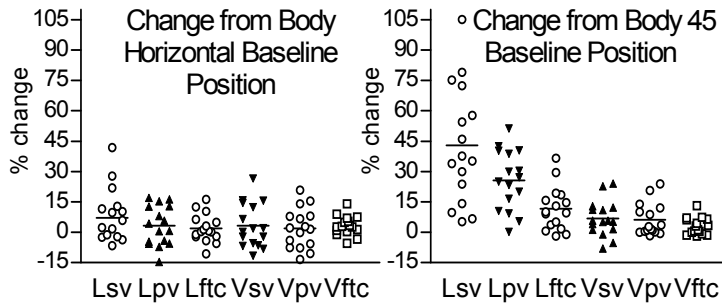


P141

AORTIC FLOW RESPONSES TO LEG LIFTING AND INSPIRATORY IMPEDANCE IN SPONTANEOUSLY BREATHING YOUNG ADULTS. P. Wall, J. Hopkins*, C. Buising*, L. Henderson*. IA Methodist Med Ctr, Des Moines, IA 50309.

Objective: To examine aortic flow responses of healthy young adults to interventions of possible use for predicting fluid responsiveness of critically ill patients. Methods: In 16 volunteers, aortic variables were monitored by transcutaneous continuous wave Doppler during 1) horizontal upper body with or without legs elevated 45° and horizontal whole body with or without use of an inspiratory impedance threshold device and 2) 135° angle of upper body to legs with either legs or upper body horizontal and with legs horizontal with or without inspiratory impedance. Results: No consistent changes occurred with a horizontal whole body baseline position or with the addition of inspiratory impedance in either body position. Position change from upper body 45° elevated to legs 45° elevated increased ($p > 0.01$) stroke volume (Lsv 5-105%), peak velocity (Lpv 5-51%), and corrected flow time (Lftc 2-37%). (Inspiratory impedance valve variables: Vsv, Vpv, Vftc) Conclusions: While horizontal, neither a 45° leg raise nor adding inspiratory impedance sufficiently enhances preload for stroke volume changes to indicate ventricular preload responsiveness in spontaneously breathing adults. A baseline upper body position of 45° may be preferable when using leg raising to assess preload responsiveness in spontaneously breathing patients. (Funding: IA Space Grant, Eagles, Drake U)

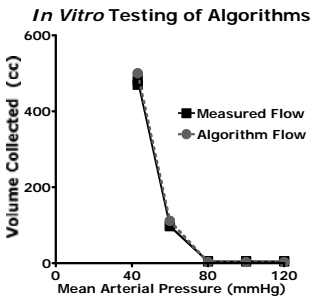
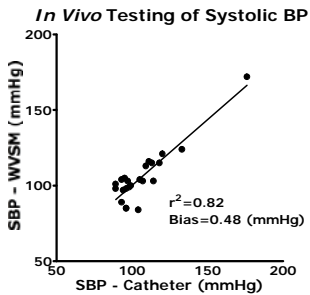


P142

DEVELOPING DECISION-ASSIST (D-A) FOR FLUID

RESUSCITATION IN HUMANS. M. Salter*, S. Henkel*, R. Seeton*, C. Mitchell*, G. Kramer and M. Kinsky, University of Texas Medical Branch, Galveston, TX 77555

There is lack of expertise for guiding fluid resuscitation efforts in combat casualties and civilians in remote locations that are in shock. Consequently, over and under resuscitation occurs and contributes to morbidity and mortality. **Objective:** To advance the concept of fluid resuscitation using a novel blood pressure (BP) system coupled with expert based fluid infusion algorithms. **Methods:** *In Vivo* testing – systolic blood pressure (SBP) data was recorded from patients fitted with wireless vital signs monitor



(WWSM, Athena GTX) and compared to intra-arterial BP (upper fig). *In Vitro* testing with WWSM was performed by inducing hypotension using a BP simulator. BP data from WWSM, captured every 5 min, generated a fluid infusion rate from embedded D-A algorithms (Trauma Tablet, RSI). The semi-automated pump controller (Power Infuser, Zoll) infused fluid into a graduated cylinder. Algorithm rate was compared to volume collected over each 5 min BP period (lower fig).

