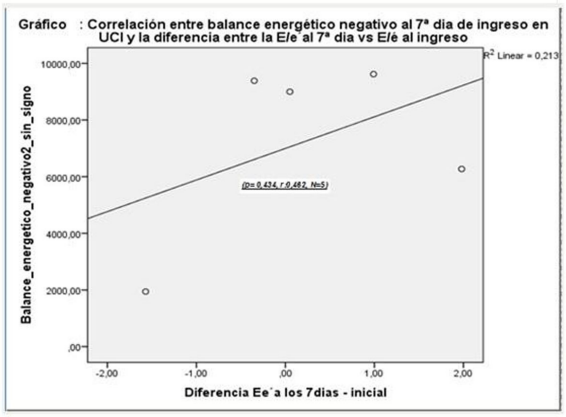

Figure 2(abstract P31) BEN AND DF

Methods: We made an observational, analytical, prospective, longitudinal pilot study.

Dependents variables:

LS, echocardiographic parameter used to assess myocardial deformity (contraction). We considered as an improvement an increase ≥10%.

E/é ratio, parameter used to assess DF. A reduction of E/é ratio ≥10% was considered DF improvement.

I. V,:

NEB during the first week of admision.

Improvement in the NS: assessed by an increase in at least one level of prealbumin nutritional scale (PNS) after 10 days of receiving 100% of estimated energy (EE) requirements (H. Benedict).

(PNS: Normal>18 md/dl, mild undernutrition: 17.9-15 , moderate: 14.9-10 severe <10).

Convenience nonprobability sample.

S. analysis: The results were expressed as means with their ST deviations, %. Linear regression (LR) and Fisher test (FT) were used to analyze possible statistics associations, expressed with their CI and p values.

TTE were performed to patients admitted from July to October, 2013, in the first 24 h of admision, at 7th and 10th days of receiving enteral and/or parenteral nutrition with 100% of EE. Acoustic catches are done in HQ digital format, f.r.> 100 Hz, for further analysis "of line" of LS. (Blind analysis).

Exclusion crit.: nephrotic syndrome, cirrhosis, chronic renal and HF.

PCR, MV (PEEP), PVC were recorded.

Results: 10 patients, 60% male, mean age: 54 (27-75). 30% normal NS, 30% mild, 10% moderate and 30% severe undernutrition. 40% traumatic and 30% spontaneous ICH , 10% thoracic trauma , 10% cardiac arrest and 10% septic shock . 70% required MV, 20% norepinephrine.

KS test: p = 0.595. We observe a tendency to an inverse relationship (**p = 0.375**, r = - 0.315, N = 10) between NEB and LS but not s. significant. 40% of those who had improvement in at least 1 level of the PNS showed a 10% increase in LV LS at 10 days receiving 100% EE (FT: **p = 0.714, OR: 0.667, 95% CI : 0.025 to 18.059**).

As in the Hammer et al study [1], in which acute progressive caloric restriction in young healthy men correlated with impaired DF, we observed a direct relationship (r = 0.462 , p = 0.434 , N = 5) between NEB and E/é, but

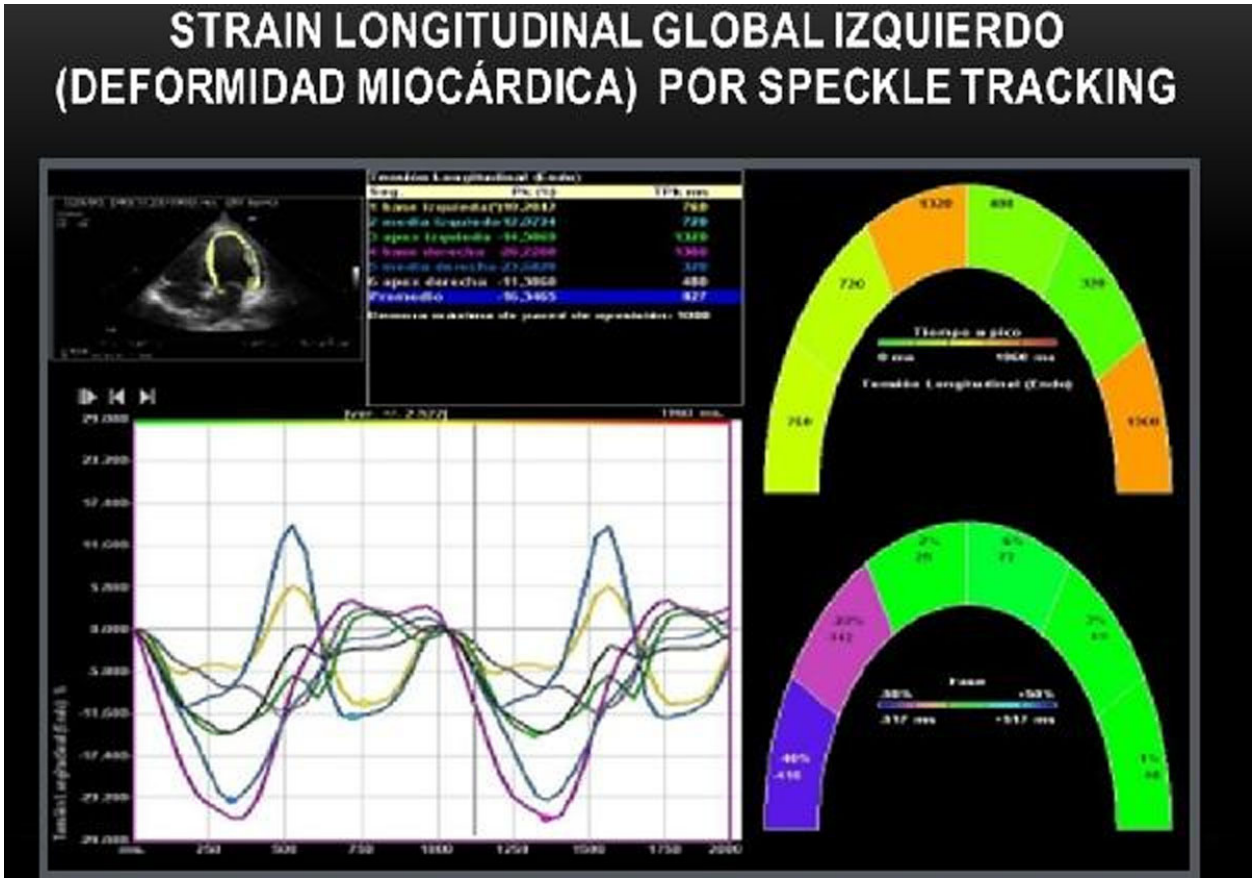

Figure 3(abstract P31) Global LS

not s. significant. The 50% who had an improvement in NS showed a 10% reduction in E /é (FT: **p = 1.00, OR 1.00 , 95% CI : 0.03 to 29**).
Conclusions: Patients with higher cumulative NEB during the first week of ICU had a decrease in LS and an increase in E/é but not s. significant. Given the limitations of this research (being a pilot study of a topic not addressed in ICU with few patients) should be carried further study with sufficient power to test this hypothesis.
Reference

1. Hammer *et al*: Progressive caloric restriction induces dose-dependent changes in myocardial triglyceride content and diastolic function in healthy men. *J Clin Endocrinol Metab* 2008, **93(2)**:497-503.

MONITORING & ASSESSMENT OF RESPIRATORY FUNCTION

P32

0548. Effects of sedation and muscle paralysis on inflammation during mechanical ventilation

LG Ayala[1], M Abreu[1*], M Avila[2], BC Bergamini[1], AC Neto[1], WA Zin[2], A Giannella Neto[1], AR Carvalho[1,2]
[1]Federal University of Rio de Janeiro, Laboratory of Pulmonary Engineering, Rio de Janeiro, Brazil; [2]Federal University of Rio de Janeiro, Carlos Chagas Filho Institute of Biophysics, Rio de Janeiro, Brazil
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P32

Introduction: A significant reduction in mortality was observed in severe ARDS patients when neuromuscular blockage (NMB) was used in the acute phase (Papazian L. et al, 2010).
Objectives: We aim to evaluate pro- and anti-inflammatory markers immediately after two hours ventilation in lung-injured rats with different sedation protocols.
Methods: Thirty male Wistar rats were divided into five groups (6 animals each): low sedation, high sedation (with or without NMB) and a healthy, non-ventilated control group. Animals were anesthetized with 2.8 or 1.4%vol isoflurane and 2.5 or 0.5 mg/kg midazolam in high and low sedation groups, respectively; and 10 mg/kg +10 mg/kg/h atracurium in NMB group. After intubation and stabilization period (baseline settings: VT= 8 mL/kg, FR=70 rpm, FiO2=50%, PEEP=3, I:E = 1:2), lung injury was induced by intratracheal injection of LPS (15 mg/kg) + ventilation induced lung injury (2.5xVT, FR=32, PEEP=10 cmH_2O for 45s) (Dixon et al, 2009). Thereafter animals were ventilated for 2 hours with baseline settings. Airway (Paw), esophagic (Peso) were continuously recorded (200 Hz sampling frequency). The mean power of Peso was then calculated, by means of the spectral density estimation (Welch's periodograms, 400 samples window, 50% overlap). At the end of ventilation, mean arterial pressure (MAP) and gas analysis were evaluated in all ventilated groups. Lungs were extracted for cytokines (IL-6, IL-10) measurement. Euthanasia was performed by exsanguination associated to isoflurane overdose. Data were compared with Shapiro-Wilk, ANOVA and Bonferroni post-hoc tests, $p \leq 0.05$.
Results: No differences in mean MAP and heart rate were observed. PaO_2/FiO_2 and PCO_2 were higher in non-paralized groups compared to paralyzed groups (p=0.01). Peak and mean Paw were significantly lower (p≤0.01) whereas the mean power of Peso was higher (p=0.004) in the low sedation without NMB. IL-6 concentration was also significantly lower in this group (p< 0.001) and presented a negative correlation (r = -0.59, R^2=0.34 and p= 0.002) with Mean power of Peso. Furthermore, IL-10 concentration was higher in the low sedation without NMB compared to the others (p=0.002) and also correlates with the mean power of Peso (r = 0.60, R^2= 0.36 and P = 0.002).
Conclusions: Animals with a superficial sedation scheme in absence NMB might have more recruited lungs (lower Ppeak and Pmean), less inflammation and higher anti-inflammatory markers. The long-term consequences must be further evaluated.
Grant acknowledgment: This work was supported by CAPES, CNPq and FAPERJ.
References

1. Papazian L, Forel J-M, Gacouin A, *et al*: Neuromuscular blockers in early acute respiratory distress syndrome. *N Engl J Med* 2010, **363**:1107-16.
2. Dixon D, De Smet H, Berstena AD: Lung mechanics are both dose and tidal volume dependant in LPS-induced lung injury. *Resp Physiol Neurobiol* 2009, **167**:333-340.

CARDIOVASCULAR DYNAMICS: EXPERIMENTAL

P33

0588. Effects of norepinephrine on tissue perfusion in a sheep model of intraabdominal hypertension

G Ferrara[1], VS Kanoore Edul[1], JF Caminos Eguillor[1], E Martins[1], C Canullán[1], HS Canales[1], C Ince[2], A Dubin[1*]
[1]Facultad de Ciencias Médicas, Universidad Nacional de La Plata, Cátedra de Farmacología Aplicada, La Plata, Argentina; [2]Academic Medical Center, Department of Translational Physiology, Amsterdam, Netherlands
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P33

Introduction: Intraabdominal hypertension (IAH) produces detrimental effects on tissue perfusion. A putative underlying mechanism is the decrease in abdominal perfusion pressure (APP = mean arterial pressure-intraabdominal pressure). Nevertheless, the benefits of increasing blood pressure on tissue perfusion are controversial.
Objectives: To describe the effects of IAH on regional and microcirculatory intestinal blood flow, renal blood flow, and urine output, as well as their responses to increases in blood pressure induced by norepinephrine.
Methods: In 24 anesthetized, mechanically ventilated, and fluid-resuscitated sheep, we measured systemic hemodynamics, left renal and superior mesenteric artery blood flows, villi microcirculation, ileal intramucosal-arterial PCO_2 (ΔPCO_2), and urine output. IAH (20 mm Hg) was generated by intraperitoneal instillation of warmed saline. After 1 h of IAH, sheep were randomized to control (n = 8) or norepinephrine (n = 8) groups for 1 h. In this last group, mean arterial pressure was increased about 20 mm Hg by means of norepinephrine. A sham group (n = 8) was also studied.
Conclusions: In this experimental model of IAH, the gut and the kidney displayed contrasting responses. While intestinal blood flow and villi microcirculation remained unchanged, renal perfusion and urine output were severely compromised. The increase in blood pressure with norepinephrine failed to improve these variables.
Grant acknowledgment: This work was supported by the grant PICT-2010-0495 from Agencia Nacional de Promoción Científica y Tecnológica, Argentina.

P34

0589. Pravastatin exerts opposite effects on splanchnic microcirculatory oxygenation during sham or septic conditions in an animal model of polymicrobial sepsis

C Beck*, F Barthel, A Herminghaus, C Vollmer, I Bauer, O Picker
University Hospital Duesseldorf, Department of Anaesthesiology, Duesseldorf, Germany
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P34

Introduction: In addition to lipid-lowering effects HMG-CoA reductase inhibitors like pravastatin also modulate the microcirculation [1]. The exact mechanisms are yet unknown and results are heterogeneous, with both positive and negative effects on endothelial microvascular function [2,3] being reported.
Objectives: The aim of this study was to evaluate the effects of pravastatin on the microcirculatory oxygenation of the colon in a rodent model of polymicrobial sepsis.
Methods: The data derive from a total of 40 experiments on rats studied with approval of the local animal care and use committee. Pravastatin (0.2 mg/kg) or NaCl were injected subcutaneously 18 h prior to sepsis induction (colon ascendens stent peritonitis) or sham operation. 24 h after induction of sepsis the animals were re-laparotomized under general anaesthesia and received ongoing fluid replacement and pressure-limited ventilation for 120 min. Macrohemodynamic variables were recorded and microcirculatory oxygen supply (μDO_2) and post-capillary oxygen saturation (μHbO_2) of the colon were measured simultaneously via laser Doppler and tissue reflectance spectrophotometry, respectively. Data are presented as means±SD, 2-way ANOVA followed by Dunnett (vs. baseline) or Tukey (between groups).
Results: 1.) In pravastatin pre-treated sham animals the microcirculatory oxygenation μHbO_2 declined by 9.8±9.4% with no change in the NaCl group. Figure 1.

Table 1(abstract P33)

Period	Group	APP (mm Hg)	Cardiac output ($mL.min^{-1}.kg^{-1}$)	Mesenteric flow ($mL.min^{-1}.kg^{-1}$)	ΔPCO_2 (mm Hg)	Villi perfused density (mm/mm^2)	Renal flow ($mL.min^{-1}.kg^{-1}$)	Urine output ($mL.min.kg^{-1}$)
Basal	Control	83±12	122±26	392±154	7±6	22±3	1906±517	1.2±0.3
	Norepinephrine	82±7	96±17	445±318	11±7	26±3	1905±729	1.1±0.5
	Sham	76±13	113±18	405±115	4±4	23±4	1890±639	1.4±0.6
1-h IAH	Control	55±10	121±41	524±302	6±6	25±3	943±416	0.4±0.1
	Norepinephrine	53±9	118±42	633±383	12±5	27±3	552±359	0.4±0.7
	Sham	87±14*	120±28	449±88	4±6	24±3	1730±510*	0.9±0.5*
2-h IAH	Control	49±18*	134±39	522±322	8±6	24±2	869±612	0.3±0.4
	Norepinephrine	73±10	113±39	634±310	12±6	28±3	620±439	0.2±0.1
	Sham	87±15	127±24	448±108	3±5	25±4	1678±569*	1.0±0.6*

*p < 0.05 vs. the other groups (*t* test with Bonferroni correction after significant time x group interaction in two-way repeated measures of ANOVA).

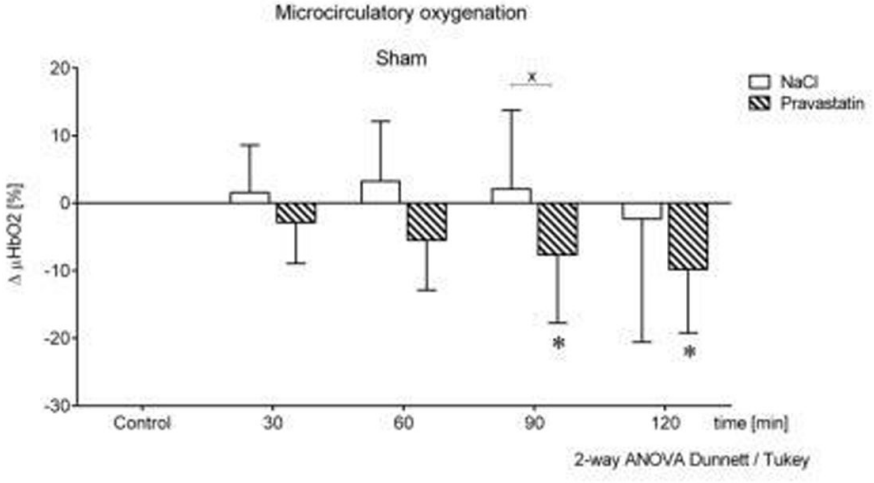

Figure 1(abstract P34) Sham

2.) During sepsis pravastatin pre-treatment ameliorated the deterioration of μHbO_2 (-5.5±8.2%), compared to a significant decrease in the NaCl group (-8.4±8.7%). Figure 2.

3.) Macrohaemodynamic variables and microcirculatory oxygen supply of the colon did not differ between the groups.

Conclusion: Pravastatin has opposite effects on splanchnic microcirculatory oxygenation depending on septic or non-septic conditions. These effects are independent of the macrocirculation or microcirculatory oxygen supply.

References

1. McGown CC, Brookes ZL: **Beneficial effects of statins on the microcirculation during sepsis: the role of nitric oxide.** *Br J Anaesth* 2007, **98**:163-175.
2. La Mura V, Pasarin M, Meireles CZ, Miquel R, Rodriguez-Vilarrupla A, Hide D, Gracia-Sancho J, Garcia-Pagan JC, Bosch J, Abraldes JG: **Effects of simvastatin administration on rodents with lipopolysaccharide-induced liver microvascular dysfunction.** *Hepatology* 2013, **57**: 1172-1181.
3. Tehrani S, Mobarrez F, Lins PE, Adamson U, Wallen HN, Jorneskog G: **Impaired endothelium-dependent skin microvascular function during high-dose atorvastatin treatment in patients with type 1 diabetes.** *Diabetes & vascular disease research* 2013, **10**:483-488.

P35

0590. Impact of arterial tone changes on dynamic arterial elastance and the arterial pressure response to fluid administration

MI Monge García[1,2*], M Gracia Romero[2], P Guijo González[2], A Gil Cano[2], J Mesquida[3], R Andrew[1], RM Grounds[1], M Cecconi[1]
[1]St. George's Healthcare NHS Trust and St George's University of London, Department of Intensive Care Medicine, London, UK; [2]Hospital SAS de Jerez, Servicio de Cuidados Intensivos y Urgencias, Jerez de la Frontera, Spain; [3]Universitat Autónoma de Barcelona, Consorci Sanitari Universitari Parc Tauli, Barcelona, Spain

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P35

Introduction: Dynamic arterial elastance (Ea_{dyn}), the relationship between pulse pressure variation (PPV) and stroke volume variation (SVV), has been suggested as a functional assessment of arterial load for predicting the arterial pressure response after volume expansion (VE)[1]. Although changes in Ea_{dyn} have been related with variations in arterial load[2], the effect of acute arterial tone changes on Ea_{dyn} and the impact on its performance for predicting the arterial pressure response after VE has not yet been determined.

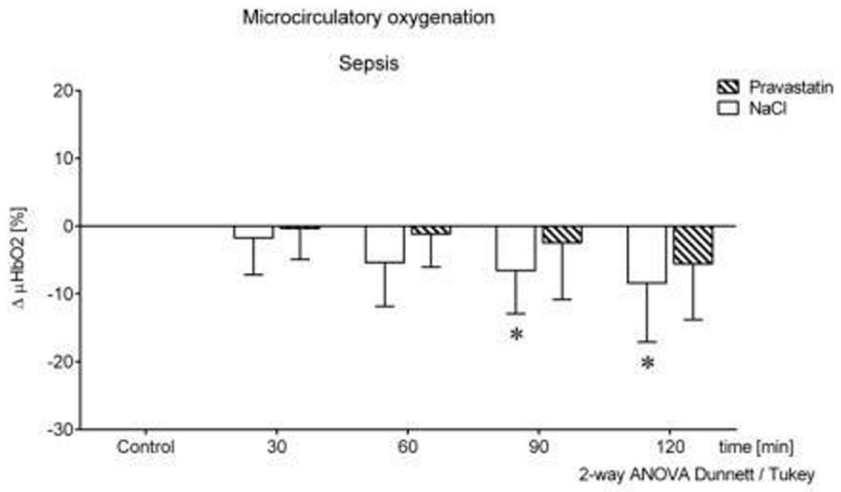

Figure 2(abstract P34) Sepsis

Objective: To evaluate the effect of acute arterial tone changes on Ea_{dyn} and the influence on its performance for predicting arterial changes after fluid administration.

Methods: 12 anesthetized and mechanically ventilated rabbits. Arterial tone changes were induced by phenylephrine (PHENY) infusion on 6 animals (HighMAP group) and by sodium nitroprusside (SNP) on the other 6 animals (LowMAP group), until reach a 50% of change on mean arterial pressure (MAP) from its baseline value. A volume challenge (10 mL/Kg) was then performed on all animals. Animals were monitored with an indwelling femoral arterial catheter and an esophageal Doppler (CardioQ-Combi). Arterial load was assess by the systemic vascular resistance, net arterial compliance and effective arterial elastance. Ea_{dyn} was calculated as the simultaneous ratio between PPV and SVV obtained from the Doppler monitor.

Results: At baseline, Ea_{dyn} and other arterial load parameters were similar on both groups. In the LowMAP group, SNP significantly decreased arterial load, reduced MAP by 44%, and consistently increased Eadyn by 75% (Figure 1). In the HighMAP group, PHENY increased arterial load, raised MAP by 58%, and significantly reduced Ea_{dyn} by 41% (Fig. 1 and 2). Overall, VE increased cardiac output by 10%, stroke volume by 21% and MAP by 15%, and decreased Ea_{dyn} from 1.08 ± 0.67 to 0.88 ± 0.45 (Fig.1). There was a significant relationship between Ea_{dyn} after arterial tone changes and increases in all components of arterial pressure after VE: systolic (R^2=0.89), diastolic (R^2=0.41), mean arterial (R^2=0.61) and pulse pressure (R^2=0.67), respectively. Animals with a MAP increase ≥ 10% after VE had a higher preinfusion Ea_{dyn} value (1.54 ± 0.49 vs. 0.46 ± 0.15; P < 0.001).

Conclusions: In this experimental settings acute modifications on arterial tone induced significant changes on Ea_{dyn}: arterial vasodilation increased Ea_{dyn}, whereas vasoconstriction decreased it. Nevertheless, preinfusion Ea_{dyn} still determined the arterial pressure response after volume administration.

References

1. Monge Garcia MI, Gil Cano A, Gracia Romero M: Dynamic arterial elastance to predict arterial pressure response to volume loading in preload-dependent patients. *Crit Care* 2011, 15:R15.

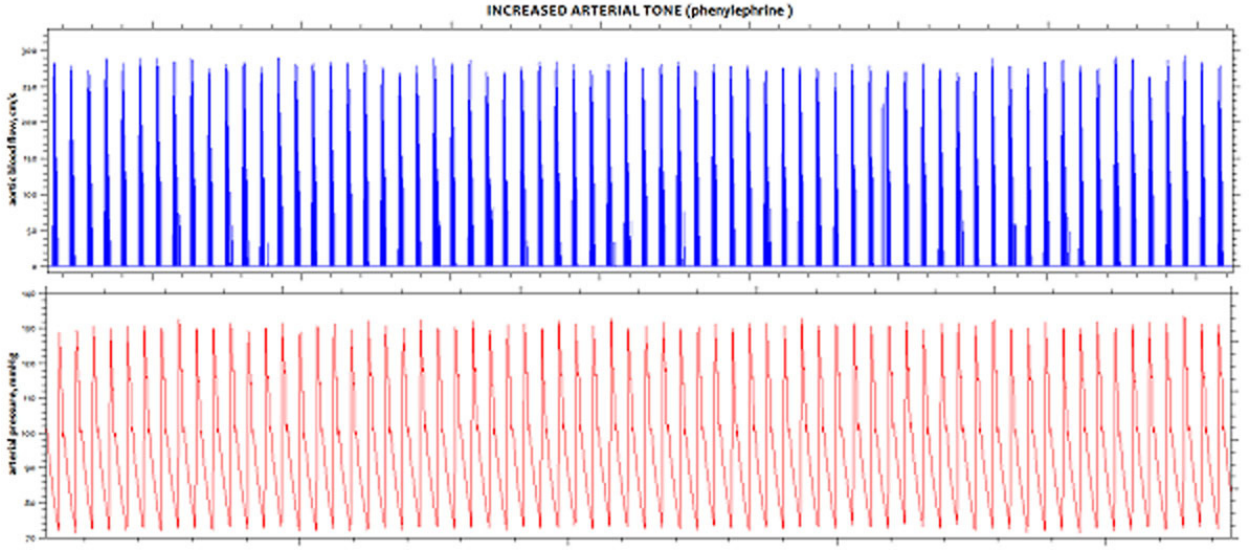

Figure 1(abstract P35) SVV and PPV example on HighMAP group.

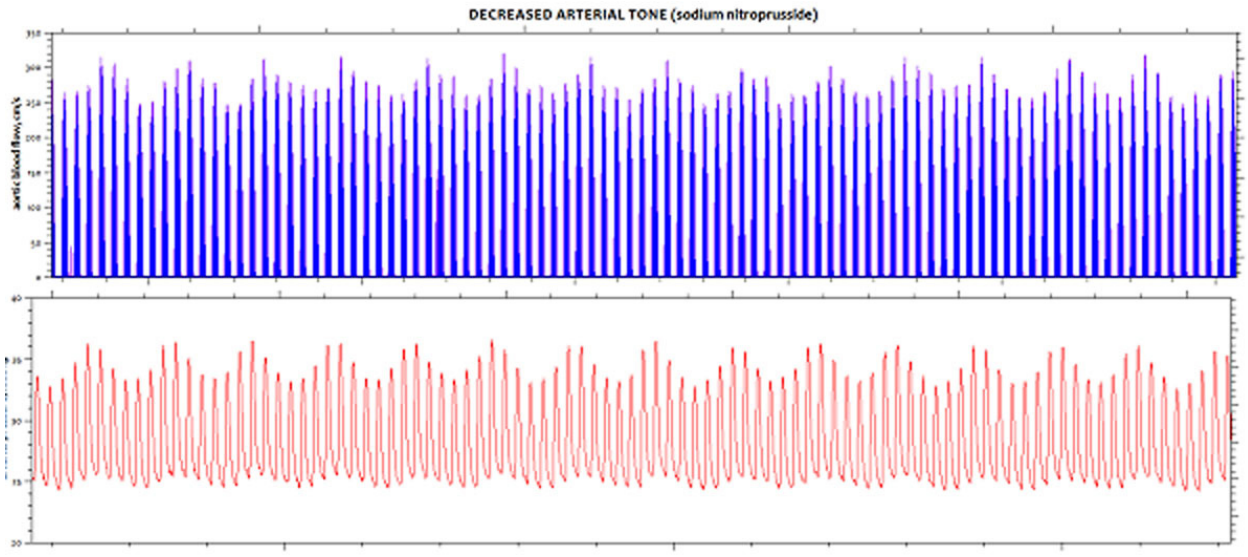

Figure 2(abstract P35) SVV and PPV example on LowMAP group.

Table 1 Comparison of arterial load parameters during different experimental conditions in HighMAP abnd LowMAP groups (n=6 on both experimental arms).

	Baseline	After change in arterial pressure	Postinfusion	P value[a]
EA_{dyn}				
HighMAP	0.92 ± 0.12	0.52 ± 0.23*	0.49 ±.0.18	<0.001
LowMAP	0.91 ± 0.16	1.64 ± 0.44*	1.26 ± 0.22†*	
Ea, cmHg/mL				
HighMAP	3.99 ± 0.93	5.42 ± 1.46*	4.45 ± 1.11†	<0.001
LowMAP	4.50 ± 1.44	1.80 ± 0.63*	1.52 ± 0.50†*	
C, mL/cmHg				
HighMAP	0.66 ± 0.13	0.41 ± 0.10*	0.48 ± 0.08†*	<0.001
LowMAP	0.53 ± 0.16	1.92 ± 0.86*	1.83 ± 0.58*	
TVSR, MPa's/m^3				
HighMAP	1514 ± 453	3157 ± 1299*	3372 ± 1306*	<0.001
LowMAP	1814 ± 585	709 ± 215*	720 ± 218*	

C: new arterial compliance; Ea: effective arterial elastance; Eadyn: dynamic arterial elastance; TSVR: total systemic vascular resistance. Data are presented as mean ± standard deviation. Data normally distributed according to Kolomogorov-Smirnov test. Note that pressure units are expressed as cmHg for convenience. *p<0.05 vs baseline. †p<0.05 vs. change in arterial pressure. *p value refers to ANOVA test for time and group interaction

2. Hadian M, Severyn DA, Pinsky MR: **The effects of vasoactive drugs on pulse pressure and stroke volume variation in postoperative ventilated patients.** *Journal of critical care* 2011, **26(328)**:e321-328.

P36

0592. Metabolic acidosis induced by haemorrhage and hydrochloric acid generates different cardiorespiratory responses

G Sabbatini*, A Dyson, M Singer
University College London, Bloomsbury Institute of Intensive Care Medicine, London, United Kingdom

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P36

Introduction: Metabolic acidosis is classically thought to induce an enhanced ventilatory pattern, irrespective of the underlying aetiology.
Objectives: To induce a similar level of acidaemia in a rat model, by either infusion of an acidic solution or by blood withdrawal, and to assess the physiological responses to these insults.
Methods: Isoflurane-anaesthetised, tracheotomized rats were instrumented with left common carotid arterial and right jugular venous lines for blood sampling/BP monitoring and fluid/blood administration, respectively. Oxylite™ probes (Oxford Optronix, UK) placed in thigh muscle were used to monitor tissue oxygen tension (tPO_2). Animals were subjected to either continuous 0.1 M hydrochloric acid (HCl) infusion or 60% withdrawal of estimated blood volume in six 10% steps over three hours to induce an equivalent fall in arterial base excess (BE). All animals (including a control group) received n-saline throughout. Hourly measurements were made of haemodynamics, tPO_2 and arterial blood gas analysis.
Results: See figure 1.
HCl induced a metabolic acidosis with arterial hypoxaemia yet a preserved muscle tPO_2, no tachypnoea nor fall in $PaCO_2$. By contrast, haemorrhage to achieve a similar acidaemia, resulted in significant falls in blood pressure and tPO_2, hyperlactataemia, a small rise in SaO_2 and a decrease in respiration rate with a concomitant fall in $PaCO_2$ probably related to higher tidal volumes.

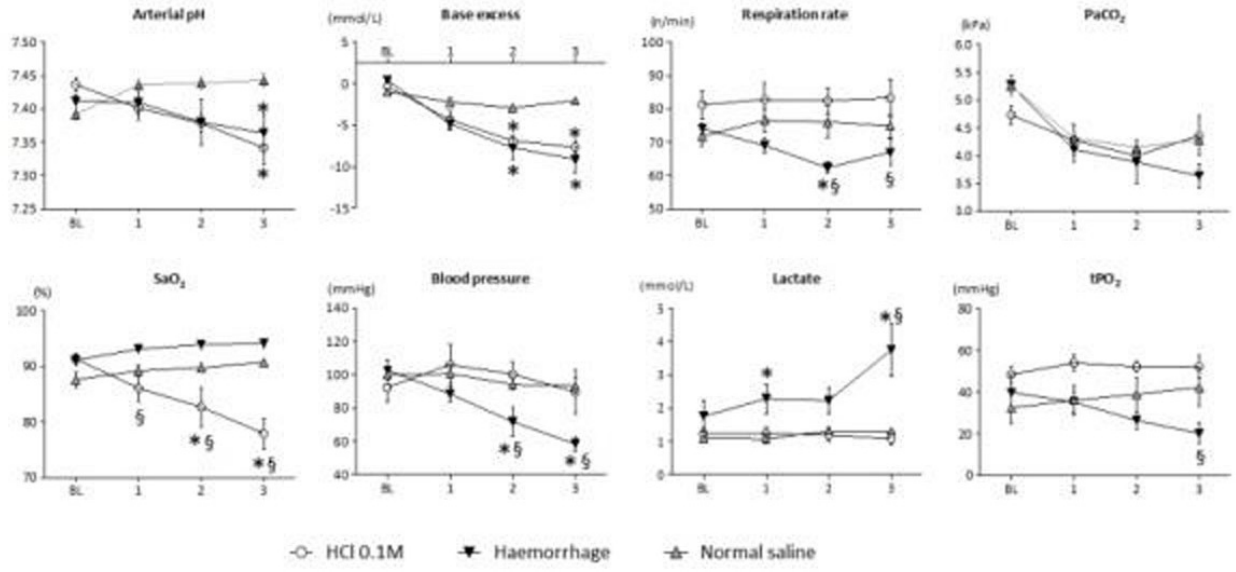

Figure 1(abstract P36) Results. BL= baseline, SaO_2= arterial oxygen saturation. Data shown as mean (SEM). Hydrochloric acid (HCl) n=6, Haemorrhage n=8, Controls n=10/group, *p< 0.05 comparing treated to controls, §p< 0.05 comparing haemorrhage to HCl. Statistics: repeated measures two-way ANOVA and Bonferroni's test for multiple comparisons.