Results: There was a strong significant correlation between intra-arterial systolic BP and the WWSM ($r^2=0.82$). There was high fidelity between fluid volumes recommended by software algorithms and actual pump volumes. **Conclusions:** D-A blood pressure devices and software can reliably provide assistance to administer fluid based on BP. Further *in vivo* and *in vitro* studies are underway to employ the use of D-A resuscitation technology. (ONR grant, 427140)

P143

HYPERTONICITY PROTECTS AGAINST OXIDANT-INDUCED ENDOTHELIAL DAMAGE BY RESTORING PHOSPHATIDYLINOSITOL-4,5-BISPHOSPHATE.

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Background: Thermal burn injury induces large scale release of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2). Excess ROS disrupts the endothelial actin cytoskeleton to breach the endothelial barrier and this contributes to the precipitation of burn-mediated multi-organ failure. Since the phospholipid PIP_2 regulates the actin cytoskeleton, we hypothesize that H_2O_2 induces actin cytoskeletal disruption by suppressing PIP_2 synthesis and that hypertonicity, which stabilizes the actin cytoskeleton by increasing PIP_2 , protects against H_2O_2 induced damages.

Methods: HUVECs were treated with H_2O_2 , hypertonic saline (NaCl) or a combination of these two sequentially for 15-20 min. Stress fibers were examined by immunofluorescence microscopy after labeling cells with fluorescent phalloidin. PIP_2 was quantitated by thin layer chromatography. The activity of phosphatidylinositol 4 phosphate 5 kinases ($PIP5Ks$, which synthesizes PIP_2) was determined by *in vitro* kinase assay.

Results: H_2O_2 decreases actin stress fibers and PIP_2 levels in HUVECs. Hypertonic solutions added after exposure to H_2O_2 restored actin stress fibers and increased PIP_2 level. Hypertonicity activates $PIP5K\beta$ and increases cellular PIP_2 by promoting its ser/thr dephosphorylation.

Conclusions: Oxidative and hypertonic stresses have opposite effects on $PIP5K\beta$ behavior, PIP_2 homeostasis and the cytoskeleton. Our finding that hypertonic solutions added after exposure to H_2O_2 restores PIP_2 synthesis and the actin cytoskeleton may partly explain why small volume hypertonic resuscitation, which is currently in clinical trial for the treatment of several types of traumatic injuries (The ROC Consortium, NHLBI), may be effective in protecting against complications of burn injury.

P144

UPDATE ON THE SHOCK SOCIETY BIOINFORMATICS INITIATIVE ON COLLABORATIVE CURATION AND AUTOMATED INFORMATION EXTRACTION.

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Introduction: Managing the volume and scope of biomedical research is a significant challenge. Advances in bioinformatics aid the organization and potential utilization of this knowledge, but the permeation of these advances into the general research community has been slow. In 2004 a project was initiated within the Shock Society to introduce a novel method of integrated knowledge curation and utilization using scientific societies as modular units of applied bioinformatics. To date this project has involved the development of a translational grammar and implementation of a named entity recognition program to process meeting abstracts. **Methods:** Abstracts submitted to Shock 2009 were processed using a program based on the NIH MetaMap tool (<http://skr.nlm.nih.gov/>). Emails were sent to authors with links to their abstracts in which named entities recognized by our NER tool were annotated. Collaborative curation was carried out via this web interface and terms categorized based on 6 Unified Medical Language System (UMLS) semantic type groups corresponding to the previously developed translational grammar. **Results:** Extracted terms were added to the existing Shock lexicon, and a web interface was created allowing user to navigate terms, relationships between terms and abstract number of related submissions. **Discussion:** This ongoing project of knowledge integration positions the Shock Society at the forefront of functional bioinformatics. It is hoped that the results from this project will provide a substantial benefit to the Shock research community in terms of organizing its knowledge, making it accessible to its membership and promoting cross-talk and collaboration within the community.

P145

NOVEL SYNTHETIC SUGARS INHIBIT BACTERIA-ENTEROCYTE INTERACTIONS.

M. Henry-Stanley,

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Both methicillin-sensitive (MSSA) and resistant (MRSA) *Staphylococcus aureus* colonize the intestine and increasingly pose a risk for clinical infection in the community as well as in the ICU. The GI tract has been implicated as a reservoir for MSSA and MRSA, and GI colonization may lead to disease transmission and systemic infection. *S. aureus* interacts with heparan sulfate (HS), a negatively charged glycosaminoglycan (GAG) abundantly expressed (as the proteoglycan, syndecan-1) on the basolateral enterocyte surface. Because soluble heparin (an HS mimic) and HS are known to inhibit *S. aureus* internalization by enterocytes, we designed and synthesized three sulfated GAG-like oligosaccharides (oligos) for potential use as novel antimicrobial agents. In gentamicin protection assays (used to assess bacterial invasion of mammalian cells), two of the three oligos (50 µg/ml) displayed antimicrobial properties and inhibited internalization of two strains of *S. aureus* by Caco-2 and HT-29 enterocytes (table below).