Conclusions: Tissue hypoperfusion (and not just acidaemia per se) is an important component that triggers an enhanced ventilatory drive.

P37

0593. Reelin mediates the human neutrophil peptide-induced endothelial dysfunction and platelet aggregation

AAL Luo[1*], B Han[1], K Quinn[2], E Tullis[3], A Reheman[3], H Ni[3], A Slutsky[3], H Zhang[3]
[1]St. Michael's Hospital, Anaesthesia, Toronto, Canada; [2]University of Toronto, Toronto, Canada; [3]St. Michael's Hospital, Toronto, Canada
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P37

Introduction: Atherosclerosis is an inflammatory disease with fundamental primitive events including endothelial dysfunction and platelet aggregation [1]. Human neutrophil peptides (HNP) are the most abundant cationic proteins in neutrophils and are released into the extracellular milieu upon activation [2]. HNP have previously been detected in atherosclerotic lesions, and are in high concentrations in blood of patients with acute coronary syndrome [2]. We have previously demonstrated that HNP can induce foam cell formation, platelet aggregation and leukocyte recruitment through the LRP8 signaling pathway [3], but there is no direct interaction between HNP and LRP8.
Objectives: We examined if HNP-induced endothelial dysfunction and platelet aggregation through the LRP8-signaling pathway is mediated by the LRP ligand reelin.
Methods: Human coronary artery endothelial cells (HCAEC) were stimulated with HNP or recombinant reelin (rRLN) to assess reelin protein expression and release, and endothelial and inducible nitric oxide synthase (eNOS and iNOS, respectively) protein expression, respectively. Human platelet-rich plasma (PRP) was primed with ADP, followed by stimulation with vehicle control, or HNP in the presence or absence of rRLN. Platelet activation and aggregation were determined by flow cytometry and Chronolog Aggregometer, respectively. The specificity of reelin-induced responses in HCAEC and PRP were confirmed by using a reelin neutralizing antibody (CR50).
Results: HNP stimulation of HCAEC resulted in increased reelin protein expression and release. rRLN-stimulated HCAEC increased iNOS and decreased eNOS protein expression, and induced nitrotyrosine production. rRLN-treated PRP was able to produce a wave of platelet activation and aggregation that was not observed in PRP treated with ADP alone. The endothelial dysfunction and platelet aggregation responses mediated by reelin were reverted upon treatment of a reelin neutralizing antibody.
Conclusions: HNP-induced endothelial dysfunction, platelet activation and aggregation were mediated by the LRP8-ligand, reelin. Blocking reelin maybe a potential therapeutic target in atherosclerosis.
Grant acknowledgment: Canadian Institute of Health Research
References

1. Lusis AJ: *Atherosclerosis. Nature* 2000, **407(6801)**:233-41.
2. Zhao H, *et al*: **Acute ST-segment elevation myocardial infarction is associated with decreased human antimicrobial peptide LL-37 and increased human neutrophil peptide-1 to 3 in plasma.** *J Atheroscler Thromb* 2012, **19(4)**:357-68.
3. Quinn K, *et al*: **Human neutrophil peptides: a novel potential mediator of inflammatory cardiovascular diseases.** *Am J Physiol Heart Circ Physiol* 2008, **295(5)**:H1817-24.

P38

0595. Quantitating granulocyte reactive oxygen species production by flow cytometry in a clinical setting

B Smit[1*], MC de Waard[1], YM Smulders[2], HM Oudemans-van Straaten[1], C Boer[3], AB Vonk[4], D Veerhoek[5], JJ García Vallejo[6], S Kamminga[7], AR Girbes[1], AM Spoelstra-de Man[7]
[1]VU University Medical Center, Intensive Care, Amsterdam, Netherlands; [2]VU University Medical Center, Internal Medicine, Amsterdam, Netherlands; [3]VU University Medical Center, Anaesthesiology, Amsterdam, Netherlands; [4]VU University Medical Center, Cardiothoracal Surgery, Amsterdam, Netherlands; [5]VU University Medical Center, Clinical Perfusion, Amsterdam, Netherlands; [6]VUmc, Molecular Cell Biology & Immunology, Amsterdam, Netherlands; [7]VU University Medical Center, Amsterdam, Netherlands
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P38

Introduction: Oxidative stress is an important part of a wide range of pathologies and therefore an interesting parameter to determine. However, the detection of reactive oxygen species (ROS), is not straightforward. Sample heterogeneity, delayed analysis and sample preparation, reduces specificity and sensitivity due to probe activation by light, air, probe leakage from cells or cellular activation by sample manipulation. Hence, samples should be processed minimally and analysed immediately if possible. Since clinical research can be subject to uncontrollable timetables and only few

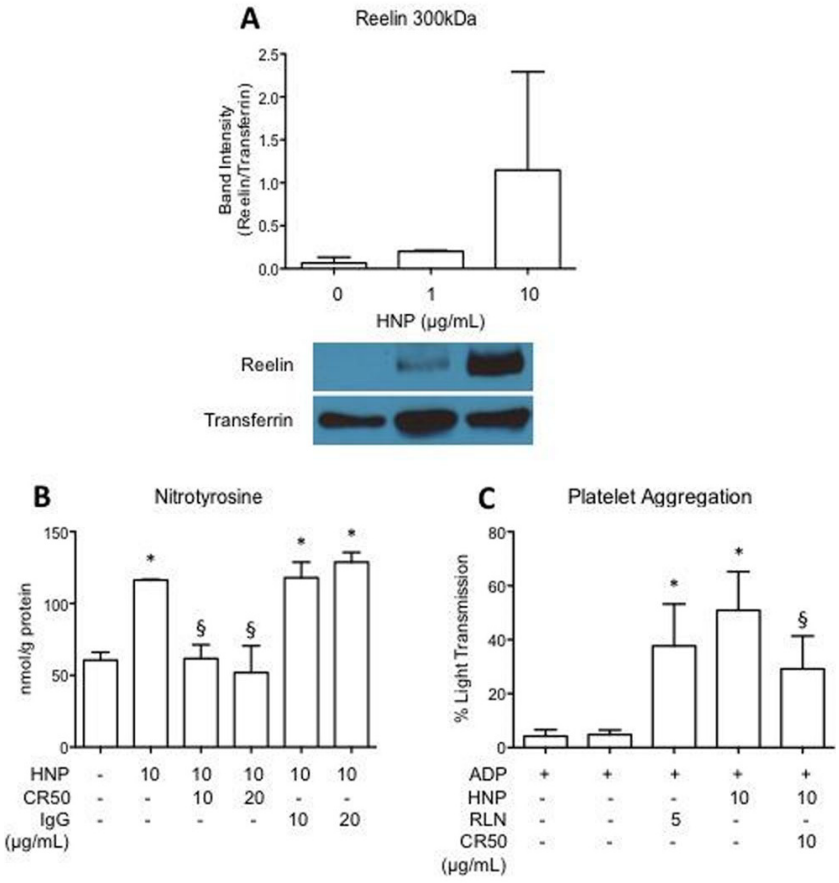

Figure 1(abstract P37) The role of reelin in HNP-induced nitrotyrosine production and platelet aggregation.A. HNP indues reelin (RLN) protein release in HCAEC. Released reelin was determined by western blot from cell culture medium. **B.** Reelin neutralizing antibody (CR50) reduced HNP-induced nitrotyrosine production. **C**. CR50 attenuates HNP-stimulated platelet aggregation in ADP-primed human platelet rich plasma. *p<0.05 *vs* control; §p<0.05 *vs* HNP 10 µg/mL.

departments have access to a dedicated laboratory, the quantification of ROS is challenging.

Objectives: For the purpose of a randomized-controlled trial involving Coronary Artery Bypass Graft surgery (CABG), we sought to establish a flow cytometric assay to detect ROS production in granulocytes (PMN) that is easy to perform, does not require a specialised laboratory on-site and is relatively insensitive to delay between sample preparation and analysis. The fluorescent probe applied is CellROX® Green (CrX; Life Technologies, Eugene, Oregon, USA), which becomes fluorescent after oxidation and subsequently binds to DNA.

Methods: Whole blood samples were collected from healthy volunteers and from patients before (baseline) and during CABG surgery (after 1 hour of cardiopulmonary bypass, CPB). 30 µl whole blood was incubated for 1 hour with 10µM CrX immediately after sampling. To compensate for the surgery induced PMN count increase, 1 hour CPB samples were incubated with 10, 20 and 30µM CrX. Following incubation, erythrocytes were lysed by hypotonic shock and the sample was washed three times with Phosphate Buffered Saline (PBS). Finally, leukocytes were stored in 200µl of PBS at room temperature, in the dark, until analysis. Positive and negative controls were obtained by additional incubation with phorbol-12-myristate-13-acetate and N-acetyl-cysteine. Mean Fluorescence Intensity (MFI) of the PMN population, which is proportional to the ROS produced, was measured in the FL1 channel of a BD FACSCalibur as soon as possible after incubation of the second CABG sample (~5 hours). PMN were identified based on their forward and sideward scatter characteristics to avoid fluorescence compensation corrections and aspecific activation of neutrophils by fluorescent cell-specific antibodies.

Results: In line with our expectations, little production of ROS was demonstrated in healthy volunteers (n=4) and baseline samples from CABG surgery patients, while a marked increase was found after one hour of CPB (n=8, figure 1). PMA induces a NAC-inhibitable increase of MFI in CABG baseline samples. Interestingly, we were also able to detect what appears as separate ROS producing neutrophil populations, which warrants further investigation.

Conclusions: Our current method is easy to perform, implementable into clinical research and is able to detect different ROS responses.

P39

0596. Plasma "drug" of cardiovascular dysfunction in the rat model of hemorrhagic shock

N Sennoun[1,2*], M Toussaint-Hacquard[1], A Lecomte[3], L Chevreux[1], T Lecompte[1], S Gross[1], B Levy[3]

[1]French Blood Establishment-Lorraine Champagne, Vandoeuvre-lès-Nancy, France; [2]University of Sciences and Technology Houari-Boumediene, Cellular and Molecular Biology, Alger, Algeria; [3]Shock Research Group - Avenir Inserm U 961, Vandoeuvre-lès-Nancy, France

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P39

Introduction: Hemorrhagic shock (HS) often cause coagulopathy and cardiovascular dysfunction. The extent of these alterations correlates with the severity of HS. Fresh Frozen Plasma use is usually designed to restore coagulation proteins levels. Clinical and experimental studies have shown

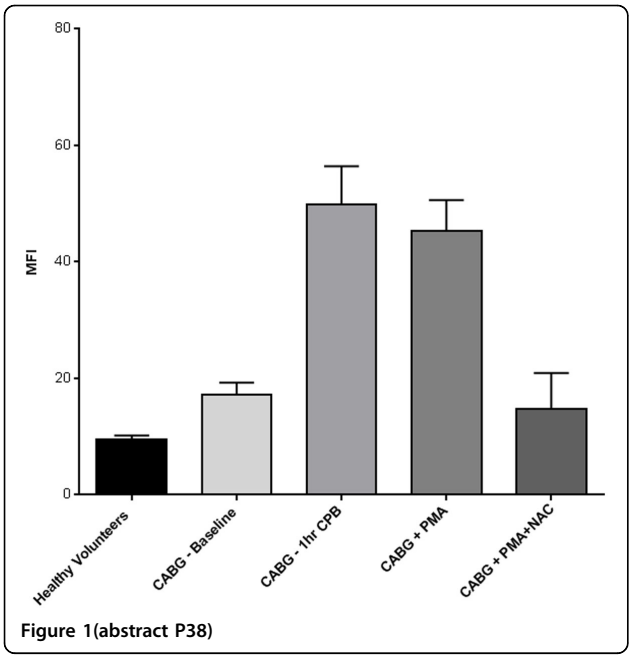

Figure 1(abstract P38)

that resuscitation with fresh plasma is associated with improved outcome after severe hemorrhagic shock.

Objectives: The benefical effect of FFP on coagulopathy is well documented but its effect on vascular dysfunction is not clear. We hypothesized that in addition to its effects on hemostasis, FFP has other protective effect on cardiovascular dysfunction.

Methods: A Wistar rat model of Hemorrhagic shock (HS) was used. Rats were anesthetized and instrumented for continuous monitoring of blood pressure, heart rate and body temperature.

Rats with cannulated jugular and femoral veins were subjected to withdrawals of 20mL/kg body weight of blood for 15min; resuscitation fluids (vol/vol blood loss) were infused over 45 minutes after HS. Different treatments were studied : RBC+plasma of rats or RBC+HEA. Four groups were studied : control (without HS), HS (without treatment), HS+RBC+rat plasma and HS+RBC+HEA. We evaluated survival to 300 min and hemodynamic parameters. eNOS, P-eNOS, Bax and Bcl2 levels in the aorta tissue were determined by Western Blot.

Results: Plasma and HEA treatment improved significantly survival up to 300min and restored hemodynamics parameters (arterial pressure, heart rate, carotid blood flow). These results are in agreement with P-eNOS/eNOS ratio. The protein level showed that HS increased apoptosis in aorta as determined by Bax/Bcl2 ratio expression ($p < 0.05$). Plasma infusion resulted in significantly less apoptosis than HEA resuscitation. These results suggest that plasma may have a protective effect on vascular function.

Conclusions: Plasma and HEA resuscitation have the same beneficial effects on survival and haemodynamic parameters. However, plasma seems to have additional benefical effect on apoptosis as compared to HEA, but the clinical significance remains to be determined.

HS: Hemorrhagic Shock, Voluven®: HEA, RBC: Red Blood Cells.

P40

0597. The relation between intestinal intramucosal ph and stress hormones in pig hemorrhagic shock model

M Arai[1*], S Ito[2], Y Kosaka[2], M Toda[2], M Kuroiwa[2], H Okamoto[2]
[1]Kitasato University, Anesthesia and Intensive Care Medicine, Sagamihara, Japan; [2]Kitasato University, School of Medicine, Anesthesiology, Sagamihara, Japan

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P40

Introduction: It is known that intestinal intramucosal pH (pHi) is a good parameter of tissue perfusion in critical patients. However, physiological meaning of pHi value related to other physiological parameter, such as stress hormone, is not well known. The purpose of the study is to clarify the importance of monitoring pHi during hemorrhagic shock in pig model. The relation between pHi, oxygen supply and demand balance at intestinal region and stress hormones are discussed.

Methods: The studies were performed on 6 pigs (body weight 25±4 kg). Anesthesia was induced by inhalation of isoflurane 3-5% and after endotracheal intubation, pigs were placed on a positive pressure ventilation. Anesthesia was maintained with pancronium and isoflurane 2%. A catheter was inserted through carotid artery to measure aortic blood pressure and to sample blood. Ringer's Lactated solution was infused throughout the experiment at 10 ml/kg/hr. The pig's abdomen was opened through a midline incision and the electromagnetic flow probe was placed on the root of superior mesenteric artery (SMA). Tonomitor® (Tonometrics) was inserted from antimesentric region of jejunum. A polyvinyl tube was inserted to superior mesenteric vein (SMV) to sample blood.

Protocol: Control data were obtained after 30 min stabilization period (Baseline; S1). Then the pigs were bled gradually and MAP was maintained at 40mmHg for 1 hours (Shock Phase; S2). Pigs were resuscitated with whole blood (Resuscitation Phase 1; S3). Additional 1 hour was evaluated for post-resuscitation phase (S4).

Measurements: Jejunal pHi was calculated by the luminal PCO2, obtained with a balloon tonometer, and arterial bicarbonate concentration. Arterial blood samples were taken for analysis of adrenaline (ADR), noradrenaline (NA), angiotensin II (Ang II) and arterial blood gas. SMV blood samples were taken for analysis of lactate/pylvate acid (sma L/P), and blood gas (PsmvCO2).

Results: At shock phase, SMA flow decreased to 40% from the baseline ($p < 0.01$) and pHi decreased from 7.4±0.2 to 6.8±0.2 ($p < 0.01$). The correlation between pHi and other parameters are as follows, pHi vs PsmvCO2; y=-0.02x+8.22 (r2=0.72) (Fig 1), pHi vs smv L/P ; y=-0.02x+7.64 (r2=0.76) (Fig 2), pHi vs ADR (pg/ml); y=-0.09ln(x)+7.74 (r2=0.72), pHi vs NA (pg/ml); y=-0.09ln(x)+7.79 (r2=0.74), pHi vs Ang II (pg/ml); y=-0.11ln(x)+7.71 (r2=0.75).

Discussion: Changes of pHi value during hemorrhagic shock was correlated with PsmvCO2, smv L/P and stress hormones (ADR, NA, Ang II). It is considered that change in pHi show the anaerobic metabolism status of the intestinal tissue which indicate tissue hypoxia of the intestine induced by hemorrhagic shock and increase in sympathetic nerve activity. pHi was strongly correlated to other physiological parameters which indicate that pHi is significance parameter of hemorrhagic shock.

Conclusions: pHi is significant parameter of tissue hypoperfusion and sympathetic nerve activity during hemorrhagic shock.

P41

0600. Altered electrical activity of the heart in pigs during apnoea

B-A Tudor[1], KH Wodack[2], D Lebherz-Eichinger[1], CJ Trepte[2], GA Roth[1], DA Reuter[2], CG Krenn[1*]
[1]Medical University of Vienna, Vienna, Austria; [2]University Hospital Hamburg-Eppendorf, Hamburg, Germany

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P41

Introduction: Cardiac arrhythmias are a major cause of morbidity and mortality. Their overall incidence is reported to vary between 14 and 19 percent in adult patients admitted to intensive care units [1]. Even in patients without prior heart pathologies, an electrical instability could be detected during repolarisation shortly before cardiac arrest with a high resolution ECG, not recordable with a conventional ECG [2]. Furthermore the effects of disease pattern and intensive care measures e.g. sepsis and elevated intracranial pressure and the administration of pressure support on heart rate and variability is poorly described and understood. In contrast even minor circadian beat to beat variations might precede the occurrence of potentially life threatening arrhythmias. Thus circadian haemodynamic profiles might thus be a marker of potentially life threatening arrhythmias and an early predictor of prognosis. It seems therefore of importance to recognize even small changes of the circadian cardiac rhythm as early as possible.

Objectives: Changes in ventilation such as hypoxia are a major cause of haemodynamic alterations. We analysed changes of beat-to-beat cardiac activity during experimental apnoea in swine with a high resolution ECG. Obtained results may offer new insights in the development of alterations

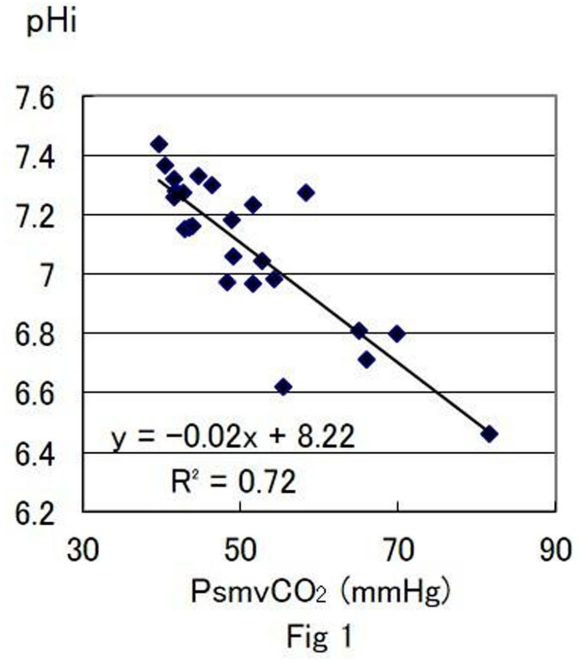

Figure 1(abstract P40)

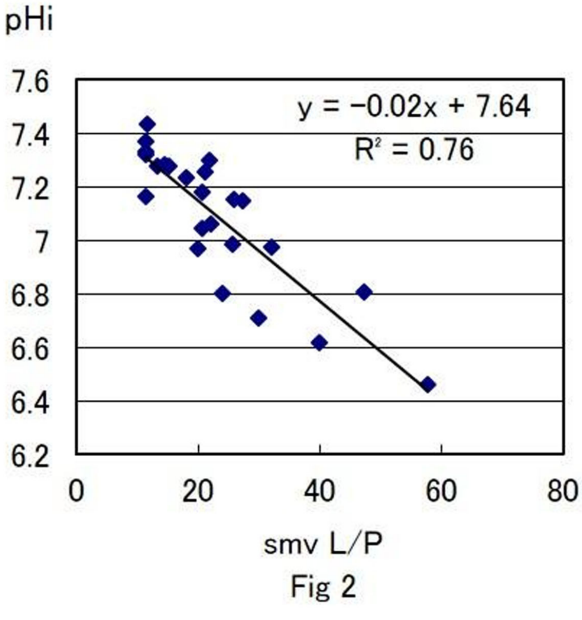

Figure 2(abstract P40)

in cardiac electrical activity and thus heart lung interactions in critically ill patients.

Methods: Four healthy pigs (36kg) were anesthetized with Sevofluran and Fentanyl according to protocol and electrodes were fixed on the prepared skin. To analyse the cardiac electric activity during normal anaesthesia and phases of three-minutes-apnoe the leads record I, II and III and reconstruct pursuant to Einthoven's equation aVR, aVL, aVF, at a 1000 Hz sampling rate, obtaining continuous ten-minute recordings (Lab SystemTh Pro - Bard electrophysiology U.S.A.).

Results: Results obtained from 12 apnoea-phases demonstrate that from the beginning of the apnoea the R-peak increases beat-to-beat additionally up to 0.25 mV (p< 0,05), reaching its maximum after nine seconds. This variation persisted for the entire duration of the apnoea. After restarting ventilation, the following R-peaks decreased within the next eleven seconds to their pre-value. Furthermore a reduction of the heart rate during the phase of apnoea could be observed. Other ECG data of the pigs remained unchanged during the time of apnoea.

Conclusions: In otherwise healthy pigs, haemodynamic alterations could already be detected seconds after the onset of apnoea. With regard to comorbidities of ICU patients, it seems reasonable that changes in cardiac electric activity based on heart lung interactions in humans during their ICU stay might be observed even earlier. They furthermore might serve as early markers for the occurrence of arrhythmias and disease severity.

References

1. Muller JE: **Circadian variation in the frequency of sudden cardiac death.** *Circulation* 1987, **75(1)**:131-138.
2. Burri H, Sunthorn H, Shah D: **Simulation of anteroseptal myocardial infarction by electrocardiographic filters.** *Journal of Electrocardiology* 2006, **39(3)**:253-258.

P42

0601. An inhibitor of pro-inflammatory enzyme soluble epoxide hydrolase improves hippocampus dependent memory function after cardiac arrest and cardio-pulmonary resuscitation in mice

T Fujiyoshi*, S Hoka
Kyushu University, Department of Anesthesiology, Fukuoka, Japan

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P42

Introduction: Promotion and improvement of cardiopulmonary resuscitation (CPR) technique contributes to increase of the survivors from unexpected cardiac arrest (CA). However, many patients have some neurological deficits, because of few effective treatment to improve the outcome. Hippocampus damage causes a memory dysfunction which suffers the survivors. Microglia activation caused neuron damage following brain stroke and inhibition of pro-inflammatory soluble epoxide hydrolase (sEH) reduced the infarction size in partial ischemia mice model.

Objectives: We investigated whether therapeutic inhibition of sEH suppresses neuronal damage and improve memory dysfunction.

Methods: Under isoflurane anesthesia, male adult C57Bl/6 mice were orally intubated and were connected to the ventilator. A jugular vein catheter, thermometer and electrocardiography (EKG) was placed. CA was induced by potassium chloride injection. When cardiac arrest was confirmed by EKG, isoflurane and mechanical ventilation were disconnected. Brain temperature was maintained at 37.5 ± 0.5 °C and rectal temperature is decreased to 28 ± 0.5 °C during CA. Mechanical ventilation was restarted 30 seconds before CPR and CPR, chest compression and epinephrine administration, was initiated at 8 minutes after CA onset. The sEH inhibitor 4-phenylchalcone oxide (4-PCO, 5 mg/kg ip) or vehicle was injected at 5 minutes after CACPR and repeated daily. Neuro-scoring examination was performed 3 days after CAPR. Surviving CA1 hippocampal neurons were counted 1, 3 or 10 days and microglia activation was assessed by immunostaining for the activation marker Mac-2 at 1, 3, and 10 days after CACPR Delay fear conditioning was tested 10 days after CA/CPR.RESULTS; Succeeded CPR rate was 86.4 % and 65.9 % of CACPR mice survived for 10 days after CACPR. CACPR mice showed some neurological deficits on day 3, whereas sham-operated mice showed no deficits. Delayed neuron death appeared on day 3 and persisted 10 days after CACPR in hippocampus CA1 area of CACPR mice. Microglia activated on day 1, migrated to the ischemia-induced damaged hippocampus area on day 3 and persisted on day 10 in CACPR mice. In CACPR mice, 4-PCO activated microglia on day 3, but surprisingly, 4-PCO reduced damaged neurons on day 3, not on day 10. CACPR mice showed reduced freezing to context, not to cued and CACPR mice treated by 4-PCO showed more freezing to context, significantly. This result of fear conditioning test indicated that CACPR caused hippocampus dependent memory deficit, which was improved by 4-PCO treatment.

Conclusions: Experimental CACPR caused hippocampal neuronal damage, microglial activation, and memory deficit in mice. Inhibition of sEH activated microglia but protected ischemia-induced neurons and improved memory function in hippocampus in mice. The inhibition of sEH will be a key of new therapeutic approach to global ischemia-induced damaged brain.

SHOCK RESUSCITATION

P43

0633. Time-resolved spectroscopy using non-invasive monitoring may detect hapatic ischemia

Y Tomotsugu*, Y Kakihana, K Yamaguchi, M Nakahara, T Futatsuki, J Taniguchi, K Nakamura, N Okayama
Kagoshima University Hospital, Intensive Care Medicine, Kagoshima University, Japan
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P43

Introduction: The aim of the present study was to investigate whether the changes in hepatic oxygenation can be detected by time-resolved spectroscopy (TRS) placed on the skin surface above the liver.
Methods: With approval of the local Hospital Ethics Committee and informed consent, 6 healthy volunteers aged 28.8 years (25-36 years), and 5 patients with chronic renal failure aged 70.6 years (58-81 years) were studied. In 6 healthy volunteers, following the echography, TRS (TRS-10, Hamamatsu Photonics K.K., Hamamatsu, Japan) probe consisting of a near-infrared light (at 760 nm, 800 nm, 835 nm) emitter and a receiver optode, was placed 4 cm apart on the abdominal skin surface above the liver or apart from the liver at least 10 cm. In 5 patients with chronic renal failure, following the echography, TRS probes were placed 4 cm apart on the skin surface above the liver during hemodialysis (HD).
Results: In 6 healthy volunteers, the values of abdominal total hemoglobin concentration (tHb) was significantly higher in the liver area than in the other area (80.6±26.81mM vs 44.6±23.1mM, P=0.0017), while the value of abdominal SO_2 in the liver area was nearly the same as that in the other area (71.5±3.6% vs 73.6±4.6%, P=0.19). The values of mean optical path length and scattering coefficient (μ´s) at 800 nm in the liver area were significantly different from those in the other area (21.3±4.9 cm vs 29.2±5 cm, p=0.0004, and 7.97±1.14cm-1vs 9.02±0.51cm-1, P=0.015). One of 5 patients with chronic renal failure complained of the severe abdominal pain during HD, and the abdominal SO_2 was decreasing from 53% to 22%, but the pain relief occurred following cessation of HD, and SO_2 recovered to the baseline.
Conclusions: Our data suggest that the optical properties of the liver may be measured by the TRS placed on the skin surface, and the hepatic oxygenation may act as a non-invasive monitoring for early detection of intestinal ischemia.

ECMO

P44

0671. Extended extracorporeal lung support in a porcine acute lung injury model. Feasibility and preliminary data

A Bruhn[1*], P Cruces[2], P Tapia[1], P Garcia[3], L Alegria[1], J Araos[1], D Soto[1], D Hurtado[4], F Rodriguez[1], M Amthauer[1], T Salomon[5], D Rodriguez[1], ME Rucán[1], G Castro[1], B Erranz[2], R Cornejo[6], G Bugedo[1]
[1]Pontificia Universidad Catolica de Chile, Departamento de Medicina Intensiva, Santiago, Chile; [2]Universidad Andrés Bello, Centro de Investigación de Medicina Veterinaria, Santiago, Chile; [3]Pontificia Universidad Catolica de Chile, Escuela de Kinesiología, Santiago, Chile; [4]Pontificia Universidad Catolica de Chile, Departamento de Ingeniería Estructural y Geotécnica, Santiago, Chile; [5]Clinica Alemana de Santiago, Unidad de Cuidados Intensivos Pediátricos, Santiago, Chile; [6]Universidad de Chile, Departamento de Medicina, Santiago, Chile
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P44

Introduction: During the last years there has been a renewed interest in using extracorporeal membrane oxygenation (ECMO) to treat severe ARDS. ECMO may correct hypoxemia but may also aid in protecting the lungs [1]. However, there is scarce data about the optimal way to ventilate the lungs during ECMO. Experimental models of acute lung injury with ECMO are usually too short.
Objectives: To develop an extended severe ARDS model supported with ECMO suitable to assess the impact of different ventilatory strategies during ECMO.
Methods: Eighteen domestic pigs (27-35 kg) were anesthetized, mechanically ventilated (Vt 10 ml/kg, PEEP 5, O2 1.0) and invasively monitored. Thereafter they were randomized into 3 groups: sham, lung injury, and lung injury + ECMO. SHAM animals were ventilated for 24 hours with standard ventilation (Vt 10 ml/kg, PEEP 5, FiO2 1.0 and RR adjusted to keep PaCO2 30 to 50 mmHg) and then euthanized. In the other groups lung injury was induced by saline lavages (30 ml/kg per lavage) performed repeatedly in both supine and prone position until PaO2/FiO2 dropped below 250. They were then subjected to a 2-hour injurious ventilation with PCV, PEEP = 0,Pinsp = 40 cmH2O, RR = 10/min, I:E = 1:1, one hour in prone and the other in supine. After completing lung injury (time 0) animals were subjected to 24 hours of standard ventilation. In the ECMO group, animals were immediately connected to a saline primed-MEDOS Hilite ECMO circuit by inserting a AVALON 23F double-lumen cannula through the external jugular vein. Blood flow was set at 60-70% of cardiac output. Respiratory and hemodynamic data, as well as plasma and BAL samples were collected at times 0,3, 6, 12, 18 and 24h. After euthanizing animals at time 24h tissue samples were extracted from the lungs.
Results: The two-hit lung injury model resulted in severe hypoxemia, decreased compliance, and with extensive lung inflammation on histology, with only 6/12 animals surviving up to 24h. Lung tissue revealed extensive inflammation. ECMO resulted in improved survival, increased oxygenation, lower pulmonary artery pressures, but no change was observed on compliance. Wet/dry ratio in lung tissue was 4.1±0.9, 7.2±0.1, and 7.2±0.5 in the SHAM, lung injury and lung injury+ECMO groups, respectively. ECMO setup had minimal recirculation with high O2 and CO2 transfer rates. Although protective ventilation was not applied in this feasibility study in pilot experiments we confirmed that the ECMO setup has the potential to provide extended full lung support.
Conclusions: An extended lung injury model supported with ECMO is feasible. The two-hit model produced a steady compromise in compliance despite partial recovery of hypoxemia.
Grant acknowledgment: CONICYT, Fondecyt 1130428
Reference
1. Gattinoni L, *et al*: Towards ultraprotective mechanical ventilation. *Curr Opin Anesthesiol* 2012, **25**:141-147.

P45

0681. Effects of veno-venous co2 removal therapy on pulmonary circulation in an ARDS model

P Morimont[1,2*], T Desaive[2], J Guiot[1], V Tchana-Sato[1], N Janssen[1], A Cagnina[1], S Habran[2], S Kosta[2], D Hella[1], F Blaffart[1], P Kolh[1,2], J-O Defraigne[1,2], B Lambermont[1,2]
[1]University Hospital of Liege, Liege, Belgium; [2]University of Liege, GIGA-Research, Cardiovascular Sciences, Liege, Belgium
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P45

Introduction: Acute respiratory distress syndrome (ARDS) is responsible for injuries to the alveolar epithelium and microvascular endothelium resulting in hypoxemia, decreased pulmonary compliance and increased pulmonary vascular resistance. Beneficial effects resulting from protective lung ventilation are counterbalanced by deleterious hemodynamic effects. Indeed, hypercapnia resulting from ventilation at lower tidal volume enhances pulmonary hypertension and is associated with right ventricular failure in ARDS [1].
Objectives: The aim of our study was to determine if low flow CO2 removal therapy used at early stage of ARDS could have beneficial hemodynamic effects on pulmonary circulation.
Methods: This study was performed on an experimental model of ARDS obtained in 6 pigs. After sedation, analgesia and endotracheal intubation via a cervical tracheostomy, the pigs were connected to a volume-cycled ventilator. A micromanometer-tipped catheter was inserted into the main pulmonary artery and into the left atrium. Systemic arterial blood pressure (AP) was monitored via a micromanometer-tipped catheter inserted into the abdominal aorta through the left femoral artery. A conductance micromanometer-tipped catheter was inserted into the right ventricle. ARDS was obtained by repeated bronchoalveolar lavage (0.9% saline solution). Protective ventilation at lower tidal volume was then achieved. 12 Fr aspiration and 10 Fr injection cannulas were inserted into the inferior and the superior vena cava, respectively. These cannulas were connected to a pump-driven extracorporeal membrane oxygenator (PALP, MAQUET, Germany) in order to achieve CO2 removal therapy.

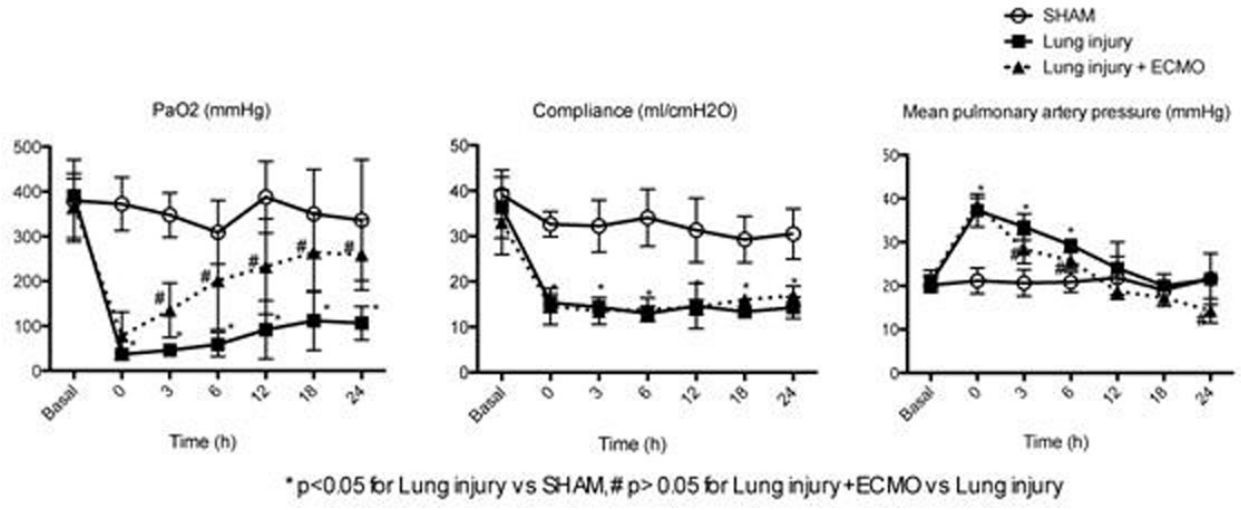

Figure 1(abstract P44)

Results: ARDS induced severe hypercapnic acidosis (PaCO2 = 82.5 ± 10.8 vs. 46.3 ± 10.7 mm Hg and pH = 7.14 ± 0.07 vs. 7.46 ± 0.07, p < 0.01). Systolic pulmonary artery pressure (PAPs) significantly increased from 27.4 ± 4.3 to 43.0 mm Hg, p < 0.01. After the PALP was started, acidosis was corrected (pH = 7.39 ± 0.08) and normocarbia was maintained (PaCO2 = 41.9 ± 12.6 mm Hg, p < 0.01) despite protective ventilation (tidal volume = 6 vs. 10 mL/kg). At the same time, PAPs significantly decreased to 30.8 ± 5.0 mm Hg, p < 0.01. Cardiac output, left atrial pressure as well as AP did not significantly change. Mean PALP blood flow was 645 mL/min.
Conclusions: Veno-venous removal therapy enabled protective ventilation while maintaining normocarbia during ARDS. CO2 removal decreased pulmonary hypertension and improved right ventricular function. This technique may be an effective lung- and right ventricular- protective adjunct to mechanical ventilation.
Grant acknowledgment: Leon Fredericq Foundation.
Reference
1. Lheritier G, Legras A, Caille A, Lherm T, Mathonnet A, Frat JP, *et al*: Prevalence and prognostic value of acute cor pulmonale and patent foramen ovale in ventilated patients with early acute respiratory distress syndrome: a multicenter study. *Intensive Care Med* 2013, **39**:1734-42.

ELECTROLYTES, MICRONUTRIENTS & VITAMINS

P46

0714. Interaction between adipokines and the metabolic stress response: angpt l2 cxcl5 and visfatin in patients undergoing cardiac surgery

T Meyer[1], E Nitschmann[2], U Schurr[3], A Boltres[1], C Bläsi[1], L Wykes[2], H Pargger[1], A Kopp Lugli[1*]
[1]University Hospital Basel, Department of Anesthesia & Surgical Intensive Care, Basel, Switzerland; [2]McGill University, School of Dietetics and Human Nutrition, Montreal, Canada; [3]University Hospital Basel, Department of Cardiac Surgery, Basel, Switzerland
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P46

Introduction: Adipose tissue plays an intriguing role in the endocrine system by producing adipokines [1]. In patients with obesity, metabolic syndrome, and type 2 diabetes (DM2), insulin resistance is influenced by adipokines.[1]
From a metabolic point of view, the stress response caused by surgical injury imitates the diabetic state. Stereotypical endocrine-metabolic and inflammatory reactions result in catabolism and pronounced insulin resistance, which is related to outcome [2].
Objectives: Three adipokines including angiopoietin-like protein 2 (ANGPTL2), CXC-chemokine ligand 5 (CXCL5), and Visfatin that are known to interact with insulin resistance [1] were evaluated in patients undergoing cardiac surgery.
Methods: Sixty-six patients scheduled for elective aortocoronary bypass surgery and/or valve repair were consecutively enrolled receiving standardized perioperative care. Serum adipokine concentrations were assessed before anesthesia induction as baseline values, upon arrival on the intensive care unit, on the first (POD1) and third (POD3) day postoperatively.
Results: Patients' baseline characteristics are shown in table 1. ANGPTL2 increased from baseline until POD1 and remained elevated on POD3 (table 2). In contrast, CXCL5 levels decreased during surgery and returned to baseline by POD3. Visfatin levels increased during surgery, showed a decrease by POD1 only to increase again by POD3. DM2 patients appear to exhibit a marked Visfatin increase during surgery, as well as a pronounced increase in CXCL5 from POD1 to POD3.
Conclusions: This exploratory study assessed for the first time the perioperative levels of ANGPTL2, CXCL5, and Visfatin. Further studies are warranted to correlate adipokine concentrations with insulin resistance and surgical outcomes and to investigate the potential role of adipokines as biomarkers or therapeutic targets.
Grant acknowledgment: Research Fond, Department of Anesthesia and Surgical Intensive Care, University Hospital Basel, Switzerland.
References
1. Ouchi N, *et al*: Adipokines in inflammation and metabolic disease. *Nat Rev Immunol* 2011, **11**:85-97.
2. Coursin DB, *et al*: Perioperative diabetic and hyperglycemic management issues. *Crit Care Med* 2004, **32**:S116-25.

P47

0716. Does intravenous iron induce oxydative stress in critically ill patients? A comparison with healthy volunteers

S Lasocki[1*], P Piednoir[2], C Couffignal[3], E Rineau[1], C Schilte[4], G Dufour[2], X Duval[4], F Driss[5]
[1]CHU de Angers, Département d'Anesthésie-Réanimation, Angers, France; [2]CHU Bichat-Claude Bernard-Paris Diderot, Departement d'Anesthésie Réanimation, Paris, France; [3]CHU Bichat-Claude Bernard-Paris Diderot, Biostatistiques, Paris, France; [4]CHU Bichat-Claude Bernard-Paris Diderot, CIC, Paris, France; [5]INSERM U 1149, Unniversité Paris Nord Val de Seine, Paris, France
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P47

Introduction: Anaemia is frequent in critically ill patients. Iron deficiency, secondary to blood losses or prior to admission, is in part responsible for this

Table 1(abstract P46)

Variable	All patients (66)	Non-diabetics (46)	Diabetics (20)
Sex (M/F)	45/21	33/13	12/8
Age (yrs)	65.5 ± 13	64 ± 14	68.7 ± 9.8
ASA (2/3/4)	13/46/7	9/35/2	4/11/5
BMI (kg/m2)	27.5 ± 4.4	26.4 ± 4.2	30.1 ± 3.9
HbA1c (%)	6.3 ± 0.9	5.9 ± 0.4	7.3 ± 1.1
EuroSCORE	4.8 ± 4.6	4.0 ± 2.6	6.5 ± 7.1
SAPS II Score	28.3 ± 8.3	27.7 ± 8.7	29.9 ± 7.1
Type of surgery AKB/valve/multiple	23/22/21	16/17/14	7/5/7
Duration of surgery (min)	212.5 ± 47.2	206.2 ± 46.2	226.8 ± 47.5

Table 2(abstract P46)

Variable		Preop	Arrival ICU	POD 1	POD 3
ANGPTL2 (ng/ml)	All	66 ± 36	86 ± 30	96 ± 35	90 ± 35
	Non-DM2	71 ± 38	85 ± 32	97 ± 36	90 ± 38
	DM2	56 ± 29	87 ± 26	93 ± 31	90 ± 30
CXCL5 (pg/ml)	All	1022 ± 503	662 ± 357	742 ± 456	936 ± 595
	Non-DM2	1009 ± 479	620 ± 331	695 ± 380	890 ± 532
	DM2	1051 ± 563	756 ± 402	855 ± 598	1043 ± 723
Visfatin (ng/ml)	All	4.9 ± 2.5	13.2 ± 7.1	7.7 ± 3.4	12.6 ± 5.8
	Non-DM2	4.6 ± 2.4	12.5 ± 7.2	7.6 ± 3.6	12.6 ± 6.2
	DM2	5.3 ± 3.2	14.6 ± 6.9	7.8 ± 2.9	12.5 ± 4.9

anaemia. Iron may thus be proposed to critically ill patients (CI). However, iron may promote oxidative stress, which is potentially deleterious. In a mouse model, we previously demonstrated that iron induces less oxidative stress in inflamed mice than in control ones [1], but no data are available in the CI.

Objective: To compare the oxydative stress induced by an intravenous infusion of iron in CI and healthy volunteers (V).

Methods: Adult critically ill patients (from 2 ICUs) and healthy Volunteers were included after informed consent. Blood samples were drawn before (T0) and 2, 6 and 24 hours (T2, T6, T24) after an intravenous infusion of 100 mg of iron sucrose (Venofer©) over 60 minutes. Markers of lipid oxidation -8α-Isoprostanes (8ISO)-, protein oxidation -Advanced Oxydized Protein Product (AOPP)- as well as glutathion reduced/oxidized (GSH/GSSG), Non-transferrin bound iron (NTBI) have been measured at these time points. Variations of area under the curves from T0 to T6 ($\Delta AUC_{0\text{-}6}$) have been compared using a Wilcoxon test. Data are expressed as n(%), mean±SD or median[min-max].

Results: 38 CI have been studied (25(66%) males, aged 67.9[19-85]yrs, 38(100%) ventilated, SAPSII 48.5[21-80], Hb 8.4[6.6-11.8] g/dl) and 39 V (18(46%) males, aged 42.1[21-78] yrs, Hb 13.9[11.9-17.2] g/dl). Iron treatment indications for CI were (many causes possible): 18(45%) elevated soluble transferrin receptor (sTfR), 14(35%) ferritin < 100 µg/l + TSat< 20%, 12(30%) blood loss > one blood mass, 9(22%) elevated sTfR/log Ferritin ratio. At T_0, [8ISO] was higher in CI than in V 8.48[3.1-63.4] vs. 4.51[2.05-13.33] pmol/l), but the $\Delta AUC_{0\text{-}6h}$(8ISO) was not different (p=0,38). Only the $\Delta AUC_{0\text{-}6h}$ (GSH) was lower in V (p=0,009), arguing for a more important decrease in anti-oxidant defences. The table summarized all the results. Eight CI had a second set of dosages (after the 4th iron infusion) showing no difference in any markers compared to the first set of dosages.

Discussion: We haven't seen any increase in lipid (8ISO) or protein (AOPP) oxidation in CI compared to V. On the contrary, V had a greater decrease in anti-oxidant (ie GSH), suggesting higher oxidative stress. Iron

Table 1(abstract P47) Oxidative stress markers dosages.

	Critically ill (n=38)	Voluntaries (n=39)	P
ΔAUC0-H6 (8ISO)	0.06 [-86.51 - 29.45]	2.01[-16.60 - 17.95]	0.38
C0 AOPP (µg/l)	36.57 [17.46 - 98.90]	19.07 [10.49-34.92]	
ΔAUC0-H6 (AOPP)	14.8 [-172.3 - 110.1]	12.4 [-92.7 - 63.7]	0.69
C0 GSH (µg/l)	3.97 [1.49 -7.31]	1.22 [0.66 -2.12]	
ΔAUC0-H6 (GSH)	-8.34 [-615.4 - 329.36]	-142.4 [-1640.1 - 989.1]	0.009
C0 GSSG (µg/l)	94.90 [31.80 - 349.00]	414.2 [266.3 -845.5]	
ΔAUC0-H6 (GSSG)	1.44 [-163.17 -77.26]	-11.79 [-152.85 -116.36]	0.43
C0 NTBI (µg/L)	0.23 [-1.44 - 0.75]	.22 [-1.35 -0.89]	
ΔAUC0-H6 (NTBI)	0.74 [-3.75 - 9.53]	0.33 [-4.53 -9.77]	0.35

in the CI doesn't induce more oxidative stress than in V, but other studies are needed to confirm the absence of clinical toxicity.

Reference

1. *Heming CCM Crit Care Med* 2012, **40(7)**:2141-8.

SEPSIS: EXPERIMENTAL STUDIES

P48

0726. The effects of carnitine on renal tissue damage and metabolic alteration among the rat intraabdominal sepsis model

S Cetin*, S Deniz, A Sezer, H Sen, S Ozkan
Gulhane Military Medical Academy, Haydarpasa Training Hospital, Department of Anesthesiology and Reanimation, Istanbul, Turkey
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P48

Introduction: Sepsis is a systematic inflammatory reaction that causes renal damage frequently. Carnitine is considered as an essential mediator of metabolic pathway during sepsis and critical conditions. It has an important role in facilitating medium- and long-chain fatty acid transport from the cytosol into mitochondria for β-oxidation and energy generation [1].
Objectives: We investigated the effects of carnitine usage on renal tissue damage and metabolic changes on rats with sepsis model. We hypothesized that carnitine decreases systemic inflammation and renal tissue damage.
Methods: 21 male rats randomly divided into 3 groups: sham operated (SO; n=7), sepsis (S; n=7) and sepsis + carnitine (S+C; n=7). In SO group only laparatomy was performed. In S and S+C groups caecal ligation and puncture was performed. At the postoperative 6th, 30th and 54th hours 1ml saline was administered intraperitoneally to both PO and S groups. Similarly 100mg/kg carnitine was administered intraperitoneally to S+C group at the postoperative 6th, 30th and 54th hours. After gathering blood and renal tissue samples, all rats were sacrificed at 60 hours postoperatively. Tissue malondialdehyde (MDA) levels were measured for oxidative stress and inflammation. Serum cytokines (TNF-α, IL-1, IL-6 and IL-10), neutrophil gelatinase-associated lipocalin (NGAL) and anti-caspase 3 were measured for inflammation markers, renal tissue damage and renal apoptosis, respectively.
Results: In histopathological examination, S+C group has achieved better results in mononuclear cell infiltration score than the S group. Also, the increase in TNF-α, IL-1, IL-6, MDA, NGAL and anti-caspase 3 levels in S+C group were found to be lower than those of S group.
Conclusion: Using carnitine in sepsis decreases the serum cytokine levels and renal damage and apoptosis via supressing the oxidative stress and inflammation both in blood and tissue.

Reference

1. Hatamkhani S, *et al*: Carnitine and sepsis: a review of an old clinical dilemma. *J Pharm Pharm Sci* 2013, **16(3)**:414-23.

P49

0727. Normal saline versus ringer´s lactate in experimental sepsis

D Orbegozo Cortes*, S Fuhong, C Santacruz, K Hosokawa, H Xinrong, J Creteur, J-L Vincent, D De Backer
Erasme Hospital, Dept of Intensive Care, Brussels, Belgium
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P49

Introduction: The development of hyperchloremic acidosis associated with normal saline (NS) administration may be deleterious in septic shock.
Objectives: To compare the time-course of hemodynamics, organ dysfunction and survival with NS or Ringer's lactate (RL) in an experimental model of severe peritonitis.
Methods: Fourteen adult sheep (24-34 Kg) were anesthetized (midazolam, ketamine and morphine), mechanically ventilated and invasively monitored. A cecotomy was performed to collect autologous feces that were later re-injected into the peritoneal cavity through the abdominal wall to create abdominal sepsis. RL was administered during the surgical procedure. After baseline measurements, animals were randomly allocated to receive only NS or RL titrated to maintain pulmonary artery occlusion pressure at baseline level. Neither vasoactive agents nor antibiotics were used during the experiment. Animals were followed until death or for a maximum of 30 hours. Time-evolution for repeated measurement data was analyzed using a Generalized Estimating Equations approach in SPSS 19.0 (IBM) with a $p < 0.05$ considered as significant. Data are presented as median with inter-quartile range.
Results: See tables 1, 2 and 3.
Survival time was significantly shorter in the NS group than in the RL group (17 [14-20] hours vs 26 [23-29] hours, p Logrank=0.003).
Conclusions: In this sheep model of severe abdominal sepsis, NS-induced hyperchloremic acidosis was associated with an increased development of organ dysfunction and greater mortality.
Grant acknowledgment: Institutional funds only.

P50

0728. Removal of natural anti-galactose α1,3 galactose antibodies with GAS914 enhances humoral immunity and prevents sepsis mortality in mice

R Manez[1,2*], M Perez-Cruz[2], D Bello[2], MA Dominguez[3], C Costa[2]
[1]Hospital Universitari de Bellvitge, Intensive Care Medicine, Hospitalet de Llobregat, Spain; [2]Idibell, Hospital Universitari de Bellvitge, Hospitalet de Llobregat, Spain; [3]Hospital Universitari de Bellvitge, Hospitalet de Llobregat, Spain
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P50

Introduction: Increasing numbers of patients with immunodeficiencies, malignant diseases, advanced surgeries and prolonged stays in intensive

Table 1(abstract P49) Acid-base parameters

		T0	T4	T8	T12	T16	T20	T24	T28
Arterial pH	**RL**	7.41 (7.38-7.44)	7.43 (7.39-7.45)	7.41 (7.36-7.43)	7.37 (7.36-7.38)	7.33 (7.32-7.38)	7.24 (7.18-7.27)	7.12 (7.06-7.18)	7.12 (6.87-7.38)
	NS	7.40 (7.36-7.49)	7.39 (7.36-7.40)*	7.35 (7.25-7.35)*	7.23 (7.14-7.29)*	7.03 (6.97-7.18)*	7.10 (7.02-7.18)*	6.99 (6.99-6.99)*	-
Arterial PCO2 (mmHg)	**RL**	36 (32-36)	31 (30-31)	32 (30-32)	33 (31-35)	33 (32-34)	35 (34-37)	35 (32-36)	41 (32-50)
	NS	34 (33-37)	32 (30-34)	32 (31-34)	32 (31-34)	35 (33-36)	34 (33-34)	33 (33-33)	-
Chloride (mmol/L)	**RL**	108 (105-109)	110 (106-111)	111 (108-112)	111 (109-114)	113 (112-114)	114 (113-117)	112 (112-113)	110 (109-110)
	NS	111 (107-113)	115 (112-117)*	121 (117-123)*	126 (122-128)*	127 (124-130)*	128 (125-130)*	126 (126-126)*	-
Arterial Lactate (mmol/L)	**RL**	1.2 (1.0-1.5)	1.2 (0.9-1.5)	1.4 (1.1-1.7)	1.4 (1.2-1.8)	2.0 (1.3-3.3)	3.7 (3.2-5.4)	6.6 (5.9-10.0)	6.0 (1.1-10.8)
	NS	1.3 (0.8-1.9)	0.8 (0.7-1.2)	1.1 (0.7-1.3)*	1.5 (1.1-2.1)	5.8 (1.2-7.3)*	3.6 (2.3-4.8)	5.7 (5.7-5.7)	-

* = $p < 0.05$ compared with RL group

Table 2(abstract P49) Systemic parameters

		T0	T4	T8	T12	T16	T20	T24	T28
Mean Arterial Pressure (mmHg)	**RL**	103 (97-111)	90 (77-94)	82 (77-90)	68 (60-85)	59 (55-79)	55 (54-57)	51 (39-57)	36 (26-46)
	NS	97 (93-109)	88 (79-93)	67 (63-81)*	52 (41-63)*	37 (33-42)*	46 (36-57)*	40 (40-40)*	-
Cardiac Index (L/min/m²)	**RL**	5.1 (4.0-5.6)	4.4 (3.9-6.6)	4.7 (3.9-5.1)	4.6 (3.4-6.0)	4.3 (3.5-5.4)	4.9 (3.5-5.9)	4.6 (2.7-5.0)	3.9 (3.0-4.7)
	NS	4.9 (4.0-5.5)	4.8 (3.8-5.5)	3.2 (2.6-4.2)*	2.9 (2.7-3.3)*	2.1 (1.5-2.8)*	3.1 (2.9-3.3)*	1.4 (1.4-1.4)*	-
Diuresis (ml/Kg/H)	**RL**	1.0 (0.5-2.0)	0.9 (0.6-2.2)	1.3 (0.5-2.3)	1.2 (0.3-2.9)	0.2 (0.0-1.3)	0.1 (0.0-0.4)	0.0 (0.0-0.0)	0.5 (0.0-1.0)
	NS	1.0 (0.6-1.8)	0.9 (0.7-1.2)	1.3 (0.2-2.8)	0.2 (0.0-0.4)*	0.0 (0.0-0.1)	0.2 (0.0-0.4)	0.0 (0.0-0.0)	-
PaO2/FiO2 ratio	**RL**	462 (420-493)	482 (364-496)	458 (317-483)	352 (222-444)	266 (220-359)	251 (170-324)	281 (150-298)	138 (64-212)
	NS	388 (322-420)	385 (284-486)	268 (259-400)*	192 (173-299)	176 (160-292)	217 (177-257)	204 (204-204)	-

* = $p < 0.05$ compared with RL group

Table 3(abstract P49) Laboratory parameters

		T0	T4	T8	T12	T16	T20	T24	T28
Creatinine (mg/dL)	**RL**	0.9 (0.7-1.2)	0.8 (0.6-1.0)	0.9 (0.6-1.0)	1.1 (0.7-1.2)	1.4 (0.9-2.0)	2.0 (1.4-2.7)	3.0 (2.4-3.2)	2.3 (1.4-3.2)
	NS	0.7 (0.6-1.0)	0.7 (0.6-0.8)	0.7 (0.7-0.9)	1.2 (0.8-1.6)	2.0 (1.1-2.5)	1.5 (1.4-1.6)	2.4 (2.4-2.4)	-
PTT (sec)	**RL**	32 (28-36)	33 (32-41)	40 (35-47)	45 (40-53)	49 (40-59)	50 (40-61)	60 (49-70)	108 (67-150)
	NS	36 (24-42)	34 (27-42)	45 (37-47)	55 (46-55)	62 (50-135)	104 (58-150)*	63 (63-63)	-

* = $p < 0.05$ compared with RL group

care units are at risk for developing infections by enteric bacteria, which often are systemic (sepsis) and caused by multidrug resistance isolates. Intestinal bacteria provide also a constant antigenic stimulation for the synthesis of natural anti-carbohydrate antibodies such as those targeting blood group-associated antigens or galactose α1,3 galactose (Gal). Enteric bacteria that cause sepsis are more likely to bind anti-Gal antibodies than those isolated from stools, which appears related to the higher resistance to serum killing evidenced by the former microorganisms.

Objective: Investigate the impact of removing anti-Gal antibodies in the sepsis after cecal ligation and puncture (CLP) in α1,3 galactosyltransferase knockout (Gal-KO) mice. These mice produce from four months of age natural anti-Gal antibodies as humans without the need of any additional Gal antigen exposure.

Methods: Anti-Gal antibodies were removed *in vivo* using a soluble Gal trisaccharide-polylysine conjugate (GAS914) and determined by ELISA. Binding of serum antibodies to *Escherichia Coli* isolated from Gal-KO mice blood was performed by FACS and serum bactericidal activity was determined by a complement-mediated assay. The pattern of 40 cytokines and antibodies against 577 carbohydrate and bacterial antigens before and after GAS914 was evaluated by two different arrays.

Results: GAS914 at 10 mg/kg subcutaneously every other day for two weeks led to the removal of anti-Gal antibodies in Gal-KO mice from day one. Removal of anti-Gal antibodies with GAS914 significantly improved survival in Gal-KO mice subsequently subjected to CLP on day 3 (83% versus 35% in controls; $p < 0.01$). On day one after CLP there was a reduction of 50% in the level of anti-Gal antibodies in untreated Gal-KO mice. Blood cultures obtained 24 h after CLP were positive for *Escherichia Coli* in 83% of mice carrying anti-Gal antibodies and 87.5% of the animals lacking them. Pulse field electrophoresis did not show differences between *E. Coli* blood isolates from GAS914-treated and untreated mice. Removal of anti-Gal antibodies was associated with a significant increase in serum IgG binding and bactericidal activity to *E. Coli*. GAS914 administration was associated in the glycan array with the removal of anti-Gal antibodies and the significant augment of binding to several bacterial antigens. Finally, GAS914 also significantly reduced the level of several proinflammatory and anti-inflammatory cytokines prior to CLP in Gal-KO mice.

Conclusion: Removal of natural anti-Gal antibodies with GAS914 enhances humoral immunity against enteric bacteria expressing Gal-antigen isolated from the blood preventing sepsis mortality after CLP in mice. Depletion of existing antibodies may be a new approach to boost immunity and prevent or treat infection diseases and other disorders.

P51

0729. Interstitial changes in spleen during sepsis

ØS Svendsen[1*], L Stangeland[2], B Elvevoll[1,3], BT Gjertsen[2], J Skavland[2], H Wiig[4], O Tenstad[4], P Husby[1,3]

[1]Haukeland University Hospital, Department of Anaesthesia and Intensive Care, Bergen, Norway; [2]University of Bergen, Department of Clinical Science, Bergen, Norway; [3]University of Bergen, Department of Clinical Medicine, Bergen, Norway; [4]University of Bergen, Department of Biomedicine, Bergen, Norway

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P51

Introduction: The spleen has important functions in innate and acquired immunological responses, and splenectomy leads to risk for overwhelming infections [1]. The microcirculation in spleen is only partly understood, and knowledge of the local interstitial fluid composition and changes during sepsis can offer new insights in the systemic inflammatory response syndrome.

Objectives: To isolate prenodal lymph from spleen in pigs during a control situation and during sepsis, and analyze the composition of proteins in the lymph.

Methods: Pigs were anesthetized and monitored. Plasma volumes were measured and the changes in hematocrit were, after corrected for the fluid gain and loss, used to estimate global plasma extravasation. One pre-nodal spleen lymph-vessel was cannulated. LPS was administered to induce sepsis. Lymph and plasma were studied to investigate the specific local reaction in spleen during control situation and sepsis.

Results: Basal extravasation rate was 0.24±0.05 mL/kg*min^{-1}(n=4). Although technically demanding, we succeeded in collecting lymph from the spleen, deemed macroscopically to be prenodal. After inducing sepsis, pulmonary artery pressure increased from 19.0±2.2 mmHg to 30.4±4.6 mmHg one hour after LPS, (n=5, p=0.001). Adrenaline and Ringer's acetate were administrated to support the animals through the inflammatory response. Plasma extravasation rate increased twofold to 0.53±0.04 mL/kg*min^{-1} (n=4, p=0.003). Colloidosmotic pressure in plasma was 14.1±0.6 mmHg in control situation, decreasing to 12.9±0.5 mmHg (n=4, p=0.003) one hour after LPS.

Colloidosmotic pressure in lymph was 13.0±1.7 mmHg (n=4) in control situation. During the first hours after sepsis, colloidosmotic pressure in spleen lymph decreased to 11.8±2.4 mmHg (n=3).

Conclusions: Our model shows typically hemodynamic changes previously described to be associated with an initial endotoxin reaction in pigs [2]. We show that it is possible to isolate lymph from spleen. The colloidosmotic pressures in lymph and plasma suggest a low protein reflection coefficient in spleen microvasculature.

Grant acknowledgment: Supported by The Western Norway Regional Health Authority.

References

1. Semaeva E, *et al*: Access to the spleen microenvironment through lymph shows local cytokine production, increased cell flux, and altered signaling of immune cells during lipopolysaccharide-induced acute inflammation. *Journal of immunology* 2010, **184**:4547-4556.
2. Schmidhammer R, *et al*: Infusion of increasing doses of endotoxin induces progressive acute lung injury but prevents early pulmonary hypertension in pigs. *Shock* 2006, **25**:389-394.

P52

0730. Plasma endocan levels are associated with endothelial dysfunction during experimental human endotoxemia

LT van Eijk[1,2*], LAE Cox[1,2,3], BPC Ramakers[1,2], MJ Dorresteijn[1,2], J Gerretsen[1,2], M Kox[1,2,3], P Pickkers[1,2]

[1]Radboud University Medical Center, Intensive Care Medicine, Nijmegen, Netherlands; [2]Radboud Institute for Infectious Diseases, Nijmegen, Netherlands; [3]Radboud University Medical Center, Anesthesiology, Pain and Palliative Medicine, Nijmegen, Netherlands

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P52

Introduction: The endothelium plays a central role in pathophysiology of sepsis. Systemic inflammation results in endothelial dysfunction, contributing to the development of shock and multiple organ dysfunction. However, an easy to obtain, early, and clinically applicable marker of endothelial dysfunction is currently not available. Such a marker could guide early and targeted therapy such as selective iNOS inhibition. Endocan is a soluble proteoglycan secreted by endothelial cells in response to pro-inflammatory cytokines, bacterial endotoxins, and angiogenic factors, and enhances microvascular permeability. Plasma endocan levels are increased in septic patients, and have been shown to correlate with sepsis severity and mortality. However, the direct relationship between endocan and endothelial function has not yet been investigated in humans *in vivo*.

Objectives: To investigate the kinetics of plasma endocan levels during human endotoxemia and to examine their relation with inflammation-induced endothelial dysfunction.

Methods: Seventeen healthy male volunteers were subjected to experimental endotoxemia (infusion of 2 ng/kg *E.Coli* lipopolysaccharide [LPS]). Plasma levels of inflammatory cytokines (TNF-α, IL-6, IL-10, and IL-1RA), endocan, ICAM, and VCAM were measured at T=0, 0.5, 1, 1.5, 2, 4, 6 and 8 hrs after LPS infusion. Both before and 4 hrs after LPS administration, endothelial function was assessed by determination of the vasodilatory response of forearm blood vessels to the incremental intra-arterial infusion of endothelium-dependent (acetylcholine) or endothelium-independent (nitroglycerine/nitroprusside) vasodilators using venous occlusion plethysmography. Furthermore, correlations between the increases in plasma endocan, ICAM, and VCAM levels, and the endotoxemia-induced changes in vasodilatory responses were explored.

Results: Plasma levels of all measured cytokines, endocan, ICAM, and VCAM concentrations significantly increased after LPS administration (Figure 1). LPS administration resulted in a significantly blunted response of forearm vasculature to acetylcholine (P = 0.028, Figure 2A), whereas the response to nitroglycerine/nitroprusside was not significantly affected (P = 0.11, Figure 2B). Furthermore, there was a significant correlation between the increase in plasma endocan levels and the attenuation of vasodilatory responses to acetylcholine (Pearson's r = -0.49, P = 0.049). No correlation existed between plasma levels of ICAM or VCAM and the attenuation of the acetylcholine-induced vasodilatory response.

Conclusions: Endocan levels are related to endothelial dysfunction during systemic inflammation in humans *in vivo*. Therefore, it may prove to be a suitable marker for the early identification of endothelial dysfunction and could guide targeted therapy.

P53

0731. Effects of sildenafil in a porcine model of endotoxemia

DA Kemper[1], DA Otsuki[1], DR Maia[1], N Queiroz-Hazarbassanov[2], CO Massoco[3], JOC Auler Jr[1], DT Fantoni[1,2*]

[1]Faculdade de Medicina da Universidade de São Paulo, Laboratory of Anesthesiology (LIM08), São Paulo, Brazil; [2]Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, Surgery, São Paulo, Brazil; [3]Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, Pathology, São Paulo, Brazil

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P53

Introduction: Sepsis-induced lung injury is one of the major causes of morbidity and mortality in intensive care patients [1]. The clinical manifestations include pulmonary hypertension, formation of extravascular lung water (EVLW), and deterioration of pulmonary gas exchange. Administration of sildenafil, a selective inhibitor of isoenzyme phosphodiesterase-5, in patients with pulmonary hypertension improves oxygenation and ameliorates pulmonary hypertension [2].