* Avg ± SE log₁₀ numbers of viable internalized *S. aureus* from ≥ 3 replicate experiments. † and ‡, decreased at P<.01†, P<.05‡, respectively, compared to corresponding control enterocytes, ANOVA plus Fisher's post hoc.

Thus, these specific synthetic, sulfated, oligos may have the potential to interfere with *S. aureus* infection initiated via the enteric route.

Oligo	<i>Staphylococcus aureus</i> strain*			
	RN6390		SAC028W	
	HT-29	Caco-2	HT-29	Caco-2
None	3.1 ± 0.1	4.8 ± 0.1	3.0 ± 0.1	4.9 ± 0.1
57-B2	2.4 ± 0.1†	4.3 ± 0.1†	2.3 ± 0.2†	4.4 ± 0.1†
74-B2	2.3 ± 0.1†	4.4 ± 0.1†	2.5 ± 0.1‡	4.5 ± 0.1†
77-B2	3.2 ± 0.1	4.8 ± 0.1	3.0 ± 0.1	4.9 ± 0.1

P146

PROTEOMICS APPROACH IDENTIFIES PROTEINS WITH AGE-RELATED INCREASES IN NITRATION DURING SYSTEMIC INFLAMMATION.

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Systemic inflammatory response syndrome (SIRS) is a serious clinical condition characterized by whole body inflammation and can lead to an array of complications resulting in multiple organ failure and death. This condition is particularly threatening to elderly patients who suffer much higher mortality rates than younger patients. One of the major pathological consequences of SIRS is acute lung injury caused by oxidative damage due to the production of superoxide during the inflammatory response. Protein tyrosine nitration, a biological process known to alter the function of proteins, occurs when peroxynitrite, a toxic oxidant and nitrating agent, is produced by the reaction of superoxide with nitric oxide. Though the presence of nitrated proteins has been implicated in sepsis and SIRS, the identification of these proteins has only begun. Here, we used a proteomics approach to identify proteins that are nitrated by systemic inflammation. Acute endotoxemia was induced in young and aged mice by intraperitoneal injection with bacterial endotoxin lipopolysaccharide (LPS), mice were sacrificed 12 hours later and lung tissues harvested for protein analysis by two-dimensional gel electrophoresis and western blot against nitrotyrosine. By mass spectrometry (MALDI TOF-MS), we identified 19 nitrated proteins, of which 4 showed LPS-dependent nitration. These proteins include aldose reductase (a mediator of nitric oxide production), serine proteinase inhibitor, clade B, member 1a (lung defense enzyme against infection), beta-tropomyosin (a protein for muscle contraction) and peroxiredoxin (an antioxidant enzyme). Additionally, the first 3 proteins listed also exhibited an age-associated increase in LPS-induced nitration. Altered function of these proteins due to nitration could be related to increased oxidative damage in the lungs of aged patients with SIRS or sepsis.

P147

PROFILING MESENTERIC LYMPH IN A SHOCK MODEL BY LABEL-FREE MASS SPECTROMETRY.

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J. Eun, and A. Banerjee. University of Colorado Denver, Aurora, CO. 80045

Monitoring protein changes in the mesenteric lymph (ML), is providing insights into the system biology of hemorrhagic shock (HS). Our recent efforts characterized the ML using 2D gels to detect (by MALDI-TOF & TOF/TOF) protein-spots that changed at least 1.5 fold. We hypothesized that liquid chromatography followed by tandem mass spectrometry (LC-MS/MS), would provide better sequence coverage and allow quantification of a much larger percentage of the ML proteome, after HS. **Methods:** ML from male SD rats was collected preshock and at half hour time intervals postshock (n=3). Tryptic digests of lymph were analyzed by LC-MS/MS runs (n=2 per animal) on a LTQ-FT hybrid instrument. Data was analyzed by aligning spectral features, normalizing and quantization of individual assigned peptides between runs to provide relative quantification of proteins. **Results:** Over two hundred proteins ($p < 0.01$), were identified (versus 54 obtained by previous gel based approaches). Due to improved sensitivity we identified novel proteins not identified in our gel based studies including extracellular matrix proteins, cytokines, protease inhibitors and additional proteins from the coagulation pathway. In some cases, peptides that mapped to the same protein sequence did not change in unison. This may be due to novel splice variants, differential cleavage, or post-translational modifications. **Conclusion:** ML is amenable to MS based differential proteomics which can lead to a better understanding of the sequelae of HS as well as identification of inflammatory mediators. The LC-MS/MS approach has allowed us to detect relative protein abundance changes over a time course of HS in ML. The sensitivity and high mass accuracy of the analytical platform used allowed us to identify cleavage sites in proteins that may be specific to tissue injury after HS.