Objectives: To evaluate the effect of sildenafil on endotoxin-induced lung injury in pigs.

Methods: Twenty anesthetized and mechanically ventilated pigs were randomized after baseline (BL) measurements to Control (saline solution) or Sildenafil (100mg) group. After 30 minutes of saline/sildenafil administration, all animals were submitted to a continuous lipopolysaccharide (LPS) infusion (4mcg/kg/min) until the end of study. Hemodynamics and oxygenation parameters were evaluated at BL, 30,

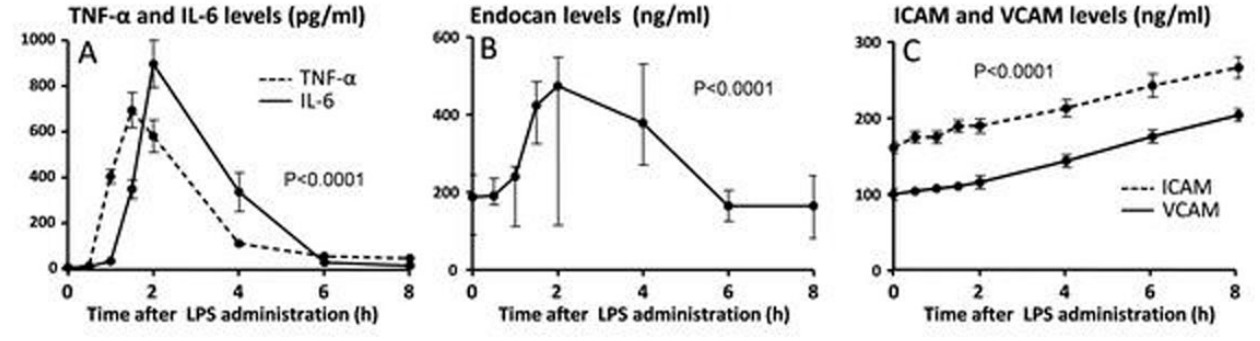

Figure 1(abstract P52) Plasma concentrations of cytokines TNF-α and IL-6 (A), endocan (B), and ICAM and VCAM (C) during endotoxemia. Data in panels A and C are expressed as mean with SEM, and analyzed using repeated measures one-way ANOVA (P-values apply to all indicated parameters). The non-depicted anti-inflammatory cytokines IL-10 and IL-1RA peaked at T=3 hrs (702 ± 183 pg/ml and 7332 ± 759 pg/ml, respectively, both P<0.0001). Data in panel B are expressed as median with interquartile range, and analzed using Friedman test.

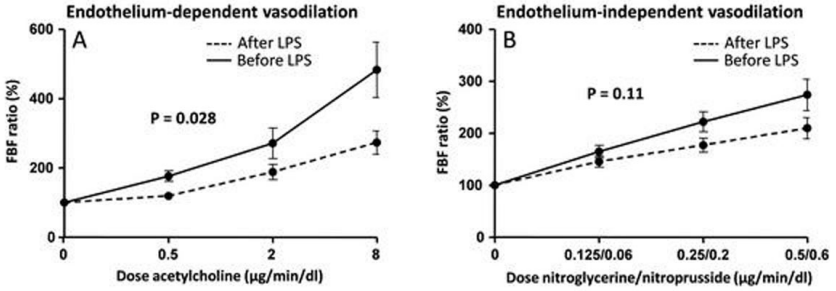

Figure 2(abstract P52) Forearm blood flow (FBF) ratios in response to the acetylcholine (A) and nitroglyverine/nitroprusside (B) before and during endotoxemia. FBF ratios were calculated by dividing the flow in the experimental arm by the concurrent flow in the non-infused (control) arm, to adjust for systemic changes in flow. At baseline this ratio was set at 100%. Data are expressed as mean with SEM. p-values indicate difference between FBF ratios before and after LPS administration calculated using repeated measures two-way ANOVA (interaction term).

60, 120 and 180 minutes after LPS (LPS60, LPS120 and LPS180). Plasma cytokines (TNF-alpha, IL-1beta, IL-6 and IL-10) were evaluated at BL and LPS180. The parametric data were analyzed using ANOVA for repeated measurements and nonparametric data with Kruskall-Wallis and the Mann-Whitney U test.

Results: Endotoxemia induced a significant pulmonary hypertension with more than a twofold increase in mean arterial pulmonary pressure and pulmonary vascular resistance index, and also a decrease in PaO_2/FiO_2. Mean arterial pulmonary and mean arterial pressures were significantly lower in Sildenafil group. Sildenafil improved arterial oxygen tension but also increased the shunt fraction (Figure1).

All cytokines increased after LPS infusion in both groups and no difference was observed between the animals receiving sildenafil and normal saline.

Conclusions: Sildenafil administration improved pulmonary hypertension and oxygenation in LPS-induced lung injury but increased shunt fraction

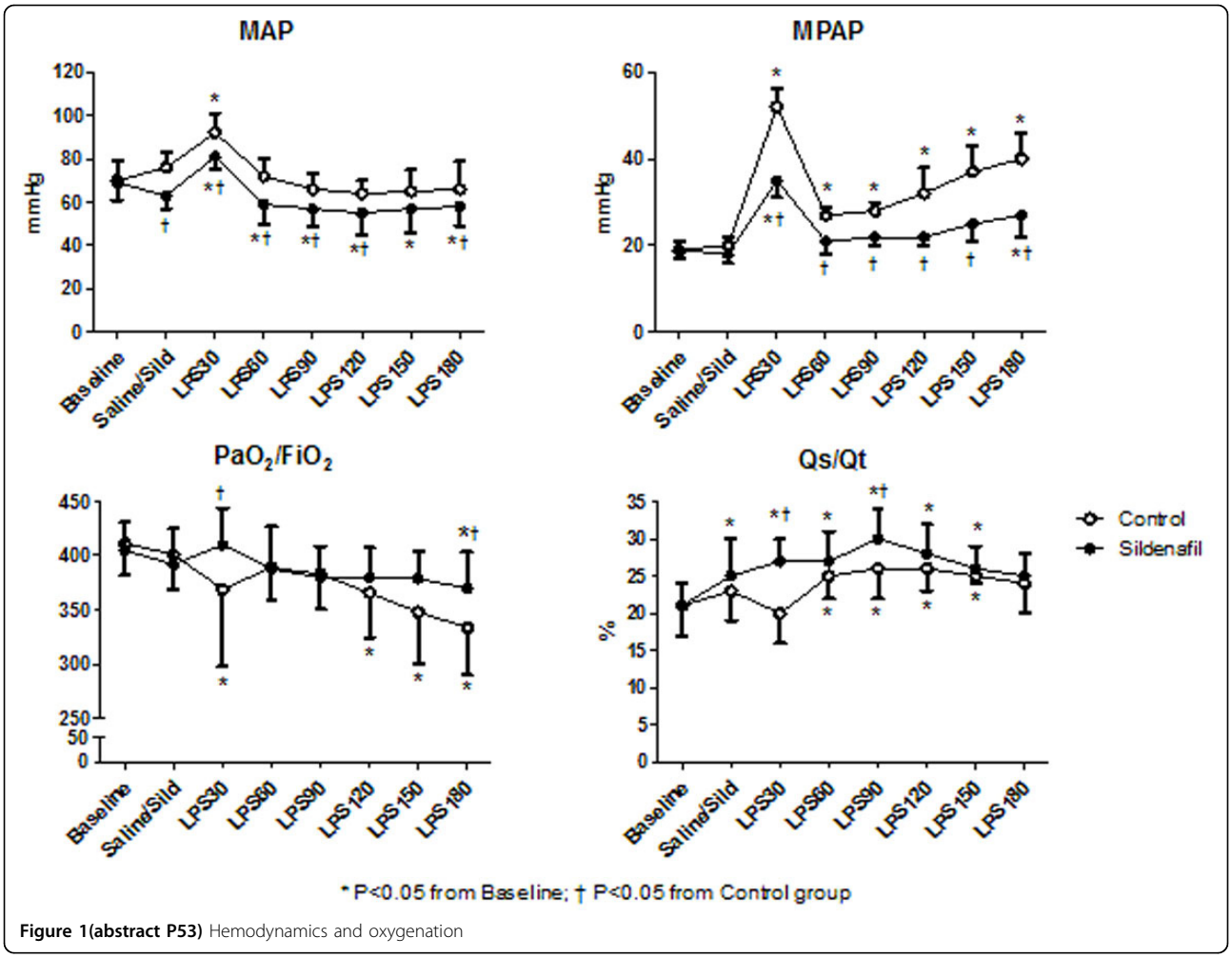

Figure 1(abstract P53) Hemodynamics and oxygenation

and promoted systemic hypotension. It remains unclear whether sildenafil may be beneficial in sepsis patients.

Grant acknowledgment: Laboratory of Anesthesiology (LIM08), Faculdade de Medicina da Universidade de São Paulo.

References

1. Rubenfeld GD, Caldwell E, Peabody E, *et al*: **Incidence and Outcomes of Acute Lung Injury.** *N Engl J Med* 2005, **353**:1685-93.
2. Galie N, Ghofrani HA, Torbicki A, *et al*: **Sildenafil citrate therapy for pulmonary arterial hypertension.** *N Engl J Med* 2005, **353**:2148-57.

P54

0732. Sequential changes in the pattern of liver architecture in the acute phase of severe sepsis under the vision of videomicroscopy. Experimental study

IHJ Koh[1*], JC Vieirra[1], J Almeida-Filho[2], RC Tedesco[3], RB Souza[3], AMA Liberatore[1]
[1]Federal University of Sao Paulo, Surgery, Sao Paulo, Brazil; [2]Federal University Foundation of Vale do São Francisco, Surgery, Petrolina, Brazil; [3]Federal University of Sao Paulo, Morfology and Genetics, Sao Paulo, Brazil
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P54

Introduction: Therapeutic grounded in the specific state of microcirculatory dysfunction in septic patients is still an impediment due to technological limitations, and consequently there is little knowledge about the pathophysiological microcirculatory dynamics associated with organ failure.
Objectives: Investigate the pattern of hepatic microcirculation during the golden period of therapy in severe sepsis.
Methods: Wistar rats underwent severe sepsis (iv. *E. coli* 2×109 CFU, DL70-80 in 26 hours3) and under general anesthesia the dynamics of microcirculatory at the liver surface was monitored by SDF1,2 at T0,T30min and T1-T6 hours and the tissue injury by histology (T0,T2h,T6h). Saline injection was used as control.
Results: At SDF-images is possible to visualize the hexagonal architecture of hepatic lobules with radiated sinusoidal blood flow draining into the central lobular vein between the columns of hepatocytes, showing the close interaction between microcirculation and hepatocytes. In sepsis, already since 30min (T30, T1) merges areas with enlargement of hepatocytes with dilated sinusoids and narrowing of sinusoids, suggesting broad cellular edema and sinusoids that make up those narrowed blood flow limitation. From 3 hours, there was a predominance of narrowed sinusoids with or without visible flow, associated with the appearance of coalescence of columns of hepatocytes. Up to six hours, lobular architecture became

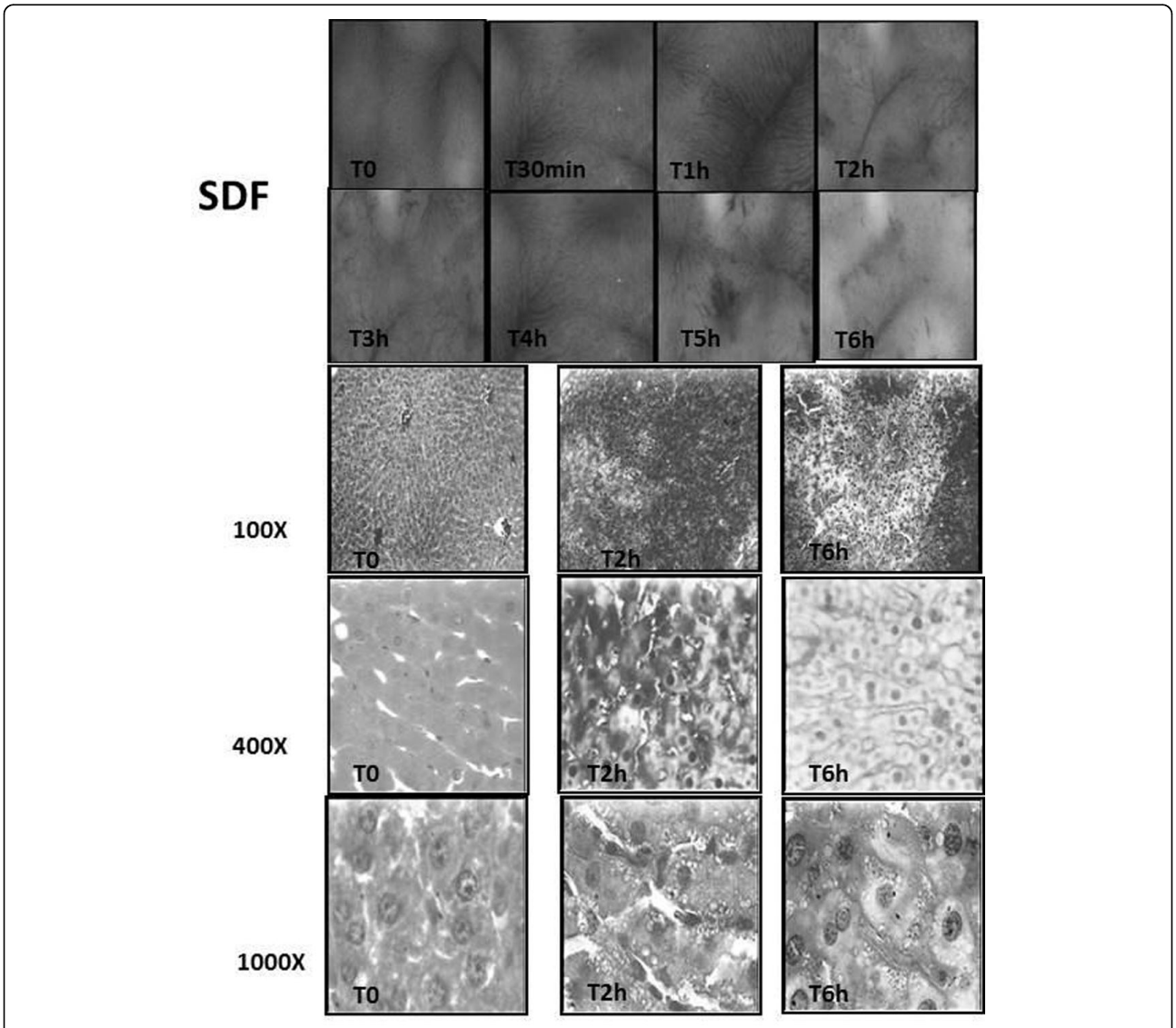

Figure 1(abstract P54) Liver images by SDF and Histology.

misshapen and disseminated in a crescent manner. At six hours of sepsis the lobular architecture was not anymore identifiable in the entire length. The blurring of the edges of the columns of hepatocytes by its enlargement with progression of sepsis, associated with narrowing of the adjacent sinusoids, suggested that a severe vascular and tissue dysfunction were in progress. Confronting these findings with histology, the enlarged perivascular areas were composed mostly of cytoplasm edema at the early sepsis phase and of varying stages of the cell necrosis process at the further periods. These processes occurred throughout the liver showing that the surface findings can be extensive to deeper areas.

These findings suggested that the process involved in the genesis of hepatic failure in sepsis could be due to the cyclical repetition of the event: sinusoidal microcirculatory dysfunction - cytopathic hypoxia of the hepatocytes - edema of the columns of hepatocytes - compression of sinusoids - hepatic lobules dysfunction.

Conclusions: The genesis of liver failure in severe sepsis appears to depend on the repetitive cyclic of sinusoidal dysfunction and subsequent adjacent hepatocytes that in turn exacerbates the progression of the liver dysfunction, thus suggesting the conjoined participation of both factors in the genesis of the solid organ dysfunction.

Grant acknowledgment: FAPESP 2011/20401-4.

References

1. Koh Ivan HJ, *et al*: *Shock* 2010, **34(7 Suppl 1)**:27-33.
2. De Backer D, *et al*: *Intensive Care Med* 2010, **36**:1813-1825.

P55

0733. Impact of endotoxin challenge on disseminated intravascular coagulation in obese minipigs

T Duburcq[1,2,3*], A Tournoys[4], F Pattou[1,2], T Hubert[1,2], V Gmyr[1,2], L Quintane[1,2], R Favory[3], J Mangalaboyi[3], M Jourdain[1,2,3]
[1]INSERM U859, Lille, France; [2]European Genomic Institute for Diabetes (EGID), Lille, France; [3]Pole de Réanimation CHRU, Lille, France; [4]Centre de Biologie Pathologie CHRU, Lille, France

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P55

Introduction: An early activation of coagulation and fibrinolysis occurs during sepsis and results in a marked increase of thrombin and fibrin formation, leading to the syndrome of disseminated intravascular coagulation (DIC). DIC is a strong predictor of death and multiple organ failure in patients with septic shock [1]. Obesity has been demonstrated to be a hypercoagulable [2] and hypofibrinolytic [3] state but its impact on coagulation and fibrinolysis during sepsis has never been studied.

Objectives: In this study, we aimed to determine if obesity impairs DIC in an acute endotoxic shock using minipigs.

Methods: This was a prospective, comparative and experimental study, approved by the Animal Ethics Committee. Pigs were chosen as a clinically relevant species, resembling to humans in coagulation reactions. Four groups of five "Yucatan" minipigs were studied: lean and obese control groups, lean LPS group receiving Escherichia Coli endotoxin (LPS) and obese LPS group receiving the same endotoxin dose. We measured standard coagulation parameters [prothrombin time (PT), platelet count and fibrinogen levels], thrombin-antithrombin complex (TAT), tissue plasminogen activator (t-PA) and plasminogen activator inhibitor-1 (PAI-1). All measurements were performed at baseline and at 30, 60, 90, 150 and 300 minutes. Results were given as median with 25-75 interquartile ranges.

Results: At baseline, platelet count (477 [428-532] vs. 381 [307-442] G/l; p=0.005) and fibrinogen levels (4.6 [3.8-5.2] vs. 2 [1.8-2.9] g/l; p< 0.001) were significantly higher whereas prothrombin time (80 [76-92] vs. 96 [89-100] %; p=0.01) was significantly lower in obese pigs compared to lean pigs. Control groups remained stable during the study-period. In LPS groups, administration of endotoxin resulted in a typical hypokinetic shock with DIC. The decrease in coagulation parameters (PT, platelet count and fibrinogen levels) and the increase in TAT complex (581 [382-1057] vs. 247 [125-369] µg/ml at 150 min; p=0.03) were significantly more important in obese LPS group compared to lean LPS group. Concerning the fibrinolytic reaction, we found a more important increase of PAI-1 in obese LPS group at 300 min (481 [365-617] ng/ml vs. 355 [209-660] ng/ml; p=0.66) without reaching statistical significance. Nevertheless, the increase of t-PA was significantly lower in obese LPS group compared to lean LPS group at 90 min (10 [8-17] vs. 5 [2-9] ng/ml; p=0.04).

Conclusions: In our model of endotoxic shock, obese pigs developed a more severe disseminated intravascular coagulation with a more serious procoagulant response.

References

1. Angstwurm MWA, *et al*: *Crit Care Med* 2006, **34(2)**:314-320, févr.
2. Stoppa-Vaucher S, *et al*: *Obes Silver Spring Md* 2012, **20(8)**:1662-1668, août.
3. Semeraro F, *et al*: *Thromb Haemost* 2012, **108(2)**:311-317, août.

P56

0734. Early changes in heart rate predict long-term survival in a rodent model of sepsis

A Rudiger[1*], M Arrigo[2], T Hauffe[1], DR Spahn[1], D Bettex[1]
[1]University Hospital Zurich, Institute of Anesthesiology, Zurich, Switzerland; [2]University Hospital Zurich, Clinic for Cardiology, Zurich, Switzerland

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P56

Introduction: In sepsis, early prognostication of outcome would be helpful both for the detection of underlying mechanisms (adaptive vs. maladaptive) and the development of novel treatments for potential non-survivors.

Objectives: We tested whether telemetry derived heart rate (HR) changes during early sepsis could prognosticate outcome in a long-term rat model of fecal peritonitis.

Methods: Male Wistar rats were instrumented with telemetry for continuous HR and temperature recording. Abdominal sepsis was induced by intraperitoneal injection of fecal slurry. A jugular catheter allowed the intravenous administration of opioids (nalbuphin 1mg/kg/h), antibiotics (rocephin 30mg/kg at 4 and 24h) and crystalloids (bolus of 20ml/kg at 4h, followed by an infusion of 10ml/kg/h, halved after 8 and 24h). The awake animals were followed for 48 hours.

Results: Septic animals became lethargic and febrile. Mortality was 30%, and all deaths occurred between 4 and 24 hours. Postmortem examination showed variable degrees of peritonitis and purulent ascites. Rats surviving 24 hours showed clinical signs of recovery.

ROC analysis revealed that the change in HR between baseline and 4h was a good prognosticator (AUC 0.84, 95% CI 0.56-1.00; p=0.03). An increase in HR of ≥50 bpm between baseline and 4 hours separated survivors and non-survivors with a sensitivity and specificity of 80% and 100%, respectively.

Conclusions: In this clinically relevant rat model of abdominal sepsis changes in HR as early as 4 hours after the septic insult predicted outcome with good sensitivity and excellent specificity. This will allow future investigation of adaptive changes in potential survivors and mechanisms of death in potential non-survivors. In addition, novel sepsis-treatments (e.g. beta-blockers) can be tested in regards of their beneficial and harmful effects in potential non-survivors and survivors.

Grant acknowledgment: ESICM ECCRN Established Investigator Award 2011 to Alain Rudiger

Table 1(abstract P56) Heart rate (bpm) in survivors and non-survivors:

	Survivors (n=14)	Non-survivors (n=6)	p-value
At BL	405 (329-471)	394 (296-439)	0.31
4 h after septic insult	389 (318-440)	452 (349-494)	0.11
6 hours post septic insult	416 (331-494)	500 (418-594)	0.02
Change BL to 4 hours	-10 (-87-49)	61 (-52-85)	<0.01

Values are median (range); BL = baseline

References
1. Dyson A, Rudiger A, Singer M: *Intensive Care Med* 2011, **37**:1192-200.
2. Rudiger A, Dyson A, Felmann K, *et al*: *Clin Science* 2013, **124**:391-401.

P57

0735. Effects of metoprolol in a porcine model of septic shock

AL Corrêa[1*], DT Fantoni[2], JOC Auler Jr[1], NG Queiroz-Hazarbassanov[3], CO Massoco[3], DA Otsuki[1]
[1]Faculdade de Medicina da Universidade de São Paulo, Department of Surgery, São Paulo, Brazil; [2]Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, Department of Surgery, São Paulo, Brazil; [3]Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, Department of Pathology, São Paulo, Brazil
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P57

Introduction: The use of cardiovascular drugs increased in recent years [1], including the use of β-blockers, such as metoprolol. And although the β-blockade is still very contradictory in patients with sepsis, both studies in experimental models and in septic patients with previous prescription of β-blockers have shown a potential role of these drugs in patients with sepsis [2,3].
Objectives: To evaluate the effect of metoprolol, a beta 1 selective beta-blocker, over echocardiographic parameters, cytokines and cardiac markers, in a porcine model of septic shock.
Methods: Twenty pigs in which septic shock was induced through intravenous *E. coli* infusion ($6x10^9$ c.f.u/kg in 2h) were randomly assigned (n=10 per group) to the Metoprolol group (214.2 μg kg^{-1} of metoprol infused in 45 minutes) or Control group, which received a correspondent volume of normal saline. Transesophageal echocardiographic (TEE) parameters were recorded at baseline, T120 (end of bacteria infusion), T180, T240, T300 and T360 minutes. Blood samples obtained at baseline, T120 and T360, were analyzed for cytokines (tumor-necrosis factor (TNF)-alpha, interleukin (IL)-1beta, IL-6 and IL-10), troponin I (TNNI) and B-type natriuretic peptide (BNP) using commercial ELISA kits. A resuscitation protocol with lactated Ringer's solution, norepinephrine and dobutamine was used during the study to maintain the mean arterial pressure > 65 mmHg, the central venous pressure between 8-12 mmHg and the mixed venous oxygen saturation > 65%. TEE and IL-10 results were compared using the two-way repeated measures ANOVA. The nonparametric data were analyzed with the Kruskall-Wallis and the Mann-Whitney U test.
Results: The TEE parameters showed a reduction of the left ventricular diastolic and end-systolic volumes ($p < 0.001$) and areas ($p < 0.001$) in both groups after sepsis induction. No alteration was observed on the measures of left ventricular ejection fraction. Both groups presented a similar behaviour of all cytokines, with a significant elevation after bacteria infusion ($p< 0.001$). Although both groups have shown an increase in TNNI, only in the Control group this increase was significant ($p=0.002$). No difference in the BNP values was observed between timepoints or between animals receiving metoprolol or normal saline.
Conclusions: The administration of metoprolol did not improve echocardiographic parameters, cytokines or cardiac markers in pigs with septic shock. And despite the lack of beneficial effects of this drug, in a scenario where the administration of β-blockers is increasing constantly, it is important to know that its administration is well tolerated in these patients.
Grant acknowledgment: FAPESP 11/21327-2 and DS-PROAP/CAPES.
References
1. Koopman C, *et al*: *Eur Heart J* 2013, **34(41)**:3198-3205.
2. Ackland GL, *et al*: *Crit Care Med* 2010, **38**:388-394.
3. Morelli A, *et al*: *JAMA* 2013, **310(16)**:1683-1691.

P58

0736. Role of $_{c}$AMP in PAF-induced intestinal endo-and epithelial dysfunction

I Lautenschläger[1*], K Zitta[1], J Sarau[1,2], H Dombrowsky[1,2], YL Wong[1], M Albrecht[1], S Uhlig[3], I Frerichs[1], N Weiler[1]
[1]University Medical Centre Schleswig-Holstein, Dept. Anaesthesiology and Intensive Care Medicine, Kiel, Germany; [2]Leibniz Centre for Medicine and Biosciences, Priority Area Asthma and Allergies, Borstel, Germany; [3]RWTH, Dept. of Pharmacology and Toxicology, Aachen, Germany
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P58

Table 1(abstract P58)

	PAF	PAF+PDE/AC	
ΔPmax (mmHg)	30.2±4.0	14.7±1.4	p<0.05
tdΔPmax (min)	1.45±0.15	0.77±0.11	p<0.05
Vloss (ml)	19.2±5.7	3.6±0.7	p<0.05
FITClym (mg/15min/g $^{\Psi}$)	0.407±0.078	0.026±0.011	p<0.05
FITClum (mg/15min/g $^{\Psi}$)	0.671±0.169	0.013±0.010	p<0.05

$^{\Psi}$, *dry weight*

cAMP levels in control and PAF treated intestines were comparable (cAMPCON 4.99±1.76 nM vs cAMPPAF 4.98±0.88 nM, p>0.1).

Introduction: Platelet activating factor (PAF) induces vascular barrier breakdown and intestinal failure that contribute to the development of sepsis. The exact cellular mechanisms are not well understood.
Objectives: We aim to analyse the role of cAMP in PAF-induced intestinal endo- and epithelial dysfunction.
Methods: An isolated model of the rat small bowel (1) was used. Intestines were stimulated with a 0.5 nmol PAF bolus via the mesenteric artery alone (PAF, n=5) or after pretreatment with IBMX (100μM) and forskolin (0.5μM) for 20 min (PAF+PDE/AC, n=4) to increase intracellular cAMP by inhibition of phosphodiesterase (PDE) and stimulation of adenylate cyclase (AC). The pressure responses, the vascular fluid loss and the transfer of FITC-labeled vascular dextran were monitored. cAMP was measured in PAF stimulated and untreated intestines (n=5) 3 min after PAF-stimulation or at the equivalent time point.
Results: The maximal pressure amplitude (ΔPmax), the time delay to achieve ΔPmax (tdΔPmax), the vascular volume loss (Vloss) as well as the macromolecule transfer to the lymph (FITClym) and to the lumen (FITClum) were reduced significantly by inibition of PDE and stimulation of AC [Table 1].
Conclusions: While drugs that increase the intracellular cAMP concentration protect the intestine from PAF-induced endo- and epithelial dysfunction, all the cellular effects of PAF can not be explained by a deprivation of cAMP in the intestine.
Reference
1. Lautenschläger , *et al*: *Am J Physiol Gastrointest Liver Physiol* 2010, **298**: G304-313.

P59

0737. Statins protect the vasculature from excessive Angpt-2 production in sepsis

K Thamm[1], C Ghosh[2], JT Kielstein[1], WC Aird[2], A Santel[3], SM Parikh[2], S David[1*]
[1]Hannover Medical School, Hannover, Germany; [2]Harvard Medical School, Center for Vascular Biology Research, Boston, USA; [3]Silence Therapeutics AG, Berlin, Germany
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P59

Introduction: Sepsis is a syndrome of systemic inflammation arising from infection that constitutes a top-ten cause of adult mortality. The recent withdrawal of a specific sepsis therapeutic has diminished pharmaceutical enthusiasm for developing novel drugs in this domain. Angiopoietin-2 (Angpt-2) is an endothelial-derived protein that potentiates vascular inflammation and permeability and may be involved in sepsis pathogenesis.
Objectives: We set out to screen well-established drugs for their Angpt-2 lowering potential to ameliorate sepsis morbidity and to analyse the underlying molecular mechanism.
Methods: In vitro: FDA-approved library screening in human umbilical vein endothelial cells (HUVECs) and confirmation via Angpt-2 ELISA and quantitative RT-PCR.
In vivo: Murine experimental sepsis was induced both by endotoxin (LPS) and cecal ligation and puncture (CLP). Mice were either treated with an Angpt-2 specific siRNA, simvastatin, or both and survival as well as the direct effect on Angpt-2 production was assessed by RT-PCR. In men: We analyzed circulating Angpt-2 levels in a retrospective matched case-control study in individuals with septic shock.

Results: We found that simvastatin reduced endothelial Angpt-2 release and transcription in a time- and dose dependent manner in HUVECs. This effect required Simvastatin's HMG-CoA reductase activity. Similarly, *in vivo* simvastatin reduced the transcription of Angpt-2 murine lungs. In septic mice, specific inhibition of Angpt-2 in the pulmonary endothelium *via* an RNAi approach improved survival by 50% (p=0.002). Simvastatin equally improved survival, but the combination of Angpt-2 siRNA and simvastatin showed no additive benefit indicating that simvastatin might act *via* Angpt-2 inhibition. To investigate a potential link between statins and Angpt-2 in humans, we performed a matched case-control study in critically ill subjects and found that prior statin use was associated with lower circulating Angpt-2.
Conclusions: In an unbiased approach Simvastatin was found to inhibit Angpt-2 production in vitro. This observation could be confirmed in vivo in different murine models of the disease. Our data indicate that a potential beneficial effect of prior statin use in septic humans might be promoted via the Angpt-2/Tie2 axis. Therefore, "point of care" screening for circulating Angpt-2 in candidates for a clinical sepsis trial might help to identify those individuals that benefit from a statin treatment.
Grant acknowledgment: SD is supported by the DFG (DA 1209/4-1).

P60

0738. Mortality is associated with early tachycardia and cardiac troponin release in a fluid-resuscitated rat model of sepsis

W Khaliq*, M Singer
Bloomsbury Institute of Intensive Care Medicine, University College London, London, UK
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P60

Introduction: Tachycardia and high troponin levels prognosticate for poor outcomes in human sepsis [1]. Reducing cardiac stress with beta-blockade has been proposed as an important therapeutic strategy as high catecholamine levels are injurious [2]. We have characterized a 72h fluid-resuscitated rat model of faecal peritonitis where prognostication can be made with high sensitivity and specificity at 6h from heart rate and stroke volume [3].
Objectives: To determine whether non-survival is associated with early changes in troponin release and circulating catecholamine levels.
Methods: Male Wistar rats (325±15g) underwent insertion of tunneled carotid arterial and jugular venous lines under isoflurane anaesthesia, followed by immediate i.p. injection of 4μl/g faecal slurry. Control animals were treated identically but without i.p. injection of slurry. Once awake, attachment to a swivel-tether system allowed animals to move freely and access food and water *ad libitum*. Fluid resuscitation (50:50 mixture of 5% dextrose/Hartmann's; 10ml/kg/h) was commenced at 2h. At 6h, echocardiography was used to measure heart rate and stroke volume. Animals were observed until 72h to assess survival. In a second experiment septic animals underwent echocardiography at 6h followed by sacrifice and blood and tissue sampling. We here report plasma catecholamine and troponin T levels (measured by ELISA) in predicted survivors and non-survivors, and sham-operated controls.
Results: Septic animals (n=16) had a mortality rate of 56%, with death occurring between 18-36h. A heart rate cut point of 460/min measured at 6h prognosticated 3-day survival with sensitivity of 0.88 and specificity of 0.92. Clinical features of illness at this timepoint were however mild. Table 1 shows significant differences in haemodynamics and troponin levels between predicted survivors and non-survivors. Catecholamine levels, while elevated over non-septic controls, were similar.
Conclusions: An association was seen between eventual non-survival and tachycardia, low stroke volume and myocardial injury (denoted by high troponin) at 6h after induction of sepsis. The impact of modulating cardiac stress on outcome merits further study.
Grant acknowledgment: UK Intensive Care Foundation and NIHR
References
1. Ammaan P, *et al*: *J Am Coll Cardiol* 2003, **41**:2004-9.
2. Morelli A, *et al*: *JAMA* 2013, **310**:1683-91.
3. Rudiger A, *et al*: *Clin Sci* 2013, **124**:391-401.

BIOMARKERS IN ACUTE LUNG INJURY

P61

0852. Selective decontamination of the digestive tract modulates the metabolic profile in a ventilator-induced lung injury model

Y Rojas[1,2], S Naz[3], JL Izquierdo[4], N Nin[5,6], A Ferruelo[1,2], P García-Hierro[7], D Molina-Arana[7], R Herrero[1,2], L Martínez-Caro[1,2], A García[3], MA de la Cal[1,2], JM Ruiz-Cabello[4], C Barbas[3], JA Lorente[1,2,8*]
[1]Centro de Investigación Biomédica en Red de Enfermedades RespiratoriaS (CIBERES), Getafe, Spain; [2]Hospital Universitario de Getafe, Intensive Care Service and Burn Unit, Getafe, Spain; [3]Facultad de Farmacia, Universidad CEU San Pablo, Centro de Metabolómica y Bioanálisis (CEMBIO), Madrid, Spain; [4]Centro de Investigaciónes Cardiovasculares (CNIC), Madrid, Spain; [5]Hospital de Torrejon, Intensive Care Service, Madrid, Spain; [6]Hospital Español, Intensive Care Service, Montevideo, Uruguay; [7]Hospital Universitario de Getafe, Getafe, Spain; [8]Universidad Europea de Madrid, Madrid, Spain
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P61

Introduction: Acute lung injury induced by mechanical ventilation [ventilator-induced lung injury (VILI)]is characterized by a particular metabolic profile in the lung and in the systemic compartment [1]. Also, VILI has been associated with an increase in intestinal permeability [2]. We hypothesized that selective decontamination of the digestive tract (SDD) can modulate the metabolic profile associated with mechanical ventilation.
Objectives: To determine (1) the metabolic profile associated with VILI to identify potential biomarkers; (2) whether SDD modifies this metabolic profile associated with VILI.
Methods: Rats were pretreated with antibiotics by oral gavage for SDD (polymyxin E 30 mg/ml, tobramycin 12 mg/ml) or vehicle (water) as control. Twenty four hours later, rats were ventilated for 2.5 h. VILI was induced by using high tidal volume (V_T= 25 ml/kg) + PEEP = 0 cm H_2O. As control, rats were ventilated with low V_T (9 ml/kg) + PEEP = 5 cm H_2O. We studied four groups: Low V_T-SDD, High V_T-SDD, Low V_T-vehicle and High V_T-vehicle (n=20 per group). Lung tissue and serum were analyzed by ^{1}H-nuclear magnetic resonance spectroscopy (H-MRS) and high pressure liquid chromatography coupled to quadruple time-of-flight (LC-MS-QTOF), respectively. Principal component (PCA) [unsupervisated] and partial least squares (PLS) [supervised] analyses were performed. Accurate masses of features representing significant differences were searched against the MELTING, KEGG, LIPIDMAPDS and HMDB databases. We followed the Principles of Laboratory Animal Care (2010/63/UE 22-09, RD 53/2013 BOE 1-02, ley 32/2007 BOE 7-11).
Results: We found different metabolic patterns between rats ventilated with low and high V_T, and also between ventilated rats with and without SDD.

Table 1(abstract P60)

	Control (n=6)	Predicted survival (n=6)	Predicted non-survival (n=6)
Heart rate (bpm)	390 ± 21	442 ± 17	488 ± 18*
Stroke volume (mL)	0.40 ± 0.03	0.25 ± 0.02	0.18 ± 0.02*
Adrenaline (ng/mL)	8.56 ± 0.42	9.44 ± 0.23	10.3 ± 0.18
Noradrenaline (ng/mL)	1.60 ± 0.25	3.21 ± 0.23	2.98 ± 0.27
Troponin (pg/mL)	171 ± 24	168 ± 15	311 ± 47*

Data shown as median ± SE; * p<0.05 ANOVA

In the lung, the main metabolic pathways affected are involved in energy metabolism (creatine, glucose, lactate, alanine, glutamate), protein synthesis (leucine) and membrane lipids (choline, phosphoethanolamine). In serum, the main affected pathways were related to conjugated bile acids, ceramide, Land´s cycle and carnitine biosynthesis.

Conclusions: (1) Mechanical ventilation can change the metabolic profile in the lung and in the systemic compartment. (2) SDD can modify this metabolic changes induced by mechanical ventilation. (3) Metabolic studies can be useful to identify biomarkers for the diagnosis of acute lung injury, and to design new therapeutic strategies.

Grant acknowledgment: FIS 12/02898, FIS 11/02791, FIS 12/02451, European Network (7th FP) ITN 264864, CA11/00260.

References

1. Izquierdo-Garcia JL, *et al*: A Metabolomic Approach to the Pathogenesis of Ventilator-induced Lung Injury. *Anesthesiology* 2014, **120(3)**:694-702.
2. Guery BP, *et al*: Ventilation-induced lung injury is associated with an increase in gut permeability. *Shock* 2003, **19(6)**:559-63.

P62

0853. Elevated levels of soluble rage predict impaired alveolar fluid clearance in a translational mouse model of acute respiratory distress syndrome (ARDS)

R Blondonnet[1,2*], M Jabaudon[1,2], G Clairefond[1], J Audard[1,2], D Bouvier[1,3], G Marceau[1,3], P Blanc[1], P Dechelotte[1,4], V Sapin[1,3], J-M Constantin[1,2]
[1]R2D2 - EA 7281, School of Medicine, Universite d'Auvergne Clermont-Ferrand I, Clermont-Ferrand, France; [2]Intensive Care Unit, Department of Anesthesiology and Critical Care Medicine, Estaing University Hospital, CHU Clermont-Ferrand, Clermont-Ferrand, France; [3]Department of Medical Biochemistry and Molecular Biology, Estaing University Hospital CHU Clermont-Ferrand, Clermont-Ferrand, France; [4]Department of Pathology, Estaing University Hospital CHU Clermont-Ferrand, Clermont-Ferrand, France

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P62

Introduction: Receptor for advanced glycation endproducts (RAGE) is a transmembrane pattern-recognition receptor of the immunoglobulin superfamily that is abundantly expressed in the lung and primarily located on the basal surface of alveolar type I cells. RAGE is implicated in ARDS as an important pathway to alveolar inflammation and, when its soluble form sRAGE is assayed in plasma or pulmonary edema fluid, as a marker of AT I cell injury [1]. Functional activity of AT 1 cells can be assessed by the measurement of alveolar fluid clearance (AFC) rate [2], but the relationship between sRAGE plasma levels of sRAGE and AFC rates has never been investigated.

Objectives: To report plasma levels of sRAGE in a translational mouse model of direct acid-induced epithelial injury, and to test their correlation with AFC rates.

Methods: Forty-one male CD-1 mice were divided in 2 groups: an "HCl" group of mice who received a tracheal instillation of hydrochloric acid on day 0, and a group of control uninjured animals. Mice were evaluated on day 0, day 1, day 2 and day 4 after a 30-minute period of mechanical ventilation : after sacrifice, blood and undiluted lung edema fluid (EF) were sampled. Before initiation of MV, all mice received a tracheal instillation of bovine serum albumin (BSA 5%) in order to detect changes in alveolar protein levels over 30 minutes. Plasma levels of sRAGE and total protein levels were measured in duplicate by ELISA and colorimetric detection, respectively. AFC rate values were corrected after measurement of mouse serum albumin in EF.

Results: Basal AFC rate was 35% over 30 min in HCl-injured mice, but it was significantly depressed on day 1 (16% over 30 min; p=0.02). Over time, AFC reached basal levels again. Plasma levels of sRAGE were higher in HCl-treated animals than in control animals on day 1 (p=0.03) and day 2 (p=0.02). Significant correlation was found between AFC rates and plasma levels of sRAGE (Spearman correlation coefficient -0.49 (IC 95 [-0.70 ; -0.19] p=0.04)).

Conclusions: The highest impairment in AFC is reported on day 1 in our animal model of acid-induced injury. sRAGE levels are also higher in injured mice and may be a good surrogate marker of AT I cell injury. This newly decribed relationship between AFC rates and sRAGE plasma level in a mouse model of direct epithelial injury confirms previous results from an ex vivo model of isolated human uninjured lungs [3]. Our results support further translational investigation on the role of RAGE in alveolar injury and recovery.

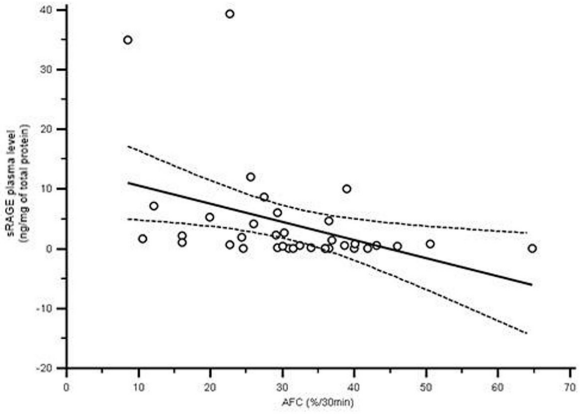

Figure 1(abstract P62) Relation between AFC rates and sRAGE plasma levels.

Grant acknowledgment: Programme de Recherche Translationnelle en santé (PRTS) 2013. Région Auvergne

References

1. *Genes Cells* 2004, **9(2)**:165-74.
2. *Eur Respir J* 2012, **39(5)**:1162-70.
3. *Chest* 2009, **135(2)**:269-275.

P63

0854. Metabolomic changes by mass spectrometry in lung tissue from septic rats with mechanical ventilation-induced lung injury

Y Rojas[1,2*], S Naz[3], N Nin[4,5], A Garcìa[3], A Ferruelo[1,2], L Martínez-Caro[1,2], M de Paula[1,6], C Barbas[3], JA Lorente[1,2,7]
[1]Centro de Investigación Biomédica en Red de Enfermedades Respiratorias (CIBERES), Getafe, Spain; [2]Hospital Universitario de Getafe, Intensive Care Service, Getafe, Spain; [3]Facultad de Farmacia, Universidad CEU San Pablo, Centro de Metabolómica y Bioanálisis (CEMBIO), Madrid, Spain; [4]Hospital Español, Intensive Care Service, Montevideo, Uruguay; [5]Hospital de Torrejon, Intensive Care Service, Madrid, Spain; [6]Hospital Universitario de Getafe, Getafe, Spain; [7]Universidad Europea de Madrid, Madrid, Spain

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P63

Objective: To identify metabolomic changes in lung tissue associated with lung injury induced by mechanical ventilation (VILI) in animals with sepsis, using for the first time a global unbiased metabolomic fingerprinting approach.

Methods: Rats received cecal-ligation and puncture (CLP) or sham operation, and 24 h later underwent mechanical ventilation for 2.5 h with either V_T=9 ml/kg, positive end-expiratory pressure (PEEP)=0 cm H_2O (n=9 and n=12, without and with CLP, respectively); or V_T=25 ml/kg, PEEP=5 cm H_2O (n=13 and n=12, without and with CLP, respectively). Lung tissue samples were obtained and analyzed by nontargeted global fingerprinting approach for lung tissue analysis, applying multiple complementary analytical techniques, including liquid cromatography-mass spectrometry (MS), gas cromatography-MS, and capillary electrophoresis-MS. We followed the Principles of Laboratory Animal Care (2010/63/UE 22-09, RD 53/2013 BOE 1-02, ley 32/2007 BOE 7-11).

Results: Metabolomic changes characteristic of sepsis and VILI were identified. Lung tissue samples from septic rats with VILI were characterized by a specific metabolomic profile as compared to samples from septic rats without VILI. Metabolomic changes indicated increased oxidative stress, and changes in purine, energy, carnitine, aminoacid, urea cycle, vitamines, collagen, ceramide-sphingomyelin and phospholipid metabolism.

Conclusion: A particular metabolomic profile can be identified in lung tissue from septic rats with lung injury induced by mechanical ventilation.

Grant acknowledgment: FIS 12/02898, FIS 11/02791, FIS 12/02451, European Network (7th FP) ITN 264864, CA11/00260.

P64

0859. Altered expression of the bone morphogenetic antagonists in the bleomycin model of acute lung injury

N Murphy[1,2*], S Coyle Rowan[3], S Frohlich[2,4], P McLoughlin[1]
[1]University College Dublin, School of Medicine and Medical Sciences, Dublin, Ireland; [2]St Vincent's Hospital, Anaesthesia and Intensive Care Medicine, Dublin, Ireland; [3]University college Dublin, School of Medicine and Medical Sciences, Dublin, Ireland; [4]University College Dublin, School of Medicine and Medical Sciences, Dubiln, Ireland

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P64

Introduction: Acute lung injury (ALI) is a devastating clinical condition, characterised by acute inflammation that often proceeds to overt pulmonary fibrosis. Basal bone morphogenetic protein (BMP) signalling is essential for normal pulmonary homeostasis. The BMP antagonist gremlin has previously been shown to be increased in hypoxic lung disease, reduce BMP signalling and thus contribute directly to lung damage [1]. BMP antagonsists also play important roles in fibrotic diseases [2].
Objectives: The aim of this study was to investigate the expresison of BMP accessory proteins in an animal model of ALI and assess any associated changes in BMP signalling.
Methods: Acute lung injury was induced in adult mice by intra-tracheal instillation of bleomycin (1U/kg); saline was instilled in control animals. Fourteen days post inoculation, animals were anaesthetised, killed and their lungs removed and snap frozen for later analysis of RNA and protein. Lungs from separate groups were isolated, fixed, and wax embedded for histological and immunohistochemical analysis.
Results: Bleomycin treated lungs showed characteristic structural changes and patchy inflammation. Gremlin, follistatin, follistatin like 1, follistatin like 3, BMPER and noggin mRNA expression was increased, the expression of BAMBI and NOV was reduced. MGP, KCP, lefty and chordin were unchanged. Expresion of Follistatin like 1, BAMBI and noggin protein was increased. Consistent with attentuated BMP signalling, BMP-2 and phospho-smad 1/5/8 was reduced.
Conclusions: These data demonstrate that BMP antagonists are altered in bleomycin injured lungs and associated with reduced BMP signalling.
Grant acknowledgment: St Vincent´s Anaesthesia Foundation, St. Vincent´s University Hospital, Dublin 4, Ireland

References

1. Cahill E, Costello CM, Rowan SC, Harkin S, Howell K, Leonard MO, Southwood M, Cummins EP, Fitzpatrick SF, Taylor CT, Morrell NW, Martin F, Mcloughlin P: **Gremlin plays a key role in the pathogenesis of pulmonary hypertension.** *Circulation* 2012, **125**:920-30.
2. Koli K, Myllarniemi M, Vuorinen K, Salmenkivi K, Ryynanen MJ, Kinnula VL, Keski-Oja J: **Bone morphogenetic protein-4 inhibitor gremlin is overexpressed in idiopathic pulmonary fibrosis.** *The American journal of pathology* 2006, **169**:61-71.

P65

0863. Lithium pharmacokinetics in the rat according to the three different modalities of human poisoning

A-S Hanak[1], L Chevillard[1], S El-Bakhli[1], P Risède[1], K Peoc'h[1], B Megarbane[2*]
[1]INSERM U 1144, Paris-Descartes University, Paris, France; [2]Lariboisière Hospital, INSERM U 1144, Paris-Diderot University, Paris, France

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P65

Introduction: Lithium-related neurological toxicity may be severe resulting in seizures, myoclonic encephalopathy, and coma. Three different poisoning presentations exist in humans, including acute poisoning in non-previously treated patients (A), acute-on-chronic poisoning (A/C), and therapeutic overdose (T). The exact reasons why severity and features are different between these three presentations are unknown, although differences in brain lithium distribution have been suggested.
Objectives: Our objective was to study lithium pharmacokinetics in blood and brain in rat models corresponding to each human presentation.
Methods: Development of three models of lithium intoxication in Sprague Dawley rats: A (one intraperitoneal injection of 185 mg/kg Li_2CO_3); A/C (800 or 1600 mg/L Li_2CO_3 in the drinking water followed by one intraperitoneal injection of 185 mg/kg Li_2CO_3 at day 28); T ($K_2Cr_2O_7$-induced acute renal failure on day 1 followed by intraperitoneal injections of 74 mg/kg/day Li_2CO_3 during 5 days); determination of plasma, erythrocyte, cerebrospinal fluid, and brain lithium concentrations using inductively coupled plasma atomic emission spectroscopy (quantification threshold: 0.6 nmol/L); modeling and determination of pharmacokinetics parameters; comparisons with non-parametric tests.
Results: Lithium followed a tricompartmental pharmacokinetics with a shortened plasma half-life in case of previous chronic exposure (1.73 vs. 3.85h). The peak lithium concentration was measured at 6h in erythrocytes, 2h in cerebrospinal fluid, and 24h in the brain in both A and A/C models; however, the elimination constants k21 (erythrocytes-to-plasma) and k31 (brain-to-plasma) were lower in the A/C model (0.36 *versus* 0.56 and 2.1 *versus* 9.2, respectively), suggesting lithium accumulation. The brain distribution was not homogeneous, with rapid entrance (as soon as 15min), peak at 24h, and delayed elimination (>78h). Lithium accumulation into the brain was more marked in the presence of previous chronic exposure (brain-to-plasma ratio at 54h: 131.27 vs. 6.42; $p < 0.0001$). Similarly, alteration in renal elimination resulted in increased brain distribution (brain-to-plasma ratio: 10.98 vs. 6.88).
Conclusions: Our experimental models suggest that the three different presentations of lithium poisonings in humans differ due to lithium blood pharmacokinetics and brain distribution. However, the hypothesis of an additional variability related to different interactions of lithium with neurological targets in each presentation could not be ruled out.

ACUTE RESPIRATORY FAILURE MISCELLANEA

P66

0866. Repercussion in hemodynamic values and extravascular lung water of alveolar recruitment maneuvers, in an experimental model of ARDS

M Varela Durán[1,2]
[1]Autonoma University, Surgery, Madrid, Spain; [2]Complejo Hospitalario Universitario de Pontevedra, Anesthesia and Critical Care, Pontevedra, Spain

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P66

Objectives: To demonstrate the hemodynamic changes caused by different conducted maneuvers of alveolar recruitment, in 17 pigs with ARDS, provoked experimentally.
Methods: Our Study was carried out in 17 Landrace pigs with ARDS, evoked through multiple bronchoalveolar lavages with saline serum. Three groups of study were established according to the maneuvers of alveolar recruitment (AR) made after the development of the ARDS.
A group: "Total AR" realizing maneuvers of recruitment to obtain PaO2>90% of the basal value, with various maneuvers of "rapid AR" and one maneuver of "sustained insufflation".
B group: Simple maneuver of "rapid AR" without objetive of oxigenation.
C group: Control group: without RA, using PEEP values bellow LIP (lower inflection point).
The hemodynamic study (Heart rate, mean BP, PCP, PAP, CI), EVLW, and lactic acid, was made at basal time and at 15, 60, 180 and 360 minutes, after the provocation of ARDS. The catheters used for hemodynamic monitoring consisted in: venous catheters, arterial catheters and pulmonary artery catheter for determination of cardiac output (Edwards Swan-Ganz thermodilution catheter). The hemodynamic monitoring equipment (Drager PM 8060 Vitara) and the PiCCO equipment was used for monitoring Cardiac output, circulatory and cardiopulmonary variables and EVLW (Extravascular Lung Water). Statistical analysis was made using ANOVA Study, T Student, X^2, and exact Test of Fisher.
Results: In our Study, the observed hemodynamic values: HR, mean BP, PCP, PAP, CI, were similar using a "Total" or "Parcial" recruitment maneuver and also using a value of PEEP below the lower inflection point (LIP). The use of a PEEP value, below the LIP,without RA maneuvers, in experimental ARDS occurred after bronchoalveolar lavage, obtained differences in the values of mPAP. No significant differences in lactic acid in the three study groups were observed. The EVLW has a tendency to decrease with the "Total" or "Parcial" alveolar recruitment maneuvers.

Conclusions: We recommended AR maneuvers associated with the use of a PEEP value below the LIP, in the ventilatory management of ARDS.

PERI-OPERATIVE INTENSIVE CARE

P67

0968. The influence of hypothermia and catecholamines on guinea pig's small bowel motility *in vitro*

M Schörghuber[1*], E Tatzl[1], P Holzer[2], W Toller[1], S Fruhwald[1]
[1]Medical University of Graz, Department of Anesthesiology and Intensive Care M, Graz, Austria; [2]Medical University of Graz, Institute of Experimental and Clinical Pharmacology, Graz, Austria
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P67

Introduction: In critically ill patients early enteral nutrition (EN) preserves gastrointestinal (GI) integrity and motility and should be started as early as possible. We know that several therapeutic strategies, e.g. catecholamines or analgosedation, exert adverse effects on GI motility.[1] What we do not know is whether therapeutic hypothermia has an influence on GI motility and thereby feeding intolerance.
Objectives: The aim of this study was to find out if guinea pig's small bowel motility is altered during hypothermia and after rewarming and if catecholamines cause alterations of peristalsis in this situation.
Methods: Guinea pig´s small bowel segments of 8 cm length were set up in organ baths containing oxygenated Tyrode´s solution. Peristalsis was elicited by luminal perfusion (0.5 ml/min) against an aboral resistance of 400 Pascal (Pa). Perfusion of the segments resulted in an increase of the intraluminal pressure up to a pressure threshold (PT; mean ±SEM), where peristaltic contractions were triggered. The pressure was recorded at the aboral end of the segments. An increase of the PT indicates an inhibition of peristalsis, while a decrease of the PT represents a stimulation of peristalsis. A PT of 400 Pa was equated with a complete block of peristalsis. PT was firstly measured at 37°C temperature of the organ bath, after rapid cooling to 20°C and after rewarming to 37°C (control). In a second setting before rewarming one of the following substances were added to the organ baths: adrenaline 100 nM, dobutamine 100 µM, noradrenaline 1 µM. At 37°C PT was evaluated again.
Results: Basic PT was 49.6 ±6.5 Pa. Lowering the bath temperature to 20°C led to a complete block of peristalsis in all tested segments (PT= 400 Pa, figure 1). During rewarming all small bowel segments started peristaltic contractions spontaneously and showed normal peristalsis at 37°C. In the second setting additional catecholamines resulted in a significantly delayed restart of peristalsis after rewarming and a persistent inhibition of peristalsis (i.e. higher PT) compared to control segments.
Conclusions: Our experimental setting demonstrates a distinct impairment of small bowel motility during hypothermia, a delayed restart and a persistent inhibition of motility in the presence of catecholamines, explaining the higher incidence of feeding intolerance in this group of patients.

Reference

1. Fruhwald S, Holzer P, Metzler H: **Intestinal motility disturbances in intensive care patients: pathogenesis and clinical impact.** *Intensive Care Medicine* 2007, **33**:36-44.

SEPSIS MISCELLANEA

P68

0976. Similar effect of albumin given either as a rapid bolus or slow infusion in a large animal model of sepsis

M von Seth[1*], P Engström[2], A Larsson[3], L Hillered[4], E Maripuu[5], C Widström[5], M Lipcsey[6], J Sjölin[3]
[1]Uppsala Universitet, Surgical Sciences, Uppsala, Sweden; [2]Hudiksvall Hospital, Department of Anaesthesia, Hudiksvall, Sweden; [3]Uppsala University, Medical Sciences, Uppsala, Sweden; [4]Uppsala University, Neurosciences, Uppsala, Sweden; [5]Akademiska Ssjukhuset, Medical Physics, Uppsala, Sweden; [6]Uppsala University, Surgical Sciences, Uppsala, Sweden
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P68

Introduction: The strategy of fluid resuscitation in severe sepsis and septic shock is a matter of ever-ongoing debate. Current sepsis guidelines recommend albumin in patients requiring large amounts of crystalloids for circulatory stability. Although the same guidelines also recommend bolus administration of fluid, concerns have been raised that fluid boluses might lead to increased extravasation of albumin leading to sustained tissue edema in patients with sepsis-induced activation of the systemic inflammatory response.
Objectives: We hypothesized that a slow infusion of 10 mL x kg^{-1} 5% albumin would lead to less capillary leakage of the given albumin compared to the same amount given as a rapid bolus. This can be studied in a porcine intensive care model of sepsis-induced systemic inflammatory response.
Methods: The systemic inflammatory response was induced by endotoxin in a dose of 1 µg × kg^{-1} × h^{-1} resulting in severe sepsis and septic shock in all animals. Thirty-two animals were monitored and ventilated with standard intensive care equipment and randomized to either a 2-hour continuous infusion (INF group; n=16) or a rapid bolus (BOLUS group; n=16) of 10 mL × kg^{-1} of 5% albumin labeled with Technetium^{99m}. Blood hemoglobin levels were used as a measure of systemic inflammatory response-induced capillary leakage. Radioactivity was monitored in plasma, urine and muscle microdialysate for a total of 6 hours. Post mortem analyses of radioactivity in liver, spleen, kidney and lung were performed and total lung water was assesed.
Results: The two groups were similar at baseline. Hemoglobin increased in both the INF and the BOLUS groups with no differences between the two (19±8 vs. 19±9 gxL^{-1}; mean±SD, p< 0.001 for both). Radioactivity in plasma differed between the two groups during the infusion, corresponding to the pattern of albumin administration (Figure 1). During the 3 hours following the fluid administration, there were no differences

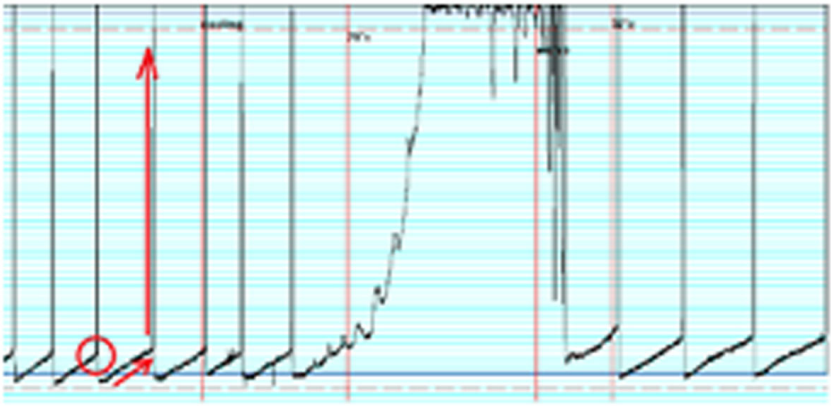

Figure 1(abstract P67) Alteration of peristalsis during hypothermia → incease of intraluminal pressure. O PT. ↑ peristaltic reflex

Table 1(abstract P67) PT and start of peristalsis after admission of catecho/amines compared to control. [1]two-sided t-test. [2]Kruskal-Wallis.

			Start of peristalsis after rewarming (%)			
	PT (Pa) after rewarming	**p[1]**	Immediately	<5 minutes	>5 minutes	**P[2]**
Control	70.4 ± 8.3					
Adrenaline	182.7 ± 39.3	0.019	10	60	30	<0.001
Dobutamine	264.2 ± 46.6	0.001	0	55	45	<0.001
Noradrenaline	223.9 ± 48.4	0.008	0	40	55	<0.001

in the rate of decrease in plasma radioactivity between the INF and BOLUS groups (-3.1x10^5±7.2x10^4 vs. -3.2x10^5±6.4x10^4; mean±SD, n.s.). There were no differences between groups in muscle microdialysate/blood radioactivity ratio at 6 hours (1.9 [0.6-26] vs. 2.7 [1.2-4.2] %; median [IQR]; n.s.; INF and BOLUS groups, respectively). No intergroup differences were seen in the urine radioactivity during the study period or in lung dry/wet ratio or radioactivity of viscera at 6 h. Finally, there were no differences between the two groups in hemodynamics, respiration, hematology or renal function during the experiment.

Conclusions: In an intensive care large animal model of sepsis, administration of significant volume of 5% albumin as rapid bolus compared to slow infusion did not lead to greater extravasation of albumin.

ALI & ARDS: EXPERIMENTAL STUDIES

P69

0984. Perfusate from lungs ventilated *ex-vivo* with high tidal volumen induce *in vitro* endotelial dysfunction reversed by superoxide dismutase and tempol

L Martínez-Caro[1,2*], I Ortiz[1,3,4], A Sanchez-Ferrer[1,2], Y Rojas[1,2], L Smit[5], B de Olaiz-Navarro[1,3], A Ferruelo[1,2], N Nin[6,7], A Esteban[1,2], JA Lorente[1,2]
[1]Centro de Investigación Biomédica en Red de Enfermedades Respiratorias (CIBERES), Getafe, Spain; [2]Hospital Universitario de Getafe, Intensive Care Service and Burn Unit, Getafe, Spain; [3]Hospital Universitario de Getafe, Getafe, Spain; [4]Hospital Virgen de la Salud, Pediatric Intensive Care Service, Toledo, Spain; [5]Universidad Alfonso X, Madrid, Spain; [6]Hospital de Torrejon, Intensive Care Service, Madrid, Spain; [7]Hospital Español, Intensive Care Service, Montevideo, Uruguay
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P69

Introduction: Ventilator-induced lung injury (VILI) has been related not only to pulmonary injury but also to systemic damage. We performed a bioassay using ex vivo models of VILI and of vascular function in order to determine the role of pulmonary-derived factors in ventilator-induced endothelial dysfunction. The involvement of nitro-oxidative stress was also examined [1].

Objectives: (i) To demonstrate that the release of soluble factors derived from the lung induces vascular endothelial dysfunction.
(ii) To define the role of nitro-oxidative stress in ventilator-induced endothelial dysfunction.

Methods: Ex vivo ventilated and perfused lungs (Harvard Apparatus, MA) from male Sprague-Dawley rats (weight 325-375 grams) were subjected to high tidal volume (V_T=25 mL/kg + PEEP=0 cm H_2O) mechanical ventilation for 2.5 h (n=22). Lungs were perfused (4 mL/min) with Krebs solution + 4% albumin (bubled with 5% CO_2 and 20% O_2) that was recirculated throughout the experiment. Aortic rings extracted from healthy rats were incubated in an organ bath for 60 minutes with the perfusate collected from the ventilated lungs. Endothelium-dependent relaxation was measured in norepinephrine precontracted rings (acetylcholine, 10 nM-10 uM). Superoxide dismutase (SOD 100 u/ml) or tempol (10^{-4} M) (extracellular and intracellular superoxide scavengers, respectively) or MnTMPyP (10^{-5} M) (a superoxide and peroxynitrite scavenger), were added to the organ bath in order to explore the role of nitro-oxidative stress in vascular dysfunction. Dose-response curves were compared by repeated-measurements ANOVA. We followed the Principles of Laboratory Animal Care (2010/63/UE 22-09, RD 53/2013 BOE 1-02, ley 32/2007 BOE 7-11).

Results: High V_T mechanical ventilation was associated with an increase in peak airway pressure (PIP), as well as increased levels of LDH, CK and lactate in the perfusate at the end of the experiment, in approximately half of the high V_T ventilated lungs (n=10), whereas half of the isolated-perfused lungs did not show any changes in PIP, LDH, CK and lactate after 2.5 h of high V_T mechanical ventilation (n=12). The perfusate collected from the lungs that showed increased PIP induced an impairment in vascular responses in vitro. On the contrary, the perfusate collected from lungs that did not show an increase in PIP did not induce significant changes in vascular responses in aortic rings. Impaired-responses to acetylcholine were improved by the administration of tempol and SOD, but not by MnTMPyP, to the organ bath (n=12-15 rings per treatment).

Conclusions: (i) Factors released from injured lungs ex vivo are able to induce endothelial dysfunction in vitro.
(ii) Oxidative stress is involved in endothelial dysfunction induced by high V_T mechanical ventilation.

Grant acknowledgment: FIS 12/02898, FIS 11/02791, FIS 12/02451, European Network (7th FP) ITN 264864, CA11/00260.

Reference

1. Martínez-Caro L, *et al*: *Int Care Med* 2009, **35**:1110-9.

P70

0985. Open lung ventilation improves conditions for right ventricle performance by decreasing pulmonary vascular wave reflections in an experimental model of ARDS

A Santos[1,2,3*], E Gomez Peñalver[2], JB Borges[1], J Retamal[1], MI Monge García[4], G Tusman[5], A Larsson[1,6], G Hedenstierna[1], F Suarez-Sipmann[1,6]
[1]Uppsala University, Hedenstierna Laboratory, Surgical Sciences Department, Uppsala, Sweden; [2]Fundación Jiménez Diaz, Intensive Care Medicine, Madrid, Spain; [3]Insitituto de Investigación Sanitaria, IIS-FJD, Madrid, Spain; [4]Hospital del SAS de Jerez, Intensive Care Medicine, Jerez de la Frontera, Spain; [5]Hospital Pirvado de Comunidad, Anesthesiology, Mar del Plata, Argentina; [6]Uppsala University Hospital, Anesthesiology and Critical Care Medicine, Uppsala, Sweden
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P70

Introduction: Impaired right ventricle (RV) function is associated with worse outcome in ARDS. Pulmonary artery pressure waveform analysis provides information about phenomena that affect RV performance. In particular, pulmonary vascular wave reflection (WR) is directly related with RV stress. We hypothesised that open lung ventilation (OLV), compared with conventional ARDS-net ventilation (CV), would improve conditions for RV performance in an ARDS model. This hypothesis was tested by measuring pulmonary vascular wave reflection (WR).

Objectives: To evaluate the effect of two mechanical ventilation (MV) strategies on WR in an experimental model of ARDS.

Methods: 8 anesthetized and muscle relaxed pigs were submitted to a two-hit lung injury model combining repeated lung lavages with injurious MV. After lung injury was induced, animals were randomized (4 pigs in each group) to one of two strategies of MV: OLV, PEEP 2cmH2O above the PEEP corresponding with the maximal dynamic compliance in a decreasing PEEP trial after a recruitment manoeuvre; or CV, PEEP adjusted according to the ARDSnetwork table. In both groups tidal volume was 6ml/kg, respiratory rate to maintain PaCO2 between 55-65 mmHg and FIO2 to maintain PaO2 55-80 mmHg.

Pulmonary artery (PA) flow and pressure waveforms (1000Hz sampling rate) were acquired by a high-fidelity microtip manometer and an instantaneous transonic pulmonary flow probe placed in the main PA by a

small lateral thoracotomy. These signals were used to separate the forward and backward components of the pressure waveform[1] and quantify WR. The following indexes of WR were calculated: Backward wave amplitude (APbw); Reflection index (RI) which is the ratio between the backward wave amplitude and the sum of backward and forward wave amplitude. Evaluation was done before (BL) and after lung injury (ARDS) and after 4 hours of management in the respective MV strategy.

Results: We did not find any significant changes by induction of ARDS but both APbw (5.28±1.35 vs 10.85±2.16 mmHg, p=0.021) and RI (0.28±0.04 vs 0.39±0.04, p=0.021) were lower in OLV comparing with CV.

Conclusions: In this experimental ARDS-model OLV decreased WR in the pulmonary vascular system comparing with CV, indicated that OLV could reduce the stress on the RV and improve conditions for RV performance.

Grant acknowledgment: This study is part of a project awarded by the ECCRN of the ESICM (Basic Science Award 2012).

Reference

1. Laxminarayan S: **The calculation of forward and backward waves in the arterial system.** *Med Biol Eng Comput* 1979, **17(1)**:130.

P71

0986. The effect of eritoran on the severity of lung injury in two different rat lung injury models

A Kundakci*, H Verdi, E Ozturan Ozer, O Ozen Isiksacan, FB Atac, S Turkoglu, A Pirat
Baskent University Medical School, Ankara, Turkey

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P71

Toll like receptors (TLR) play an important role in noninfectious problems in critically ill patients. The central role of TLR4 in sepsis has been clearly demonstrated. However, the role of TLR as a therapeutic target in acute respiratory distress syndrome (ARDS) has not been studied.

The goal of this study is to compare the effects of eritoran, a TLR4 antagonist, on severity of ARDS in two different (pulmonary versus extrapulmonary) ARDS rat models.

Forty rats were randomized to sham (n=8), extrapulmonary control (n=8), extrapulmonary eritoran (n=8), pulmonary control (n=8), and pulmonary eritoran (n=8) groups. Intestinal ischemia-reperfusion (I/R) via ligation of superior mesenteric artery was used to induce the extrapulmonary ARDS model. Intratracheal instillation of hydrochloric acid was used for the pulmonary ARDS model. Rats received either eritoran 100 μg/kg or an equal volume of placebo solution. Serum tumor necrosis factor- α (TNF-α), interleukin-1β (IL-1β), and IL-6; lung tissue malondialdehyde (MDA), glutathione (GSH), and myeloperoxidase (MPO) levels; lung histopathologic examination; arterial partial oxygen pressure (pO_2); and lung wet-to-dry weight ratio (w/d) measurements were used to compare and evaluate the severity of lung injury between the groups. TLR-4 and nuclear factor-κB (NF-κB) gene expressions were determined from lung tissue using polymerase chain reaction technique.

Compared to its control group, extrapulmonary eritoran group exhibited significantly less severe lung injury, as indicated by lower mean values for MDA ($p < 0.05$), MPO ($p < 0.01$), lung histopathologic injury score ($p < 0.05$), lung w/d weight ratio ($p < 0.01$), and higher mean values for pO_2 ($p < 0.01$) and GSH ($p < 0.05$). Likewise mean values for serum TNF-α, IL-1β, and IL-6 levels were significantly lower than the control values in extrapulmonary eritoran group ($p < 0.05$ for all). TLR-4 and NF-κB gene expressions were significantly more pronounced in extrapulmonary control group than extrapulmonary eritoran group ($p < 0.05$ for both). Pulmonary control and eritoran groups were not significantly different in terms of mean values for MDA, GSH, and pO2 measurements ($p > 0.05$ for all). However, mean values for MPO ($p < 0.05$), w/d weight ratio ($p < 0.05$), and lung histopathologic injury score ($p < 0.05$) were significantly higher in pulmonary control group than pulmonary eritoran group. Mean values for serum TNF-α, IL-1β, and IL-6 levels were not significantly different between pulmonary control and eritoran groups. The expression of NF-κB gene was not significantly different between the pulmonary control and pulmonary eritoran groups ($p > 0.05$). However, compared to pulmonary control group the expression of TLR-4 gene was significantly less pronounced in the pulmonary eritoran group ($p < 0.05$).

In conclusion eritoran reduced the severity of lung injury both in pulmonary and extrapulmonary ARDS models. This effect of eritoran was more pronounced in the I/R lung injury model.

P72

0987. FAS activation alters tight junction proteins in pulmonary alveolar epithelial cells

R Herrero[1,2*], F Puig[2,3], R Guillamat[2,3], L Prados[4], Y Rojas[1], A Artigas[2,3], A Esteban[1,2], JA Lorente[1,2,5]
[1]Hospital Universitario de Getafe, Servicio de Cuidados Intensivos, Getafe, Spain; [2]CIBERES (CIBER Enfermedades Respiratorias), ISC III, Madrid, Spain; [3]Corporació Sanitária i Universitária Parc Taulì-UAB, Area de Cuidados Críticos, Sabadell, Spain; [4]Hospital Universitario de Getafe, Laboratorio de Análisis Clínicos, Getafe, Spain; [5]Universidad Europea de Madrid, Madrid, Spain

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P72

Introduction: Active soluble Fas ligand (sFasL) accumulates in lung fluid of patients with acute respiratory distress syndrome (ARDS), and causes apoptosis and inflammation in lung epithelial cells [1]. Alveolar epithelial damage induced by Fas receptor activation results in protein-rich lung edema [2]. Dysfunction of the tight junction proteins may contribute to the formation of lung edema.

Objectives: Determine whether sFasL increases protein permeability of the alveolar epithelium by mechanisms involving disruption of the tight junction proteins in ARDS.

Methods: Primary human pulmonary alveolar epithelial cells were cultured in permeable transwell chambers. After reaching maximal confluency, the cells were incubated for 0.5, 1, 2 or 4 h with medium with or without human recombinant sFasL (rh-sFasL). Protein permeability of the cell monolayer was measured by using fluorescein-labeled albumin (FITC-Albumin). C56BL/6 wild-type mice and *lpr* (Fas deficient) mice were treated with an intratracheal dose of rh-sFasL (25 ng/g b.w.) or PBS, and the lungs were studied 16 h later. We performed immunofluorescence double staining for the detection of tight junction proteins (ZO-1 and Occludin) and apoptosis (Terminal Transferase dUTP Nick End Labeling assay).

Results: In vitro, human sFasL increased protein permeability of the alveolar epithelial cell monolayer (medium only: 17.17 ± 2.4% vs rh-sFasL: 28.0 ± 3.6%, means ± SD, $p < 0.05$, t-test), altered the distribution of the tight junction proteins ZO-1 and Occludin, and induced apoptosis. In vivo, intratracheal instillation of rh-sFasL, which increases pulmonary protein permeability in wild-type but not in *lpr* mice, altered the distribution of ZO-1 and Occludin, and induced apoptosis in cells of the alveolar walls only in wild-type but not in *lpr* mice.

Conclusions: Activation of the Fas/FasL system increased protein permeability of the pulmonary alveolar epithelium *in vitro* and *in vivo*. This increased permeability was associated with disruption of tight junctions and apoptosis. These results provide a mechanism that could be targeted for the prevention of lung edema in ARDS.

Grant acknowledgment: FIS 12/02451, FIS 12/02898, FIS 11/02791.

References

1. Matute-Bello G, *et al*: **Soluble Fas ligand induces epithelial cell apoptosis in humans with acute lung injury (ARDS).** *J Immunol* 1999, **163(4)**:2217-25.
2. Herrero R, *et al*: **The biological activity of FasL in human and mouse lungs is determined by the structure of its stalk region.** *J Clin Invest* 2011, **121(3)**:1174-90.

P73

0988. Human neutrophil peptides play an important role in the pathogenesis of ARDS

F Pozzi[1,2*], M Parotto[1], R Molinaro[1], D Islam[1], A Pesenti[2], AS Slutsky[1], H Zhang[1]
[1]Keenan Research Centre for Biomedical Science of St. Michael's Hospital, Toronto, Canada; [2]University of Milan - Bicocca, Milan, Italy

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P73

Introduction: Acute respiratory distress syndrome (ARDS) and ventilator induced lung injury (VILI) are characterized by neutrophil recruitment in the lung, and human neutrophil peptides (HNP) are the most abundant cationic proteins that are released into the extracellular matrix upon neutrophil activation. We and others have previously shown that high concentrations of HNP can lead to capillary-epithelial barrier damage in a mouse model of ARDS. We also demonstrated that VILI can lead to lung

fibrotic development. We now extended the studies to examine the effects of HNP in lung remodeling.

Objective: Examine the role of HNP on lung remodeling in a mouse model of ARDS followed by mechanical ventilation.

Methods: Mice endogenously expressing HNP ($HNP^{+/+}$) were generated using a human neutrophil elastase promoter. The $HNP^{+/+}$and the FVB wild type mice received either HCl (pH = 1.0, 3 mL/kg) intratracheally, or vehicle solution as a control. Respiratory system compliance, protein concentrations and differential cell counting in bronchoalveolar lavage fluid (BALF) were determined 48 h after HCl. In a parallel experiment, the mice were randomized into 3 different groups 48 h after HCl: 1) no mechanical ventilation; 2) low pressure mechanical ventilation for 2 h; and 3) high pressure mechanical ventilation for 2 h. The mice were then observed for 14 days for evaluation of epithelial damage, Ashcroft score for fibrosis and inflammation.

Results: The $HNP^{+/+}$ mice showed significantly body weight loss (Fig 1A), increased neutrophil count (Fig 1B) and enhanced total protein (Fig 1C) in BALF and decreased lung compliance (Fig 1D) at 48 h after HCl challenge, as compared to the FVB mice. The lung compliance remained low in $HNP^{+/+}$ mice 14 days after HCl instillation followed by mechanical ventilation at either low pressure or high pressure (Fig 1E).

Conclusion: HNP can accelerate inflammation at an early phase in a mouse model of HCl-induced ARDS, which may contribute to lung remodeling at late phases, magnifying the deleterious effects of ventilator-induced lung injury. We are hoping to present a full picture regarding lung remodeling 14 days after receiving HCl/mechanical ventilation at the ESICM conference in September.

Grant acknowledgment: Canadian Institute of Health Research.

P74

0989. Accuracy of delivered airway pressure during proportional assist ventilation +. A bench study

F Beloncle[1,2*], N Rittayamai[1,3], E Akoumianaki[4], L Brochard[1,5]
[1]Critical Care Department, St Michael's Hospital and Keenan Research Centre, Toronto, Canada; [2]Département de Réanimation Médicale, CHU d'Angers, Université d'Angers, Angers, France; [3]Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand; [4]Intensive Care Unit, Klinik für Herzchirurgie, Herzzentrum, Leipzig, Germany; [5]Interdepartmental Division of Critical Care, University of Toronto, Toronto, Canada
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P74

Introduction: Proportional assist ventilation (PAV+) is a partial ventilatory support mode delivering airway pressure (Paw) in proportion to patient effort, enhancing patient-ventilator interactions. The ventilator estimates muscular pressure by using the respiratory system equation of motion with the instantaneous volume (V) and flow (V´) and the automatically

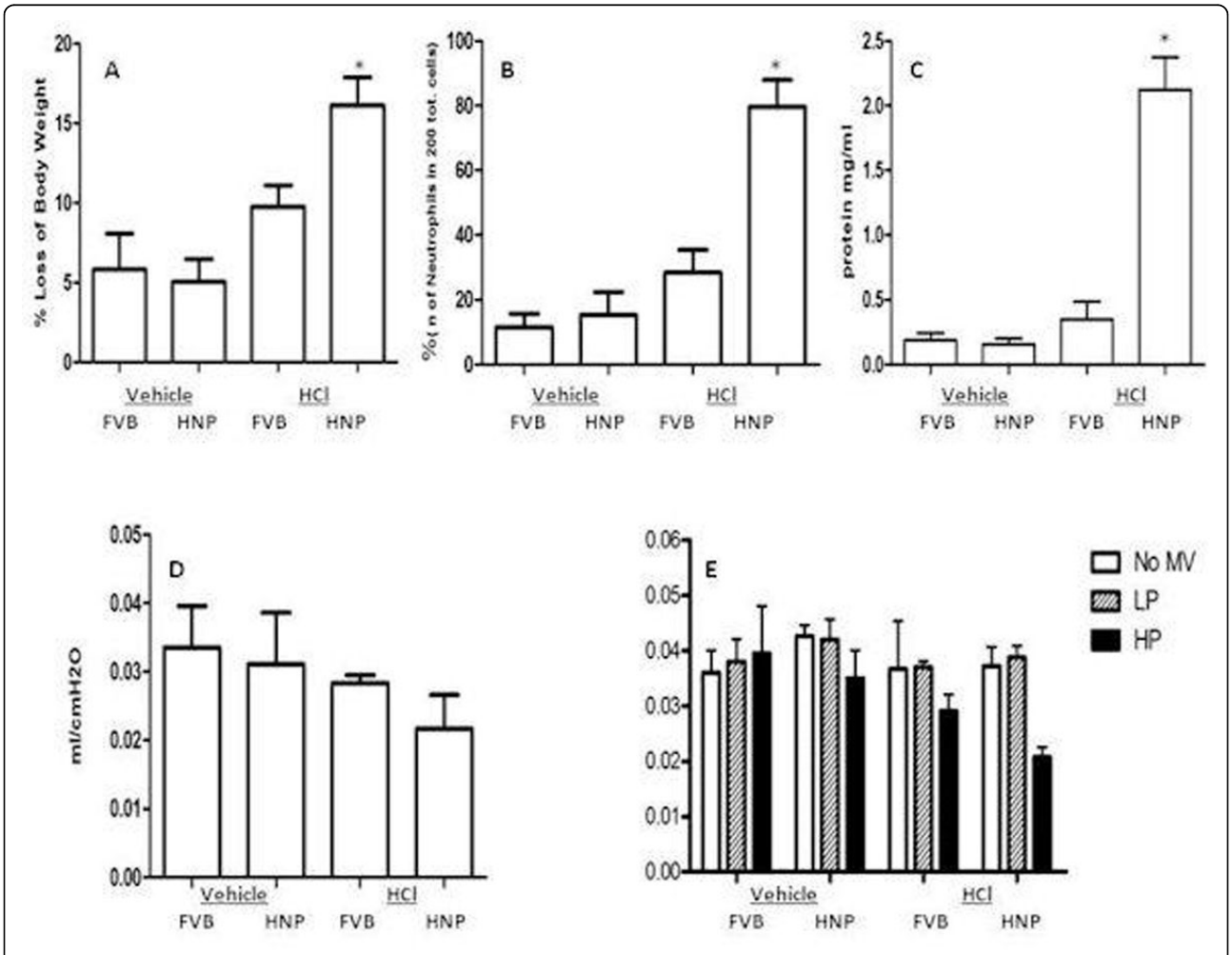

Figure 1(abstract P73) HNP contributes to lung injury. A. Loss of body weight. B. higher neutrophil count. C. increased total protein concentration in BALF, and D. decreased lung compliance in $HNP^{+/+}$ mice 48h after HCl (3 ml/kg) challenge. E. Lung lung compliance remained low in $HNP^{+/+}$ mice 14 days ater HCl installation and mechanical ventilsation (MV) at either low pressure (LP) or high pressure (HP). *p<0.05 as compared to other groups.

calculated compliance and resistance. The mode gains in popularity but the accuracy of the delivered Paw by PAV+ is unknown.
Objectives: To assess the accuracy of PAV+ by comparing the delivered Paw by the ventilator (Paw_{meas}) to the theoretical Paw as defined by the equation of motion (Paw_{Th}) and to examine the factors influencing this accuracy.
Methods: An active servo lung (ASL5000) was programmed to resemble 4 respiratory mechanics: normal (Compliance (C)=60mL/cmH_2O, Resistance (R)=10cmH_2O/L/sec), obstructive (C=60, R=20), restrictive (C=30, R=10), and mixed (C=30, R=20). A Puritan-Bennett 840 ventilator with PAV+ was used. PAV+ was tested varying gain (30 and 60%), inspiratory trigger (IT) (0.8, 5 and 15 L/min), muscular pressure (Pmus) (10 and 15 cmH_2O), positive end-expiratory pressure (PEEP) (0 and 5 cmH_2O), and respiratory rate (RR) (10 to 30/min) to simulate intrinsic PEEP (PEEPi). PEEPi was measured using the Pmus curve. Paw_{Th} was calculated as follows: $Paw_{Th}=[(V/C)+(R\times V')]\times Gain$ + total PEEP.
The inspiratory time was defined from the start of Pmus to the end of inspiratory V´. We calculated the difference between the mean Paw_{meas} and the mean Paw_{Th} during inspiration and between Paw_{meas} and Paw_{Th} at 25, 50, 75 and 100% of the inspiratory time. The percentage of difference between Paw_{meas} and Paw_{Th} was calculated as follows: $\%\Delta=(Paw_{meas}-Paw_{Th})/Paw_{Th}\times 100$.
Results: Irrespective of respiratory mechanics and gain, mean Paw_{meas} was lower than mean Paw_{Th}, Table 1.
This underassistance by the ventilator was greatest at the beginning (25%) of the cycle and decreased later (75%) in inspiration. These findings were replicated under different IT, Pmus or PEEP settings. A high IT led to greater underassistance at the end of inspiration versus a low IT. A high Pmus was associated with a greater underassistance during the entire inspiration versus a low Pmus. A decrease in PEEP was associated with a major underassistance at the start of the inspiration. A higher RR resulted in a higher %Δ, showing that PEEPi increases total trigger delay and affects PAV+ accuracy, fig. 1. Combining the data from all conditions, PEEPi was correlated with the mean %Δ (R^2=0.61, $p < 0.001$).
Conclusions: PAV+ assistance is globally accurate compared to Paw_{Th} even if underassistance is often observed, especially at the start of inspiration. PEEPi leading to increased trigger delay is a major factor contributing to PAV+ inaccuracy. Clinical recommandations should include using a high trigger sensitivity and a careful PEEP titration when PEEPi is suspected.
The research laboratory has received grants from Covidien

P75

0990. Role of amplitude and rate of deformation in ventilator-induced lung injury

A Protti[1*], DT Andreis[2], M Milesi[2], P Pugni[2], F Nicosia[2], T Maraffi[2], V Melis[2], M Monti[2], E Votta[3], L Gattinoni[1,2]
[1]Fondazione IRCCS Ca' Granda - Ospedale Maggiore Policlinico, Milan, Italy; [2]Università degli Studi di Milano, Milan, Italy; [3]Politecnico di Milano, Milan, Italy

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P75

Introduction: Increasing both Tidal Volume (V_T) (*amplitude* of lung deformation) and Inspiratory Flow (V') (*rate* of lung deformation) augments incidence of Ventilator-Induced Lung Injury (VILI) [1].
Objectives: To clarify whether increasing V' at constant V_T augments incidence of VILI.
Methods: Twenty-eight healthy piglets were mechanically ventilated for up to 54 hours. Each animal was assigned to one of three groups of V_T (300-400 ml; 500-600 ml; 750 ml) and one of two groups of V'. Lower and higher V' were obtained by setting inspiratory-to-expiratory time ratio as high as 1:2 or as low as 1:9. Respiratory rate was always 15 breaths per minute. Interplay between V_T and V' was assessed at the beginning of the study as airway pressure-volume loop area (or dynamic respiratory system hysteresis). VILI was defined as pulmonary oedema (lung weight gain ≥10% across the study period).
Results: Main findings are reported in Table 1.
Conclusions: Increasing V' (*rate* of lung deformation) while maintaining V_T (*amplitude* of lung deformation) constant augments incidence of VILI. Further studies are needed to clarify whether dynamic respiratory system hysteresis is an independent predictor of VILI.
Grant acknowledgment: This study was supported in part by an Italian grant provided by Fondazione Fiera di Milano for Translational and Competitive Research (2007, Luciano Gattinoni) and by GE Healthcare.
Reference
1. Protti A, *et al*: Lung stress and strain during mechanical ventilation: any safe threshold? *Am J Respir Crit Care Med* 2011, **183**:1354-1362.

P76

0991. Nebulized heparin reduces pulmonary inflammatory responses in a rat model of acute lung injury

L Chimenti[1*], R Guillamat[1], MN Gomez[1], J Tijero[1], T Lebouvier[2], L Blanch[1,3], A Artigas[1,3]
[1]Corporació Sanitária i Universitária Parc Taulì-UAB, Critical Care Center, Sabadell, Spain; [2]Pontchaillou University Hospital, Surgical Intensive Care Unit, Rennes, France; [3]CIBERES, Enfermedades Respiratorias, Bunyola, Spain
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P76

Introduction: Sepsis is a mayor cause of acute lung injury (ALI). Pulmonary coagulopathy is intrinsic to ALI and in sepsis, anticoagulant system is impaired, due to consumption and downregulation by inflammatory mediators. Several studies, in ALI patients and in ALI experimental models, inconsistently suggest beneficial effects of systemic anticoagulants which affect systemic coagulation. Nebulization of anticoagulants might allow for higher pulmonary concentration and reduce the risk of systemic bleeding.
Objectives: To assess the effects of local heparin treatment in a rat model of acute lung injury.
Methods: Adult male Sprague-Dawley rats (250-300 g; n=6/group) were anesthetized with isofluorane and subjected to intratracheal administration (IA) of LPS (10 μg/g b.w.). Saline or heparin (1000 IU/kg) were nebulized at 4 and 8h after LPS instillation. Animals were sacrificed 24h after the injury. Inflammatory cells and total proteins were assessed in bronchoalveaolar lavage fluid (BALF). IL-6, GRO-κC, TNF-α and IL-10 were measured in lung

Table 1(abstract P74)

Gain (%)	Mechanics	Mean Pawmeas (cm H2O)	Mean PawTh (cm H2O)	Difference mean Pawmeas - mean PawTh (cm H2O)	mean Δ% (%)
30	**Normal**	6.6	9.4	-2.8	-29.8
	Obstructive	8.4	10.8	-2.4	-22.2
	Restrictive	7.0	8.5	-1.5	-17.6
	Mixed	6.7	8.8	-2.1	-23.9
60	**Normal**	9.6	13.7	-4.2	-29.9
	Obstructive	9.5	13.6	-4.1	-30.1
	Restrictive	10.2	13.0	-2.8	-21.5
	Mixed	10.3	13.5	-3.2	-23.7
All conditions		8.5	11.4	-2.9	-25.4

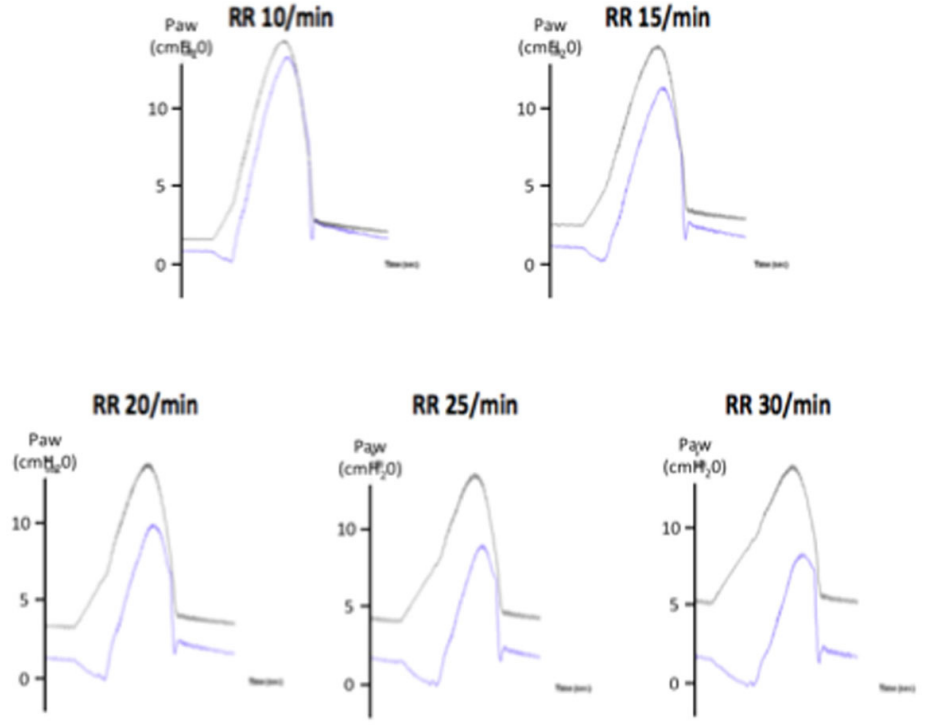

Figure 1(abstract P74) Representative tracings of measured airway pressure (Paw_{meas}) and theoretical airway pressure (Paw_{Th}) with progressive increases in rate (RR) and PEEPi. *Black lines:* Paw_{Th} wareforms. *Blue lines*: Paw_{meas} waveforms. Inspiratory trigger = 5 L.min; PEEP = 0 cmH_2O; gain = 60%; Pmus = 10 cmH_2O; Resistance = 20 cmH_2O/L/s and compliance = 60 mL/cmH_2O.

homogenate by multiplex assay (Luminex, Merck Millipore, Darmstadt, Germany). Data are reported as mean±SD. One-way ANOVA was used for multigroup comparisons.
Results: In BALF, nebulized heparin significantly reduced neutrophils in animals instilled with LPS (12.4 ± 4.3 × 107 cells/ml) compared to animals administrated with LPS and nebulized with saline (18.1 ± 6.1 × 107 cells/ml, $p < 0.05$). Total BALF proteins were found to be lower in rats nebulized with heparin (578.3 ± 89.8 μg/ml) than in rats treated with saline (914.6 ± 250.1 μg/ml, $p < 0.005$). In lung homogenate, IL-6, TNF-α, GRO-κC levels significantly decreased in animals nebulized with heparin compared to those ones nebulized with saline (IL6: LPS+Sal: 147.7 ± 1.3 ng/ml, LPS+Hep: 47.3 ± 20.2 ng/ml, $p < 0.005$; TNF-α: LPS+Sal: 1.1 ± 0.2 ng/ml, LPS+Hep: 0.3 ± 0.1 ng/ml, $p < 0.005$; GRO-κC: LPS+Sal: 45.9 ± 22.9 ng/ml, LPS+Hep: 40.9 ± 9.8 ng/ml, $p < 0.005$). IL-10 did not show any significant difference among groups.
Conclusions: Our results show that local heparin administration reduces pulmonary inflammatory responses in a rat model of acute lung injury.
Grant acknowledgment: FIS-PI12/02548, CIBERES and Fundació Parc Taulí, AGAUR GRC 532.

P77

0992. Effects of inhaled aerosolized insulin on acutely injured lungs under normoglycemia: insulin may contribute to enhance alveolar liquid clearance through epithelial sodium channel expression

M Senda[1*], W Fan[2], K Nakazawa[1], K Makita[1]
[1]Tokyo Medical and Dental University, Dept. of Anesthesiology & Critical Care Medicine, Tokyo, Japan; [2]Inner Mongolia People's Hospital, Dept. of Anesthesia, Inner Mongolia, China
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P77

Introduction: We have previously shown that hyperglycemia enhances inflammatory responses and that aerosolized insulin ameliorates inflammatory responses compared to identical doses of intravenous insulin in an experimental model of acute lung injury [1].
Objectives: The purpose of the present study is to clarify whether insulin exerts anti-inflammatory effects in acute lung injury under normoglycemia.
Methods: Twenty mechanically ventilated rabbits were randomly allocated into control group (group-C: n=10) or inhaled aerosolized insulin group

Table 1(abstract P75) Inspiratory flow and incidence of VILI

	V_T 300-400 ml		V_T 500-600 ml		V_T 750 ml	
	Lower V´	Higher V´	Lower V´	Higher V´	Lower V´	Higher V´
Tidal volume (ml)	338±48	335±42	530±27	520±27	750±0	750±0
Inspiratory flow (ml/sec)	272±36	838±105*	398±21	1278±38*	600±84	1242±95*
Hysteresis (ml*cmH_2O)	6260±2236	12938±3356*	11101±4508	34126±4508*	18415±3520	46915±7954*
Incidence of VILI	0/4	1/5	0/5	4/5*	2/5	4/4

Data are presented as mean±standard deviation. * $p< 0.05$ *vs.* Lower V' within the same V_T group (Student's *t*, Mann-Whitney Rank Sum or Fisher's exact tests). Hysteresis was associated with incidence of VILI ($R=0.68$, $p< 0.0001$) (Spearman Rank Order Correlation).

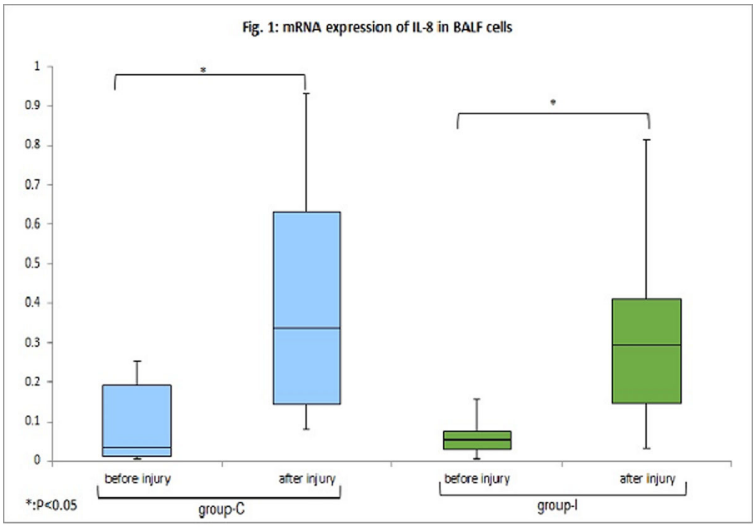

Figure 1(abstract P77) mRNA expressions of IL-8 in BALF cells.

(group-I: n=10). They were anesthetized with intramuscular ketamine and pentobarbital. Mechanical ventilation was initiated after tracheostomy (TV=10ml/kg, RR=25/min, I:E ratio=1:2, PEEP=3cmH_2O and F_IO_2=1.0). Lung lavage was performed with warm normal saline (60 ml) to produce lung injury. After induction of acute lung injury, the ventilator settings were changed as follows: TV=6 ml/kg, RR=30-40/min, and PEEP=10cmH_2O. The group-I received aerosolized insulin (1 IU/kg) diluted in 5ml normal saline through an ultrasonic nebulizer every three hours while the group-C received 5ml of aerosolized normal saline. Arterial blood samples were obtained for blood glucose and blood gas analyses at 60, 120, 180, 240, and 360 min after ventilation settings were changed. After 6 hours of treatment, the lungs and heart were excised en bloc, and then we examined interleukin-8 (IL-8), toll-like receptor 4 (TLR-4) mRNA expressions in bronchoalveolar lavage fluid (BALF) cells. We also examined mRNA expressions of endothelial sodium channel alpha subunit (ENaCα), serum/glucocorticoid regulated kinase 1 (SGK1), transforming growth factor-β1 (TGF-β1) and keratinocyte growth factor (KGF) in the lung tissue so as to examine whether insulin contributes to recovery from lung injury.

Results: The blood glucose levels maintained within normal limits (80-180 mg/dl) and identical between the two groups throughout the experi-mental course. The mRNA expressions of IL-8 in the BALF cells elevated after injury, however the levels were not different between group-C and group-I (Fig. 1). The mRNA expression of TLR-4 in BALF cells did not elevate after experiment. The tissue levels of mRNA expression of ENaCα were significantly higher in the group-I (Fig.2). However, we could not find any difference in mRNA expression of TGF-β1 and KGF in the tissue of both groups.

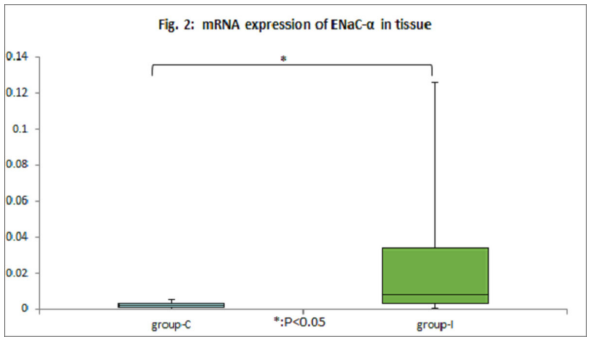

Figure 2(abstract P77) mRNA expression of ENaC-α in tissue.

Conclusions: The results failed to show insulin ameliorate inflammatory responses in acute lung injury under normoglycemia. This may partly explained by the low tidal volume ventilation with PEEP protected exacerbation of inflammatory responses in this injury model. However we found that insulin might contribute to the lung fluid clearance via expression of epithelial sodium channel.

Reference

1. Fan W, *et al*: Inhaled aerosolized insulin ameliorates hyperglycemia-induced inflammatory responses in the lungs in an experimental model of acute lung injury. *Crit Care* 2013, **17**:R83.

P78

0993. Heme oxygenase - 1 attenuates acute pulmonary inflammation by decreasing the release of segmented neutrophils from the bone marrow

F Konrad*, S Braun, I Vollmer, K-C Ngamsri, J Reutershan
University of Tübingen, Tübingen, Germany
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P78

Introduction: The stress response enzyme heme oxygenase 1 (HO-1) is expressed ubiquitous in the body tissue and mediates, besides its major biological function of heme degradation, a variety of anti-inflammatory effects. Although these effects have been described in various models, underlying mechanisms remain elusive. Few studies revealed an influence of HO-1 on the bone marrow [1].

Objectives: We investigated the particular role of the bone marrow in terms of HO-1-dependent pulmonary inflammation.

Methods: Wildtype mice inhaled LPS for 30 minutes. After 24h, PMNs were detected in lung compartments (intravascular - interstitial - alveolar) using a flow cytometry-based technique. Bone marrow and blood were analyzed by flow cytometry after four hours. HO-1 was pharmacologically induced by Cobalt(III)Protoporphyrin-IX-Chloride (CoPP) and inhibited by TinProtophorphyrin-IX (SnPP). Chemokines were measured in the BAL and bone marrow by ELISA.

Results: Immunohistochemistry revealed less lung destruction after HO-1 stimulation by CoPP, and *in vivo* migration assay showed reduced PMN migration into the BAL. Differentiation of PMNs in the BAL revealed a decrease of segmented PMNs after HO-1 activation. In this group, segmented PMNs were also decreased intravascularly and concordantly, mature and immature PMN populations were higher in the bone marrow. Inhibition of HO-1 by SnPP aggravated parameters of tissue inflammation, led to destruction and increased PMN migration into the BAL. Differential BAL counts in this group revealed an increase of segmented and banded PMNs with enhanced PMN release from the bone marrow. The chemokine SDF-1,

which mediates homing of leukocytes into the bone marrow, was increased by CoPP and reduced by inhibition of HO-1. When SDF-1 was blocked by the specific antagonist AMD3100, HO-1 activation was no longer effective in curbing PMN trafficking to the inflamed lungs. Additionally, we compared the inhibition of HO-1 by SnPP alone and in combination with CoPP. Enzyme activity was more reduced after CoPP/SnPP treatment, resulting in deteriorated lung injury with higher PMN counts in the lung interstitium, and pronounced tissue destruction.

Conclusions: We show evidence that the anti-inflammatory effects of HO-1 are largely mediated by inhibiting the release of segmented PMNs from the bone marrow rather than direct effects within the lung.

Grant acknowledgment: This work was supported by German Research Foundation Grant RE 1683/4-1 (to Jörg Reutershan).

Reference

1. Wegiel B, Gallo DJ, Raman KG, *et al*: Nitric oxide-dependent bone marrow progenitor mobilization by carbon monoxide enhances endothelial repair after vascular injury. *Circulation* 2010, **121**:537-548.

P79

0994. Development of ventilatory-induced lung injury depends on energy dissipated into respiratory system

M Gotti*, C Chiurazzi, M Amini, C Rovati, M Brioni, A Cammaroto, S Luoni, C Bacile di Castiglione, G Rossignoli, C Montaruli, K Nikolla, M Monti, D Dondossola, I Algieri, T Langer, M Cressoni, L Gattinoni
Università degli Studi di Milano, Fisiopatologia Medico-Chirurgica e dei Trapianti, Milano, Italy

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P79

Introduction: Mechanical ventilation with high volumes/pressures induces ventilatory induced lung injury (VILI). During each breath, part of the energy transmitted from ventilator to respiratory system is given back as elastic recoil and part is dissipated into the respiratory system; this amount of energy is measured by the hysteresis area of the pressure-volume (PV) curve of the respiratory system. Total dissipated energy into respiratory system, or dissipated inspiratory potency, equals to energy dissipated during every single breath multiplied by the respiratory rate (RR).

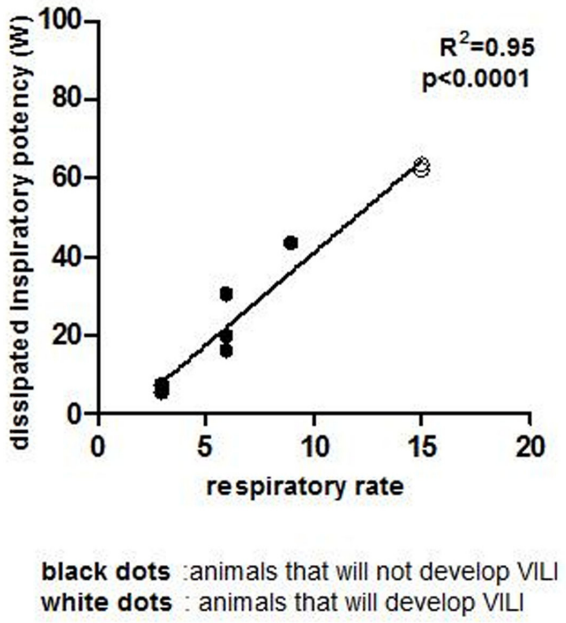

Figure 1(abstract P79)

Objectives: To measure dissipated inspiratory potency throughout lung parenchyma and to asses if the reduction of the energy load into the respiratory system, obtained reducing RR, delays the development of VILI.

Methods: Ten piglets, after general anaesthesia induction, were ventilated at lethal tidal volume (TV), defined on strain > 2.5 (which is known to cause lung oedema within 54 hours), but at different RR: 4 pigs at RR 3, 3 pigs at RR 6, 1 pig at RR 9 and 2 pigs at RR 15. We measured PaO_2/FiO_2 ratio, plateau pressure, and PV curve hysteresis in dynamic and static conditions at the beginning and at the end of the experiment and lung weight at the end of experiment.

Results: Inspiratory potency set at the beginning of the experiment had a linear relationship with RR (R^2=0.95, $p < 0.001$) (Fig.1).

There were no differences in baseline conditions and in TV setting between groups. Piglets ventilated at RR 15 developed lung oedema (final lung weight: 550±28 g vs 287±49 g, $p < 0.001$). PaO_2/FiO_2 ratio and plateau pressure at the end of the experiment were different in piglets ventilated at RR15 (177 ± 9 mmHg vs 437 ± 77 mmHg, $p < 0.01$; 33 ± 3 cmH_2O vs 22 ± 5 cmH_2O, $p < 0.05$, respectively), as compared to piglets ventilated at RR3, 6 and 9. Energy dissipated into the respiratory system increased only in pigs who developed lung oedema, from 63 ± 1 W at the beginning to 110 ± 28 W at the end of the experiment.

Conclusions: The development of VILI (lung oedema) depends on inspiratory potency dissipated into the respiratory system.

Grant acknowledgment: ESICM Bernard Drager Award 2012.

P80

0995. Aspirin reduces neutrophilic pulmonary inflammation in a human model of acute respiratory distress syndrome induced by inhaled lipopolysaccharide

U Imran Hamid*, J Conlon, S Spence, A Krasnodembskaya, A Kissenpfennig, DF McAuley, CM O'Kane
Centre for Infection and Immunity, School of Medicine, Dentistry and Biomedical Sciences, Queen's University of Belfast, Belfast, UK

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P80

Introduction: Acute respiratory distress syndrome (ARDS) is characterized by damage to the alveolar epithelial-endothelial barrier resulting in neutrophil influx and pulmonary oedema. The activation of platelet and secondary capture of neutrophils may play an important role in propagation of inflammation in ARDS [1]. Various animal studies have shown that aspirin therapy reduces pulmonary oedema and development of lung injury [2]. In observational studies, patients on aspirin therapy prior to hospital admission had a reduced incidence of ARDS [3]. By acetylating cyclooxygenase, aspirin inhibits platelet aggregation and generates anti-inflammatory molecules which modulate neutrophilic inflammation [4].

Objective: To test the hypothesis that aspirin reduces pulmonary inflammation in an *in vivo* human model of acute respiratory distress syndrome, induced by inhaled lipopolysaccharide (LPS).

Methods: Healthy subjects were enrolled in a double-blind, placebo-controlled study and were randomised to receive aspirin 75mg or aspirin 1200mg or placebo (1:1:1) for seven days prior to LPS inhalation. Measurements were performed in bronchoalveolar lavage (BAL) fluid obtained at 6 hours after inhaling 50 micrograms of LPS.

Results: 33 healthy subjects were enrolled. There was no significant difference between aspirin 75mg and aspirin 1200mg. Data for both aspirin groups were combined. Aspirin pre-treatment reduced LPS induced BAL neutrophilia (figure 1) and BAL concentrations of both the neutrophil-specific protease MMP-8, and the pro-inflammatory cytokine TNF-α. There was a non-significant trend towards reduction in a range of inflammatory cytokines (table 1).

Conclusion: This study shows for the first time that aspirin can reduce neutrophilic inflammation in the lung in humans. Further clinical studies are warranted to assess its ability to reduce neutrophil mediated inflammation in ARDS.

Grant acknowledgement: This work was funded by the UK Intensive Care Society.
ClinicalTrials.gov identifier: NCT01659307.

References

1. Kuebler WM: *J Clin Invest* 2006, **116(12)**:3106-8.
2. Looney MR, *et al*: *J Clin Invest* 2009, **119(11)**:3450-61.

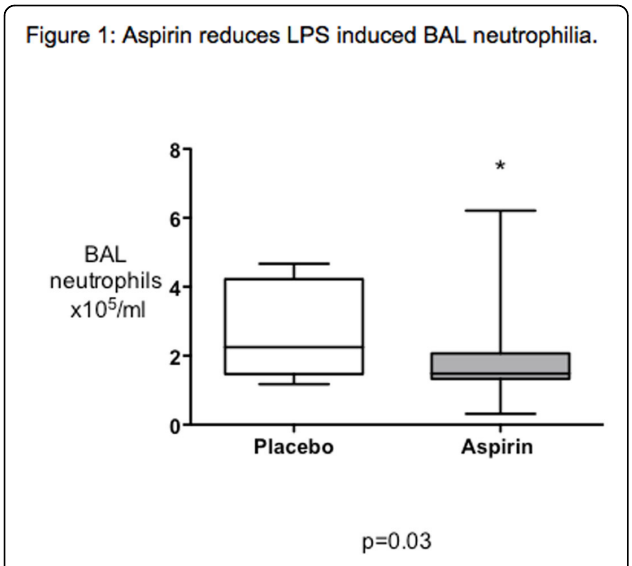

Figure 1(abstract P80) Aspirin reduces LPS induced BAL neurtophilia.

3. Erlich JM, *et al*: *Chest* 2011, **139(2)**:289-95.
4. Filep JG, *et al*: *Prostaglandins Leukot Essent Fatty Acids* 2005, **73(3-4)**:257-62.

P81

0996. Improventment of method primary cultivation and identification of rat pulmonary microvascular endothelial cells

L Yong-Jun[1*], M Jie[2], W Ping-Ping[2], O Bin[2], C Juan[2], G Xiang-Dong[2]
[1]The First Affiliated Hospital, Sun Yat-sen University, Department of SICU, Guangzhou, China; [2]The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P81

Introduction: Pulmonary microvascular endothelial cell(PMVEC) is a very important tool to study acute respiratory distress syndrome. Several methods for *in vitro* cultivation of PMVEC have been established, but all the methods damage PMVECs either by use of proteolytic emzymes or by mechanical trauma. This study was to improve the method for primary cultivation of rat pulmonary microvascular endothelial cells and identify the primary cultivated cells.
Objectives: To improve the method for primary cultivation of rat pulmonary microvascular endothelial cells and identify the primary cultivated cells
Methods: Pulmonary microvascular endothelial cells were derived from peripheral lung tissue of Sprague-Dawley rats. Primary cultivation method was improved from the procedures of animal selection, lung tissue perfusion, tissue piece pasting, culture medium selection and so on. Inverted microscope was used to observe morphological characteristics of pulmonary microvascular endothelial cells. Immunohistochemical staining for expression of VIII-related antigen and CD31, and observed by fluorescence microscope for binding with lectin from BSI (FITC-BSI binding away) to identified pulmonary microvascular endothelial cells. The cell purity was detected with flow cytometry.
Results: The PMVECs were exhibited as polygon and presented typical cobblestone-like morphology after fusion to monolayer with contact inhibition. PMVECs turned to be fusiform and presented a swirling or aggregate growth pattern after transfer of culture. Immunohistochemical staining revealed that the expression of CD31 and factor VIII-related antigen was positive. Besides, there were positive findings for FITC-BSI assay. The vitality and growth rate of PMVECs were in good condition. The cell purity was 93.2% with the improvement method of primary cultivation.
Conclusions: The improved method for primary cultivation is easy to handle, with favorable repeatability and success rate. PMVECs obtained with this method will display lower contamination, higher purity, faster growth and better status.
Grant acknowledgment: This study was supported by grant from the National Natural Science Foundation of china (81071536) and Youth Fund of the National Natural Science Foundation of china (81201452).
References
1. Magee JC, Stone AE, Oldham KT, *et al*: **Isolation, culture, and characterization of rat lung microvascular endothelial cells.** *Am J Physiol* 1994, **267**:L433-41.
2. Chen SF, Fei X, Li SH: **A new simple method for isolation of microvascular endothelial cells avoiding both chemical and mechanical injuries.** *Microvasc Res* 1995, **50**:119-28.
3. **Epoxygenase-driven angiogenesis in human lung microvascular endothelial cells.** *Am J Physiol Heart Circ Physiol* 2003, **284**:H215-24.

P82

0997. The unrecognized effects of phosphodiesterase 4 on epithelial cells in pulmonary inflammation

F Konrad[1*], A Bury[1], MA Schick[2], K-C Ngamsri[1], I Vollmer[1], J Reutershan[1]
[1]University of Tübingen, Tübingen, Germany; [2]University of Würzburg, Würzburg, Germany
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P82

Introduction: Acute pulmonary inflammation (ALI) is characterized by migration of polymorphonuclear neutrophils (PMNs) into the different compartments of the lung, passing an endothelial and epithelial barrier. Recent studies showed evidence that phosphodiesterase (PDE)4-inhibitors stabilized endothelial cells [1]. PDE4B and PDE4D play a pivotal role in inflammation, whereas blocking PDE4D is suspected to cause gastrointestinal side effects.
Objectives: To investigate the particular role of PDE4 on PMN migration and lung epithelium.
Methods: ALI was induced by inhalation of LPS in mice. PDE4-inhibitors roflumilast and rolipram were administered i.p. or were nebulized after inflammation to investigate the impact of PDE-inhibitors on pulmonary

Table 1(abstract P80) Effect of aspirin on markes of pulmonary inflammation

BAL				
	Placebo (n=13)	Aspirin (n=20)	% reduction	p-value
PMN (10^5cells/ml)	2.25 (1.18, 4.67)	1.48 (0.32, 6.21)	34.2	0.03
TNF-α (pg/ml)	105.7 (73.49, 358.5)	79.72 (37.34, 276.6)	24.5	0.02
IL-6 (pg/ml)	856.5 (535.9, 2285)	647.9 (224.6, 1582)	24.1	0.07
MMP-8 (pg/ml)	6342 (1932, 9574)	2902 (862.8, 6758)	54	0.03
MMP-9 (pg/ml)	48493 (23126, 99780)	33717 (10471, 72334)	30.4	0.03
IL-8 (pg/ml)	447.5 (236.9, 1250)	345.9 (175.5, 1522)	22.7	0.11
IL-1β (pg/ml)	42.74 (25.98, 98.76)	37.03 (18.73, 54.88)	13	0.16

epithelium in vivo. Additional, PDE4-inhibitors were tested regarding to migration of human PMNs through pulmonal epithelial barrier in vitro. Furthermore the influence of PDE4-inhibitors on PDE4B and PDE4D expression as well as on cytokines were analyzed.
Results: Both PDE4-inhibitors decreased transendothelial and transepithelial migration, whereas roflumilast showed a superior effect compared to rolipram on the epithelium. Both inhibitors decreased the chemokines TNFα, IL6, and CXCL2/3. CXCL1, the strong PMN chemoattractant secreted by the epithelium [2], was significantly more reduced by roflumilast compared to rolipram. In vitro assays with human PMNs and human pulmonary epithelium also emphasized the pivotal role of roflumilast on the epithelium. Expression of PDE4 subtypes B and D were both increased in the lungs by LPS, and PDE4 inhibitors decreased mainly PDE4B. Administration of the PDE-inhibitors still had anti-inflammatory properties after LPS inhalation and the topical administration of PDE4-inhibitors was also effective in curbing down PMN migration, both highlighting the clinical potential of these compounds.
Conclusions: We determined the pivotal role of the PDE4-inhibitor roflumilast on lung epithelium and emphasized its main effect on PDE4B in hyperinflammation.
Grant acknowledgment: This work was supported by German Research Foundation Grant RE 1683/4-1 (to Jörg Reutershan).
References
1. Schick MA, Wunder C, Wollborn J, Roewer N, Waschke J, Germer CT, Schlegel N: **Phosphodiesterase-4 inhibition as a therapeutic approach to treat capillary leakage in systemic inflammation.** *J. Physiol* 2012, **590**:2693-2708.
2. Becker S, Quay J, Koren HS, Haskill JS: **Constitutive and stimulated MCP-1, GRO alpha, beta, and gamma expression in human airway epithelium and bronchoalveolar macrophages.** *Am. J. Physiol* 1994, **266**:L278-286.

CARDIAC ARREST: MANAGEMENT & COMPLICATIONS

P83

1036. Comparison of the hemodynamic parameters of two external chest compression devices (LUCAS versus AUTOPULSE) in a swine model of ventricular fibrillation

C Pantazopoulos[1*], I Floros[1], A Mega[1], C Rigas[1], I Pavleas[1], P Vernikos[1], N Archontoulis[1], D Xanthis[1], N Iacovidou[2], T Xanthos[3]
[1]General Hospital of Athens Laiko, Intensive Care Unit, Athens, Greece; [2]University of Athens, Medical School, Neonatology, Athens, Greece; [3]University of Athens, Medical School, M.Sc. Programme in Cardiorespiratory Resuscitation, Athens, Greece
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P83

Introduction: Given the difficulty of performing efficient CPR compressions, technology has turned to automaticity. LUCAS device has a pneumatically driven piston to compress the heart and uses active decompression suction on the upstroke. AUTOPULSE is a load distributing band compressor, that is mechanically actuated and battery driven. It provides both direct compression and semi-circumferential thoracic compression.
Objectives: To compare 2 different external chest compression devices (LUCAS and AUTOPULSE) regarding the hemodynamic effects during cardiorespiratory resuscitation.
Methods: Forty (40) pigs were randomly allocated into 2 groups. Group L (LUCAS), n=20 and Group A (AUTOPULSE), n=20. After anesthesia ventricular fibrillation was induced. Five minutes post cardiac arrest without treatment, resuscitation was initiated. Electrocardiography, intra-arterial pressure (carotid artery) and Swan-Ganz catheter were used to monitor central venous pressure, cardiac output, cardiac index, systemic vascular resistance and pulmonary vascular resistance prior to ventricular fibrillation and during resuscitation in both groups.
Results: The hemodynamic parameters demonstrated that there is no statistical difference in mean arterial pressure between the 2 devices but there was statistically significant difference ($P < 0.05$) in the cardiac output with LUCAS generating higher values than AUTOPULSE.
Conclusions: The mean arterial pressure that is produced by the 2 devices is similar, while the cardiac output produced by LUCAS is higher than AUTOPULSE.
References
1. Axelsson C, *et al*: **Clinical consequences of the introduction of mechanical chest compression in the EMS system for treatment of out-of-hospital cardiac arrest-a pilot study.** *Resuscitation* 2006, **71(1)**:47-55.
2. Ong ME, *et al*: **Use of an automated, load-distributing band chest compression device for out-of-hospital cardiac arrest resuscitation.** *JAMA* 2006, **295(22)**:2629-37.

TRAUMA CARE: FROM BENCH TO BEDSIDE

P84

1068. MicroRNA-regulated immunosuppression in severely injured polytrauma patients

HC Owen[1*], HD Torrance[1,2,3], K Brohi[3], CJ Hinds[1,2], MJ O'Dwyer[1,2]
[1]Centre for Translational Medicine and Therapeutics, William Harvey Research Institute, Barts and the London School of Medicine and Dentistry, London, UK; [2]Adult Critical Care Unit, Royal London Hospital, Barts Health NHS Trust, London, UK; [3]Centre for Trauma Sciences, Blizard Institute, Barts and the London School of Medicine and Dentistry, London, UK
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P84

Introduction: We have demonstrated that traumatic injury is associated with an early immunosuppressive response, the extent of which is associated with an increased risk of developing nosocomial infections. In particular, the expression of the immunosuppressive cytokine IL-10 was up-regulated 2 hours following injury [1]. However, the mechanisms involved

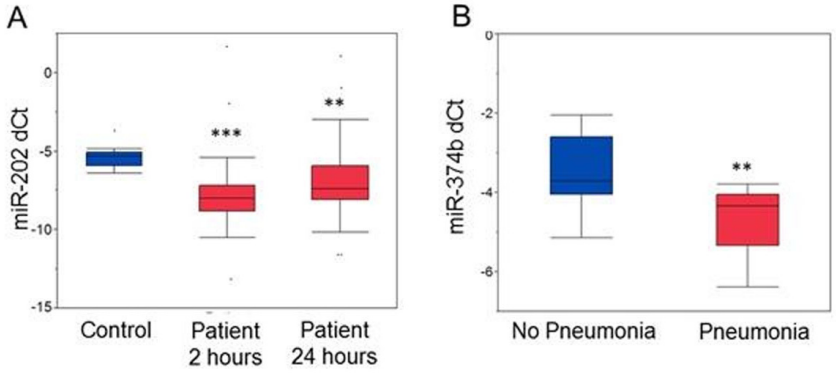

Figure 1(abstract P84) (A) miR-202 expression levels are decreased in patients vs. healthy controls at 2 and 24 hours. (B) miR-374b expression is significantly reduced in patients with pneumonia at 2 hours (**p<0.01; ***p(0.001; median and interquartline ranges)

are unclear. MicroRNAs (miRs) are short non-coding RNA molecules whose main function is to down-regulate gene expression. Although preliminary laboratory data suggest alterations in miR expression may play a role in triggering this immunosuppressive phenotype there is currently a lack of data in trauma patients [2,3].

Objectives: To explore alterations in candidate miR expression that may regulate the immunosuppressive phenotype following severe trauma and evaluate their potential role in enhancing the risk of nosocomial infections.

Methods: Following ethics approval and consent, 30 ICU patients admitted following severe traumatic injury and 16 healthy age and sex matched controls were recruited. miRs were isolated utilising PAX Gene and miRNA-Easy extraction kits (Qiagen). miRs were selected for analysis based on miRBase target prediction scores for the IL10 promoter. Candidate miRs were quantified by qPCR at 2 hours and 24 hours following injury and then normalised to the small nucleolar RNAs RNU44 and RNU48. Infections were assessed using predefined criteria.

Results: miR-202, miR-374b and miR-125a3p were selected for analysis as they were in the top 10% of miRs predicted to target the IL-10 promoter. After 2 hours, expression of miR-202 was significantly reduced (38.8%; $p< 0.001$; Figure 1A) in patients compared to healthy controls. This reduction was maintained (22.1%, $p = 0.006$) 24 hours after injury, and was associated with the extent of shock (pH ($p = 0.009$); lactate ($p = 0.012$) and base excess ($p = 0.039$)) at admission. miR-374b expression not was significantly changed at either time point but was however associated with a subsequent development of pneumonia ($p = 0.0032$; Figure 1B). miR-125a3p expression was significantly reduced by 7.6% ($p = 0.05$) 2 hours following injury. At 24 hours, miR-125a3p was associated with the later development of infections ($p = 0.015$) and in particular pneumonia ($p = 0.013$).

Conclusions: The expression of miRs with high predicted complementarity to the IL-10 promoter decrease following a severe traumatic injury. It is plausible that this reduction in inhibitory miRs is an important mechanism for the increase in IL-10 gene expression that is seen in these patients and thereby contributes to the consequent immunosuppressive phenotype and increased risk of nosocomial infections.

Grant acknowledgment: This work was funded by a Barts and the London Charity Grant.

References

1. Torrance HD, *et al*: *Crit Care* 2013, **17(Suppl 2)**:27.
2. Cheung ST, *et al*: *PLoS One* 2013, **8(8)**:e71336.
3. Wang HJ, *et al*: *Shock* 2013, **39(6)**:480-7.

P85

1069. Pharmacological preconditioning with vitamin c attenuates intestinal injury via the induction of heme oxygenase-1 after hemorrhagic shock in rats

B Zhao*, J Fei, Y Chen, X-Q Song, L Ma, L Wang, E-Z Chen, E-Q Mao
Shanghai Jiaotong University School of Medicine, Department of Emergency Intensive Care Unit, Ruijin Hospital, Shanghai, China

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P85

Introduction: Pre-induction of heme oxygenase (HO)-1, which is regarded as an effective method of "organ preconditioning", exerts beneficial effects during hemorrhagic shock (HS). However, the available HO-1 inducers exhibit disadvantages such as toxicity or complex technical requirements. Therefore, a safe and convenient HO-1 inducer would be promising and could be exploited in the treatment of foreseeable hemorrhaging, such as prior to major surgery. Recently, vitamin C (VitC) has been shown to attenuate organ injuries and inhibit inflammatory responses in hemorrhagic shock [3], but the specific mechanism remains unclear. Studies on the relationship between HO-1 and VitC are limited, and the results are controversial [4,5].

Objectives: We investigated the effect of vitamin C (VitC) on intestinal HO-1 expression and the involved mechanism. We further investigated if VitC pretreatment prevented HS related intestinal tissue injuries via HO-1 induction.

Methods: The IEC-6 were treated with grade concentration of VitC as well as SB203580, PD98059 and SP600125, the inhibitors of p38 mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase (ERK) 1/2 and c-Jun N-terminal kinase (JNK). SD rats were pretreated with VitC (intraperitoneally, 100mg/Kg), HS was induced by drawing blood from the rat femoral artery (mean arterial pressure = 30 mm Hg) for 1 hr and resuscitating with the shed blood and Ringer's solution. Some rats further received zinc protoporphyrin (Znpp, intraperitoneally, 3 mg/kg), a HO-1 inhibitor.

Results: The *in vitro* study showed HO-1 was induced in IEC-6 cell in a time- and concentration- dependent manner by VitC, and the inhibitor of ERK1/2 PD98059 inhibited the VitC induced HO-1 expression. The *in vivo* study showed the HO-1 protein (mainly observed in intestinal epithelial cells) and activity in intestine were highly induced in normal rat, and these HO-1 levels were further enhanced in HS rat model. The histological damage, apoptosis (number of TUNEL positive cell, Bcl-2/Bax ratio), neutophil infiltration (number of MPO positive cells, MPO activity and MPO protein level), and inflammatory cytokines level of tumor necrosis factor-a and interleukin-6 were all relieved by VitC pretreatment, and the protective effect of VitC was attenuated by Znpp.

Conclusions: These data suggests VitC might be applied as a safe inducer of intestinal HO-1 and VitC pretreatment attenuated HS related intestinal injuries via induction of HO-1 by activating ERK1/2 pathway.

Grant acknowledgment: This work is supported by National Natural Science Foundation of China projects 81171789.

References

1. Kubulus D, *et al*: *Shock* 2008, **29(5)**:583-590.
2. Raddatz A, *et al*: *American journal of respiratory and critical care medicine* 2006, **174(2)**:198-207.
3. Van PY, *et al*: *The Journal of trauma* 2011, **71(1)**:20-24, discussion 24-25.
4. Kim JY, Lee SM: *Life sciences* 2004, **75(16)**:2015-2026.
5. Elbekai RH, *et al*: *Cancer letters* 2007, **246(1-2)**:54-62.

P86

1070. Effects of two different mechanical ventilation strategies on lung conditions after experimental ARDS following blunt chest trauma and pulmonary contusion in pigs

S Hammermüller[1*], N Carvalho[2], S Huckauf[1], S Kobelt[1], M Baunack[1], K Noreikat[1], A Beda[2], A Reske[3], H Wrigge[1], AW Reske[1]
[1]University Leipzig, Department of Anesthesiology and Intensive Care Medicine, Leipzig, Germany; [2]University of Minas Gerais, Department of Electronic Engineering, Federal, Belo Horizonte, Brazil; [3]Fachkrankenhaus Coswig, Coswig, Germany

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P86

Introduction: Pulmonary contusion (PC) is common after blunt chest trauma, leads to inhomogeneous lung injury and can result in acute respiratory distress syndrome (ARDS) [1]. Strategies for mechanical ventilation (MV) with different physiological rationales and approaches to positive end-expiratory pressure (PEEP) and tidal volume (TV) adjustment are proposed [1-3].

Objective: To study the effects of the ARDSnetwork lower PEEP (ARDSnet) [1] and the Open Lung Concept (OLC) [2] strategies for MV on lung ventilation and function over 24 hours in pigs after experimental PC.

Methods: Pigs (n=16) were anesthetized, tracheotomized and received MV. Catheters were placed aseptically. Cefuroxime 750mg was given IV q6h. Unilateral PC was induced by a 10 kg weight dropped from 1.85 m height on a predefined location of the right chest. Chest tubes were inserted on both sides. Conditions comparable to an ICU were established. At 90 min after PC (post-PC) pigs were randomized to 24 hours of MV using ARDSnet (n=8) or OLC (n=8). Pressure controlled MV in the OLC group involved: an initial recruitment maneuver (50 cmH_2O, 10 breaths), respiratory rate 80/min, I/E 2:1, TV< 6 ml/kgBW, positive inspiratory pressure (PIP) ≤30 cmH_2O. Total PEEP of approx. 19 cmH_2O resulted from development of intrinsic PEEP on top of external PEEP of 10 cmH_2O. Cardiorespiratory, gas exchange and extra-vascular lung water (EVLW, single-indicator transpulmonary thermodilution) parameters were measured. Electrical impedance tomography was used to assess changes in lung ventilation (Vent). Vent was calculated as the number of pixels showing an impedance change of >15% of the global impedance change and expressed as % of baseline. Data are given as median and interquartile (25^{th}-75^{th}) range. Mann-Whitney-tests and General Linear Model statistics were used.

Results: Cardiorespiratory conditions were stable without significant between-group differences at pre-PC and post-PC. At 24 hours after randomization PEEP was significantly lower in ARDSnet (8 (5-10) cmH_2O) vs. OLC 19 (17-21) cmH_2O). PaO_2/FIO_2 (477 (296-514) vs. 87 (72-118) mmHg) and static compliance were significantly higher in OLC (Tab.1). Intrapulmonary shunt (28 (27-36) vs. 11 (8-18) %), $PaCO_2$ (46 (43-63) vs.

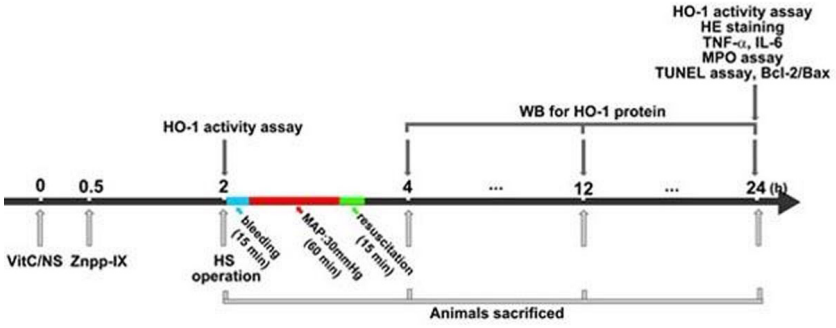

Figure 1(abstract P85) The schematic diagram of the main in vivo protocol

43 (33-45) mmHg), TV (7 (7-7) vs. 5 (5-6) ml/kg BW), driving pressure (deltaP) and EVLW were all significantly higher in ARDSnet after 24 hours, whereas Vent was significantly lower (Table 1). The difference in PIP (OLC 29 (27-30) cmH_2O vs. ARDSnet 34 (29-38) cmH_2O) was not statistically significant.

Conclusions: OLC ventilation better fulfilled common criteria for lung protection, because it facilitated MV with lower TV, deltaP, less edema (EVLW) and better lung function. It also prevented progressive derecruitment (decrease in Vent) during lung protective ventilation.

Grant acknowledgement: CNPq, CAPES, FAPEMIG, B.Braun-Foundation and DFG.

References

1. Anonymous : *NEJM* 2000.
2. Schreiter D, Reske A, Stitchert B, Seiwerts M, Bohm SH, Kloeppel R, Josten C: **Alveolar recruitment in combination with sufficient positive end-expiratory pressure increases oxygenation and lung aeration in patients with severe chest trauma.** *Crit Care Med* 2004, **32(4)**:968-975.
3. Andrew PL: *J Trauma* 2013.

P87

1071. Impact of volume resuscitation on heart rate variability in a model of hemorrhagic shock in pigs

E Salomão Jr[1], DA Otsuki[1*], AL Corrêa[1], DT Fantoni[2], JOC Auler Jr[1]
[1]Faculdade de Medicina da Universidade de São Paulo, Department of Surgery, São Paulo, Brazil; [2]Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, Department of Surgery, São Paulo, Brazil
Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P87

Introduction: Hemorrhagic shock is responsible for high mortality rates in civilian injuries and combat casualties. The initial care of these patients comprehends an early assessment of hypovolemia, bleeding management and fluid resuscitation [1], while an adequate autonomic function is essential for maintaining the hemodynamic stability during haemorrhage. The analysis of heart rate variability (HRV) has been shown as a promising noninvasive technique for assessing the cardiac autonomic modulation in trauma, and several recent studies have demonstrated an association between HRV and clinical outcome [2].

Objectives: The objective of this study was to evaluate HRV during hemorrhagic shock and fluid resuscitation, comparing to traditional hemodynamic and metabolic parameters.

Methods: Twenty pigs were submitted to acute hemorrhagic shock by removing 60% of the estimated blood volume in 15 minutes (3 mL/kg/min) and then these animals were evaluated for 60 minutes without any treatment. Surviving animals were treated with lactated Ringer solution (3:1) and evaluated for an additional period of 180 minutes. HRV metrics, as well as hemodynamic and metabolic parameters, were evaluated in survivors and non-survivors animals. Data were analysed using the repeated measures ANOVA or Friedman test (for normal and non-normal distributed date, respectively), followed by Tukey or Dunn's test as necessary. Surviving and non-surviving animals' data were compared by the unpaired t-test or Mann-Whitney U test. A multivariable logistic regression analysis was performed to estimate predictive factor for mortality resulting from hemorrhagic shock.

Results: Seven of the 20 animals have died during haemorrhage and initial fluid resuscitation. All the animals presented an increase in the LF_{nu} and a decrease in the HF_{nu} values after haemorrhage. However, non-surviving animals presented lower LF_{nu} (72.2±18.6 versus 85.9±7.1) and higher HF_{nu} values (27.8±18.6 versus 14.1±7.1), as well as lower MAP and CI and higher levels of plasma lactate and potassium. Besides these results, the fluid resuscitation was not able to reinstate the LF and HF alterations, but restored hemodynamic and metabolic parameters.

Table 1(abstract P86)

	Group	Pre-PC	Post-PC	4 hrs.	8 hrs.	12 hrs.	16 hrs.	20 hrs.	24 hrs.
Compliance (ml/cmH2O)	ARDSnet	25 (23-30)	18 (17-20)	18 (16-19)	17 (16-19)	17 (16-19)	16 (13-17)	15 (14-16)	13 (11-17)
	OLC	25 (21-34)	17 (15-18)	15 (16-19)	19 (14-23)	18 (17-23)	21 (17-26)	23 (17-26)	21 (17-24)
EVLW (ml/kg)	ARDSnet	333 (299-377)	344 (285-379)	280 (254-347)	338 (368-372)	368 (283-411)	361 (334-398)	346 (339-365)	375 (350-410)
	OLC	333 (283-345)	345 (284-411)	320 (275-378)	327 (268-365)	318 (276-326)	327 (273-372)	311 (269-359)	321 (290-397)
Ventilated area (Vent%)	ARDSnet	100	90 (72-94)	81 (77-90)	77 (73-83)	80 (76-92)	79 (76-92)	76 (71-87)	77 (72-90)
	OLC	100	90 (86-98)	93 (70-103)	94 (85-100)	98 (88-110)	99 (93-116)	99 (93-116)	104 (94-167)
deltaP (cmH2O)	ARDSnet	16 (13-22)	21 (19-23)	20 (19-23)	21 (19-25)	22 (19-25)	22 (19-26)	22 (17-28)	24 (19-28)
	OLC	21 (17-24)	26 (21-27)	9 (8-11)	10 (8-11)	10 (8-11)	10 (8-11)	10 (9-11)	10 (9-11)

Conclusions: The HRV metrics were able to discriminate survivors from non-survivors only at late stages of hemorrhagic shock. Moreover, metabolic variables along with CI and MAP were more reliable to reflect hemorrhagic shock severity than HRV metrics.

Grant acknowledgment: FAPESP.2012/00439-0.

References

1. Wilson M, Davis DP, Coimbra R: **Diagnosis and monitoring of hemorrhagic shock during the initial resuscitation of multiple trauma patients: a review.** *The Journal of Emergency Medicine* 2003, **24(4)**:413-422.
2. Winchell RJ, Hoyt DB: **Spectral analysis of heart rate variability in the ICU: a measure of autonomic function.** *The Journal of Surgical Research* 1996, **63(1)**:11-16.

P88

1072. Red blood cell transfusion in acute cerebral injuries: a systematic review of preclinical animal studies

M Laflamme[1*], A Boutin[1], H Haghbayan[1], M Shemilt[2], F Lauzier[1,2], L Moore[1,2], R Zarychanski[3], J Lacroix[4], F Lamontagne[5], DA Fergusson[6], P Desjardins[2], AF Turgeon[1,2]
[1]Université Laval, Québec, Canada; [2]CHU de Québec Research Center, Québec, Canada; [3]University of Manitoba, Winnipeg, Canada; [4]Université de Montréal, Montréal, Canada; [5]Université de Sherbrooke, Sherbrooke, Canada; [6]Ottawa Hospital Research Institute, Ottawa, Canada

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P88

Introduction: In brain-injured patients, the appropriateness of restrictive red blood cell (RBC) transfusion strategies is uncertain given the brain's vulnerability to hypoxemia. Current practice is based on small clinical trials and physiologic data from clinical and preclinical studies. Little is known regarding how preclinical data has informed RBC transfusion in this specific patient population.
Objectives: To evaluate the association between RBC transfusion strategies and important outcomes in animal models of acute cerebral injuries.
Methods: We conducted a systematic review of studies evaluating RBC transfusion strategies in animal models of acute cerebral injuries. We searched MEDLINE, EMBASE and BIOSIS for preclinical comparative studies (randomized or non randomized studies) evaluating at least two different RBC transfusion strategies, including presence/absence of transfusion. We collected data on model characteristics, baseline sample characteristics, intervention, co-interventions and outcomes. Our primary outcomes were mortality and neurological function. Risk of bias was evaluated using a tool based on recommendations from the CAMARADES group [1]. Descriptive statistics using data reported in the original publications are presented.
Results: We identified 6031 records and included 24 unique studies. Cerebrovascular injury models were used in 15 studies: stroke models (n=12), subarachnoid haemorrhage models (n=3). Traumatic brain injury models were used in 9 studies (trauma n=6, cryogenic lesion n=2, epidural lesion n=1). Twelve studies were performed using murine models. Most studies compared RBC transfusion to no transfusion (n=23) and used whole blood (n=21). Five studies reported data on mortality and 6 on neurological function; others reported surrogate endpoints such as cerebral blood flow. RBC transfusion was associated with decreased mortality in 3 of 5 (60%) studies reporting this outcome. In studies assessing neurological function, 2 of 6 studies favoured transfusion compared to no transfusion. No studies compared different transfusion thresholds. Hemodynamic and vital signs were not systematically reported in all studies. High risk of bias was observed in the majority (15/24, 58%) of studies (all studies reporting mortality and neurological function), notably lack of randomization and blinding of outcome assessment.
Conclusion: Data from animal studies are insufficient to inform transfusion practice in patients with acute cerebral injuries. The use of standardized, controlled and validated animal models, clinically relevant transfusion strategies and longer follow-up periods may help future research in the field.

Reference

1. [http://www.camarades.info].

SEDATION OF THE CRITICALLY ILL

P89

1110. Age-related effects of dexmedetomidine, an alpha-2 agonist, on coronary vasoactivity and cardiac function in guinea-pig hearts

M Hongo[1*], S Fujisawa[1], T Adachi[1], T Shimbo[1], S Shibata[2], T Ohba[1], K Ono[1]
[1]Akita University Graduate School of Medicine, Department of Cell Physiology, Akita City, Japan; [2]Iwate Medical University, Department of Critical Care Medicine, Iwate, Japan

Intensive Care Medicine Experimental 2014, **2(Suppl 1):**P89

Introduction: Dexmedetomidine is a potent and selective alpha-2 agonist, and is widely used for not only an adjunct to anesthesia but also sedation during mechanical ventilation in intensive care unit. It has been reported that, although the substance does not appear to have any direct effects on the myocardial contractility, it sometimes causes a decrease in heart rate and a dose-dependent decrease in arterial blood pressure and, in rare cases, leads to cardiac arrest or shock. However, detailed mechanisms remain uncertain. We hypothesized that effects of dexmedetomidine to coronary vasoactivity and cardiac function may differ depending on postnatal ages.
Objectives: The aim of the present study was to evaluate the effects of dexmedetomidine on cardiac performance and coronary circulation using Langendorff perfused isolated heart of young and adult guinea pigs.
Methods: Hearts from young (< 4weeks) and adult (> 4weeks) guinea-pigs were isolated and mounted on a Langendorff apparatus, and a saline-filled balloon was inserted into the left ventricle. Coronary perfusion pressure (CPP) and the left ventricular pressure (LVP) were continuously monitored and the electro-field stimulation (EFS) was applied to stimulate sympathetic nerve terminals. Also, the effects of dexmedetomidine on the ventricular action potential were evaluated in enzymatically isolated ventricular myocytes.
Results: Dexmedetomidine almost completely inhibited the increase of LVP induced by EFS with an IC_{50} value of approximately 0.2 nM in both young and adult hearts. On the other hand, the effect on the coronary artery resistance to dexmedetomidine altered during postnatal development, i.e., dexmedetomidine had little effect on CPP in young hearts whereas it increased CPP at concentrations>10 nM in adult hearts ($p< 0.05$: aged< 4weeks vs. aged 4-8weeks, $p< 0.01$: aged< 4weeks vs. aged>8weeks). The increase in CPP in adult hearts was inhibited by prazosin, an alpha-1 antagonist (10nM: p=0.0389, 100nM: p=0.0337). Dexmedetomidine had little direct effect on ventricular dP/dt and the action potential of isolated ventricular myocytes.
Conclusions: The present study confirms the antagonistic action of dexmedetomidine on the increase in ventricular contractility induced by sympathetic stimulation. In addition, the present results demonstrate that the response of coronary artery resistance to dexmedetomidine differs depending on postnatal ages, i.e., dexmedetomidine increases CPP in adult, not in young guinea-pig hearts. The alpha-1 adrenoceptor seems to be involved in the increase of CPP in adult hearts in addition to the alpha-2 adrenoceptor. Aging-associated alteration of the alpha adrenoceptor subtypes might be linked to the cardiodepressant effects of dexmedetomidine.

Cite abstracts in this supplement using the relevant abstract number, e.g.: Hongo *et al.*: 1110. Age-related effects of dexmedetomidine, an alpha-2 agonist, on coronary vasoactivity and cardiac function in guinea-pig hearts. *Intensive Care Medicine Experimental* 2014, 2(Suppl 1): P89

ESICM2014 Author index

A
Abreu M., **0548**
Adachi T., 1110
Aguilera E., 0468
Aird W.C., 0737
Akoumianaki E., 0989
Albrecht M., 0736
Alegria L., 0101, 0671
Algieri I., 0894, 0994
Almeida-Filho J., 0096, 0732
Amini M., 0894, 0994
Amthauer M., 0671
Andersson I., 0438
Andreis D.T., 0990
Andrew R., 0590
Arai M., **0597**
Arango-Dávila C.A., 0032
Araos J., 0671
Archontoulis N., 0391, 1036
Arrigo M., 0734
Artigas A., 0987, 0991
Arulkumaran N., 0035, **0371**, 0889
Atac F.B., 0986
Audard J., 0853
Auler Jr J.O.C., 0731, 0735, 1071
Avila M., 0548
Ayala L.G., 0548

B
Bachler J.P., 0101
Bacile di Castiglione C., 0894, 0994
Bakker J., 0101
Ballén-Barragán A., 0895
Barbas C., 0852, 0854
Baron R.M., 0474
Barthel F., 0589
Baudry N., 0921
Bauer I., 0589
Baunack M., 1070
Bautista D.F., 0032
Bayat S., 0897
Beards S.C., **0918**, **0920**
Beck C., **0589**
Beda A., 1070
Beilicke A., 0896
Bello D., 0728
Beloncle F., **0989**
Bergamini B.C., 0548
Bermúdez W.F., 0032
Bessems B.A.P., 0521
Bettex D., 0734
Bin O., 0996
Blaffart F., 0681
Blanc P., 0853
Blanch L., 0468, 0991
Bläsi C., 0714
Blondonnet R., **0853**
Boer C., 0595
Boerma E.C., 0020
Boltres A., 0714
Borges J.B., 0897, 0893, 0985
Boutin A., 1072
Bouvier D., 0853
Braun S., 0993
Briassouli E., **0104**, **0295**
Briassoulis G., 0104, 0295
Briedé J.J., 0521
Brioni M., 0894, 0994
Brochard L., 0989
Broche L., 0897
Brodie S., 0920
Brohi K., 1068
Brouckaert P., 0521
Bruhn A., 0032, 0101, **0671**, 0893
Buchloh D., 0896
Buensuseso Alfaro J.A., **0473**
Bugedo G., 0671
Bury A., 0997
Buurman W.A.P., 0521

C
Cagnina A., 0681
Cai J.C., 0097
Cain J., 0920
Cain J.R., 0918
Caminos Eguillor J.F., 0588
Cammaroto A., 0894, 0994
Canales H.S., 0588
Canullán C., 0588
Cardinal-Fernández P., 0895
Carvalho A.R., 0548
Carvalho N., 1070
Castells J., 0027
Castillo A.M., 0531
Castillo-Lorente E., 0531
Castro G., 0671
Cauwels A., 0521
Cazalis M.-A., 0041
Cecconi M., 0590
Ceravola E., 0371
Cetin S., **0726**
Chagnon F., 0100
Charbonney E., **0450**
Chen C.-W., 0434
Chen E.-Z., 1069
Chen J., 0034
Chen M., 0034
Chen Y., 1069
Chevillard L., 0863
Chevreux L., 0596
Chimenti L., **0991**
Chisholm K.I., **0033**, 0036
Chiurazzi C., **0894**, 0994
Choi A.M.K., 0474
Christopher K.B., **0474**
Clairefond G., 0853
Comini B., 0894
Conlon J., 0995
Constantin J.-M., 0853
Conte G., 0894
Cornejo R., 0671
Corrêa A.L., **0735**, 1071
Costa C., 0728
Couffignal C., 0716
Courtneidge H., 0889
Cox L.A.E., 0730
Coyle Rowan S., 0859
Cressoni M., 0894, 0994
Creteur J., 0092, 0354, 0727
Cruces P., 0671

D
Daikos G., 0104, 0295
Damoisel C., 0102
David S., **0737**
Davies A.L., 0033
De Backer D., 0032, 0092, 0354, 0727, 0888
De la Cal M.A., 0852, 0895
De Olaiz-Navarro B., 0984
De Paula M., 0854
De Waard M.C., 0595
Dechelotte P., 0853
Defraigne J.-O., 0681
Deloya Tomas E., 0473
Deniz S., 0726
Derosa S., 0897
Desaive T., 0681
Desjardins P., 1072
Dey N., 0108
Díaz-Martín A., **0329**
Djafarzadeh S., 0093
Dombrowsky H., 0736
Dominguez M.A., 0728
Dominguez-Herrera J., 0329
Donadello K., 0092, **0354**
Dondossola D., 0994
Dorresteijn M.J., 0730
Dos Santos C., 0450
Driss F., 0716
Dubin A., **0588**
Duburcq T., **0733**
Duchen M., 0889
Duchen M.R., 0033, 0036
Dufour G., 0716
Dugal A., 0450
Duranteau J., 0921
Durieux A.-C., 0027
Duska F., 0520
Duval X., 0716
Dyson A., 0033, 0035, 0036, 0078, 0099, 0592
Dzupa V., 0520

E
El-Bakhli S., 0863
Elvevoll B., 0729
Endeman R., 0020
Engström P., 0976
Erranz B., 0671
Esteban A., 0895, 0984, 0987

F
Faivre V., 0102
Fan W., 0992
Fantoni D.T., **0731**, 0735, 1071
Favory R., 0733
Feelisch M., 0099
Fei J., 1069
Felix N.S., 0427
Fergusson D.A., 1072
Ferrara G., 0588
Ferrer I., 0468
Ferruelo A., 0852, 0854, 0895, 0984
Fiore T., 0438, 0897
Floros I., 0391, 1036
Fredenburgh L.E., 0474
Frenzel T., 0095
Frerichs I., 0736
Freyssenet D., 0027
Fric M., 0520
Frohlich S., 0859
Fruhwald S., 0915, 0968
Fuhong S., 0092, 0727
Fujisawa S., 1110
Fujiyoshi T., **0601**
Futatsuki T., 0633

G
Gama de Abreu M., 0427
Garcia P., 0671
García A., 0852
Garcìa A., 0854
García Vallejo J.J., 0595
García-Hierro P., 0852
Garnacho-Montero J., 0329
Gattinoni L., 0894, 0990, 0994
Gazourian L., 0474
Gerretsen J., 0730
Gerretsen J.F., 0095
Ghosh C., 0737
Giannella Neto A., 0548
Gil Cano A., 0590
Girbes A.R., 0595
Gjertsen B.T., 0729
Glogauer M., 0450
Gmyr V., 0733
Goldenberg R.C., 0435
Gomez M.N., 0991
Gómez Blizniak A., **0531**
Gomez Peñalver E., 0985
Gommers D., 0449
Gordon A.C., 0094
Gotti M., 0894, **0994**
Gottin L., 0354
Gracia Romero M., 0590
Granados M., 0032
Granados R., 0895
Grandgirard D., 0353, 0356
Greco E., **0035**, **0889**

Gross S., 0596
Grounds R.M., 0590
Guan X., 0034
Guijo González P., 0590
Guillamat R., 0987, 0991
Guiot J., 0681

H
Haase J., **0896**
Habran S., 0681
Haenggi M., 0353, 0356
Haghbayan H., 1072
Hagisawa K., 0919
Hammermüller S., 0896, **1070**
Han B., 0593
Hanak A.-S., 0863
Harrois A., **0921**
Haska S., 0896
Hauffe T., 0734
Hedenstierna G., 0438, 0893, 0897, 0985
Hella D., 0681
Henriques I., 0435
Herminghaus A., 0589
Hernandez G., 0032, **0101**
Herrero R., 0852, 0895, **0987**
Hillered L., 0976
Hinds C.J., 1068
Hoka S., 0601
Holzer P., 0915, 0968
Hongo M., **1110**
Horta L.F., 0435
Hosokawa K., 0092, 0354, 0727
Hu G.X., **0097**
Hubert T., 0733
Huckauf S., 1070
Huhle R., 0427
Hurtado D., 0671
Husby P., 0729

I
Iacovidou N., 0391, 1036
Ichinose F., 0083
Ida K.K., 0033, **0036**
Imran Hamid U., **0995**
Ince C., 0588
Islam D., 0988
Ito S., 0597
Izquierdo J.L., 0852

J
Jabaudon M., 0853
Jackson A., 0918, 0920
Jakob S.M., 0353, 0356
Janssen E., 0900
Janssen N., 0681
Jaramillo C., 0895
Jarufe N., 0101
Jeger V., 0093
Jie M., 0996
Jiroutkova K., **0520**
John A., 0900
Jourdain M., 0733
Juan C., 0996
Jukka T., 0093

K
Kakihana Y., 0633
Kamminga S., 0595
Kanariou M., 0104, 0295
Kanoore Edul V.S., 0588
Karbing D.S., 0108
Katbeh U., **0042**
Kawamae K., 0232
Kemper D.A., 0731
Khaliq W., **0082**, **0738**
Kielstein J.T., 0737
Kiers D., **0900**
Kim M.J., 0355
Kinoshita M., 0919
Kissenpfennig A., 0995
Kobelt S., 1070
Koh I.H.J., 0096, **0732**
Kolh P., 0681
Konrad F., **0993**, **0997**
Koopman A.A., 0449
Koopmans M., **0020**
Kopp Lugli A., **0714**
Kornet L., 0095
Kosaka Y., 0597
Kosta S., 0681
Kox M., **0026**, **0095**, 0730, 0900
Krajcova A., 0520
Krasnodembskaya A., 0995
Krenn C.G., **0600**
Kuiper M.A., 0020
Kundakci A., **0986**
Kuroiwa M., 0597
Kurota M., 0232

L
La Rosa J., 0531
Labrador G., 0329
Lacroix J., 1072
Laflamme M., **1072**
Lambermont B., 0681
Lamontagne F., 1072
Langer T., 0894, 0994
Larraza S., **0108**
Larsson A., 0438, 0893, 0897, 0976, 0985
Lasocki S., **0716**
Lautenschläger I., **0736**
Lauzier F., 1072
Lebherz-Eichinger D., 0600
Lebouvier T., 0991
Lecompte T., 0596
Lecomte A., 0596
Leib S., 0353, 0356
Leon F., 0101
Lesur O., **0100**
Levy B., 0596
Li Bassi G., 0468
Liberatore A.M.A., **0096**, 0732
Lilles C., 0450
Lin W.-C., 0434
Lipcsey M., 0976
Litonjua A.A., 0474
Lomelí Terán M., 0473
López-Aguilar J., **0468**
Lopez-Hernández E., 0895
Lorente J.A., **0852**, 0854, **0895**, 0984, 0987
Luengo C., 0101
Lukaszewicz A.-C., 0102
Luo A.A.L., **0593**
Luoni S., 0894, 0994

M
Ma L., 1069
Madriñan H.J., 0032
Magdaleno Lara G., 0473
Maia D.R., 0731
Makita K., 0992
Malbouisson L.M.S., 0036
Manez R., **0728**
Mangalaboyi J., 0733
Mao E.-Q., 1069
Maraffi T., 0990
Marceau G., 0853
Maripuu E., 0976
Marsault E., 0100
Martí J.D., 0468
Martínez Mazariegos J.J., 0473
Martínez-Caro L., 0852, 0854, 0895, **0984**
Martins E., 0588
Massaro A.F., 0474
Massoco C.O., 0731, 0735
Matallana Zapata D.F., 0531
McAuley D.F., 0995
McLoughlin P., 0859
Meesters D.M., 0521
Mega A., 0391, 1036
Megarbane B., **0863**
Melis V., 0990
Mendoza Escorza J., 0473
Merz T.M., **0093**
Mesquida J., 0590
Meyer T., 0714
Milesi M., 0990
Minnion M., 0099
Miranda P.J., 0435
Miyawaki H., 0919
Molina-Arana D., 0852
Molinaro R., 0988
Molliex S., 0027
Mondragon Labelle T., 0473
Monge García M.I., **0590**, 0985
Monneret G., 0041
Montaruli C., 0894, 0994
Monti M., 0990, 0994
Moore L., 1072
Moraes L., **0427**, 0435
Morante A., 0531
Morel J., 0027
Moreno Eutimio M.A., 0473
Morimont P., **0681**
Muders T., 0896
Murphy N., **0859**
Murza A., 0100

N
Nakahara M., 0633
Nakahira K., 0474
Nakamura K., 0633
Nakamura N., **0232**
Nakane M., 0232
Nakazawa K., 0992
Nanas S., 0104, 0295
Naz S., 0852, 0854
Neto A.C., 0548
Ngamsri K.-C., 0993, 0997
Ni H., 0593
Nicosia F., 0990
Nikolla K., 0894, 0994
Nin N., 0852, 0854, 0895, 0984
Nitschmann E., 0714
Noguchi M., 0919
Noreikat K., 0896, 1070
Nygaard M., 0108

O
O'Callaghan D.J., 0094
O'Dea K.P., 0042, **0094**
O'Dwyer M.J., 1068
Ohba T., 1110
Okamoto H., 0597
O'Kane C.M., 0995
Okayama N., 0633
Oliveira M.V., 0435
Ono K., 1110
Onodera Y., 0232
Orbegozo Cortes D., **0092**, **0727**
Orbegozo Cortés D., 0888
Ornellas D.S., 0427
Ortiz I., 0984
Ospina-Tascón G., 0101
Ospina-Tascón G.A., **0032**
Otsuki D.A., 0036, 0731, 0735, **1071**
Oudemans-van Straaten H.M., 0595
Ouyang B., 0034
Owen H.C., **1068**
Ozen Isiksacan O., 0986
Ozkan S., 0726
Ozturan Ozer E., 0986

P
Pachón J., 0329
Pachón-Ibañez M.E., 0329
Pachot A., 0041
Padilha G.A., **0435**
Palao J.-C., **0027**
Pantazopoulos C., **0391**, **1036**
Pargger H., 0714
Parikh S.M., 0737
Park Y.S., **0355**
Parkes L.M., 0918, 0920
Parotto M., 0988
Pattou F., 0733
Pavleas I., 0391, 1036
Payen D., 0102
Pellegrini M., **0438**, 0897
Pelosi P., 0427, 0435
Peoc'h K., 0863
Perchiazzi G., 0438, **0897**
Pereira A.J., 0093
Perez d'Empaire P., 0450
Perez-Cruz M., 0728

Pesenti A., 0988
Picker O., 0589
Pickkers P., 0026, 0095, 0730, 0900
Piednoir P., 0716
Ping-Ping W., 0996
Pirat A., 0986
Plassais J., 0041
Poblano Morales M., 0473
Poeze M., 0521
Pola de Gallego M.D., 0531
Porra L., 0897
Post E.H., 0888
Pozzi F., **0988**
Prados L., 0987
Protti A., **0990**
Pugni P., 0990
Puig F., 0987
Putzu A., 0353, 0356

Q
Queiroz-Hazarbassanov N., 0731
Queiroz-Hazarbassanov N.G., 0735
Quílez M.E., 0468
Quinn K., 0593
Quintane L., 0733

R
Rahmania L., **0888**
Ramakers B.P.C., 0730
Ramos I.P., 0435
Rees S.E., 0108
Rego N., 0895
Regueira T., 0101
Reheman A., 0593
Reske A., 0896, 1070
Reske A.W., 1070
Retamal J., **0893**, 0985
Reuter D.A., 0600
Reutershan J., 0993, 0997
Rigas C., 0391, 1036
Rigol M., 0468
Rimmele T., 0041
Rineau E., 0716
Risède P., 0863
Rittayamai N., 0989
Rocco P.R.M., 0427, 0435
Rodriguez D., 0671
Rodriguez F., 0671
Rojas Y., 0852, **0854**, 0895, 0984, 0987
Ronéus A., 0438
Rossignoli G., 0894, 0994
Roth G.A., 0600
Routsi C., 0104, 0295
Rovati C., 0894, 0994
Rubenfeld G.D., 0450
Rucabado Aguilar L., 0531
Rucán M.E., 0671
Rudiger A., **0734**
Ruiz Bailén M., 0531
Ruiz Ferrón F., 0531
Ruiz-Cabello J.M., 0852

S
Sabbatini G., **0078**, **0592**
Saeed S., 0030
Saitoh D., 0919
Sakamoto T., **0919**
Salomão Jr E., 1071
Salomon T., 0671
Salvail D., 0100
Samary C., 0427
Sanchez-Ferrer A., 0984
Santacruz C., 0092, 0727, 0888
Santel A., 0737
Santos A., **0985**
Santos C.L., 0427
Santos R.S., 0427
Sapin V., 0853
Sarau J., 0736
Sarret P., 0100
Satoh S., 0919
Satoh Y., 0919
Scheffer G.J., 0900
Scheiner A., 0095
Schick M.A., 0997
Schilte C., 0716
Schörghuber M., **0915**, **0968**
Schurr U., 0714
Sen H., 0726
Senda M., **0992**
Sennoun N., **0596**
Sezer A., 0726
Shan Y., 0450
Shemilt M., 1072
Shibata S., 1110
Shimbo T., 1110
Sigurta' A., **0030**
Silva P.L., 0427, 0435
Simon V., **0099**
Sindaco A., 0897
Singer M., 0030, 0033, 0035, 0036, 0078, 0082, 0099, 0371, 0592, 0738, 0889
Sixma M., 0371, 0889
Sjölin J., 0976
Skavland J., 0729
Slutsky A., 0593
Slutsky A.S., 0988
Smani Y., 0329
Smit B., **0595**
Smit L., 0984
Smith K.J., 0033, 0036
Smulders Y.M., 0595
Somhorst P., **0449**
Song X.-Q., 1069
Soto D., 0101, 0671
Souza R.B., 0732
Soyer B., **0102**
Spahn D.R., 0734
Spence S., 0995
Spieth P., 0896
Spoelstra-de Man A.M., 0595
Springe D., 0353, **0356**
Stangeland L., 0729
Stephan J.M., 0093
Su F., 0354, 0888
Su P.-L., 0434
Suarez-Sipmann F., 0893, 0985
Sutherland S., 0450
Suzuki H., 0232
Svendsen Ø.S., **0729**
Sweep F.C.J.G., 0026

T
Taccone F.S., 0354, 0888
Tachtsidis I., 0033
Takala J., 0353, 0356
Takata M., 0042, 0094
Tam F., 0889
Tam F.W.K., 0371
Tang Z., **0034**
Taniguchi J., 0633
Tannoia A.P., 0897
Tapia P., 0101, 0671
Tatzl E., 0915, 0968
Tavares-Ranzani O., 0468
Tchana-Sato V., 0681
Tedesco R.C., 0096, 0732
Tendillo Cortijo F.J., 0473
Tenstad O., 0729
Textoris J., **0041**
Thamm K., 0737
Tijero J., 0991
Tissot S., 0041
Toda M., 0597
Todisco F.F.M., 0897
Tokuda K., **0083**
Toller W., 0915, 0968
Tomotsugu Y., **0633**
Tong L., 0097
Torrance H.D., 1068
Torres A., 0468
Torres López L., 0473
Tournoys A., 0733
Toussaint-Hacquard M., 0596
Trepte C.J., 0600
Tudor B.-A., 0600
Tullis E., 0593
Turgeon A.F., 1072
Turkoglu S., 0986
Tusman G., 0985
Tzanoudaki M., 0104, 0295

U
Uhlig S., 0736
Umaña M., 0032
Unwin R., 0371
Unwin R.J., 0889

V
Valencia J.D., 0032
Van den Wildenberg J., 0026
Van der Hoeven H., 0900
Van der Hoeven J.G., 0026, 0095
Van Eijk H.M.H., 0521
Van Eijk L.T., 0026, 0095, **0730**
Van Mourik M., 0449
Vandendriessche B., 0521
Vardas K., 0104, 0295
Varela Durán M., **0866**
Veenstra G., 0020
Veerhoek D., 0595
Vellinga N.A.R., 0020
Venet F., 0041
Verdi H., 0986
Verhaak T., 0095
Vernikos P., 0391, 1036
Vicaut E., 0921
Vicuña C., 0101
Vieira J.C., 0096
Vieirra J.C., 0732
Vincent J.-L., 0092, 0354, 0727, 0888
Vollmer C., 0589
Vollmer I., 0993, 0997
Von Seth M., **0976**
Vonk A.B., 0595
Vos de R., 0020
Votta E., 0990

W
Wang L., 1069
Watanabe T., 0232
Weiler N., 0736
Widström C., 0976
Wiig H., 0729
Wijnands K.A.P., **0521**
Wilcox E., 0450
Winding R., 0108
Wodack K.H., 0600
Wong Y.L., 0736
Wrigge H., 0896, 1070
Wu J., 0034
Wykes L., 0714

X
Xanthis D., 0391, 1036
Xanthos T., 0391, 1036
Xiang-Dong G., 0996
Xinrong H., 0727

Y
Yamaguchi K., 0633
Yong-Jun L., **0996**
Yu C.-H., **0434**

Z
Zamora Gómez S., 0473
Zarychanski R., 1072
Zhang H., 0593, 0988
Zhao B., **1069**
Ziak J., 0520
Zin W.A., 0548
Zitta K., 0736
Zuercher P., **0353**, 0356
Zwaag J., 0026