P148

RISK FACTORS FOR THE DEVELOPMENT OF SEPSIS AND SEPTIC SHOCK IN GENERAL SURGERY. L. Moore*, F. Moore, S. Jones*, S. Xu*, B. Bass*

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Purpose: Recent studies have documented poor outcomes in sepsis due to failure to rapidly provide early appropriate care. We have recently shown that aggressive sepsis screening & implementation of evidence based care bundles substantially reduced mortality in our surgical ICU. Our purpose is to identify risk factors associated with sepsis & septic shock in general surgery (GS) patients (pts). Methods: The 2005-07 NSQIP Database was queried for demographics, comorbidities, case type & mortality & grouped into no sepsis (NS), sepsis (S), & septic shock (SS). These groups were compared by chi-square tests. Logistic regression models were used to examine the association between NSQIP comorbidities & risk of developing S & SS, & 30-day mortality. Results: Of the 363,897 dataset pts, there were 349,570 (96.1%) NS, 8,350 (2.3%) S & 5,977 (1.6%) SS patients. 30 day mortality rates were as follows: NS 1.1% (3,934) vs. S 5.4% (449) vs. SS 33.7% (2,012). The SS group had more patients > 60 years of age (NS = 38.0% vs. S = 49.4% vs. SS = 67.9%, $p < 0.001$). The presence of any comorbidity increased mortality for S (5.9% vs. 0.4%, $p < 0.001$) & SS (34.4% vs. 12.6%, $p < 0.001$). The presence of any comorbidity increased the risk of developing S & SS 6 fold (OR=5.8, 95% CI: 5.5-6.2) & the risk of 30-day mortality 22 fold (OR=21.8, CI: 17.6-26.9). The presence of S & SS increased the risk of 30-day mortality by 4 (OR=3.9, CI: 3.5-4.3) & 33 (OR=32.9, CI: 30.9-35.1) fold, respectively. Conclusions: Development of S or SS in GS pts significantly increases mortality especially if pts progress into SS. Risk factors for death from S or SS in GS pts include age >60, emergency surgery, & the presence of comorbidities. These findings emphasize the need for early recognition through aggressive sepsis screening & rapid implementation of evidence based interventions for S & SS in GS pts with these risk factors.

P149

RECOMBINANT HUMAN ACTIVATED PROTEIN C INHIBITS INTEGRIN-MEDIATED NEUTROPHIL MIGRATION. M. Kim*, G. Elphick*, P. Sarangi*, Y. Hyun*, J. Hollenbaugh*, A. Ayala, W. Biffi*, H. Chung*, A. Rezaie*, J. McGrath*, D. Topham*, J. Reichner*, University of Rochester, Rochester, NY 14534.

Integrin mediated cell migration is central to many biological and pathological processes. During inflammation, tissue injury results from excessive infiltration and sequestration of activated leukocytes. Recombinant human activated protein C (rhAPC) has been shown to protect patients with severe sepsis, although the mechanism underlying this protective effect remains unclear. Using *in vitro* human neutrophil migration assay, cell adhesion assay, and *in vivo* mouse endotoxemia model, we show that rhAPC directly binds to β_1 and β_3 integrins and inhibits neutrophil migration, both *in vitro* and *in vivo*. We found that human APC possesses an Arg-Gly-Asp (RGD) sequence, which is critical for the inhibition. Mutation of this sequence abolished both integrin binding and inhibition of neutrophil migration. In addition, treatment of septic mice with a RGD peptide recapitulated the beneficial effects of rhAPC on the survival. Thus, we conclude that leukocyte integrins are novel cellular receptors for rhAPC, and the interaction decreases neutrophil recruitment into tissues, providing a potential mechanism by which rhAPC may protect from sepsis.

P150

EVIDENCE FOR TRAFFICKING OF BONE MARROW-DERIVED FIBROCYTES IN SEPTIC PERITONITIS. N. Bander*, C. Fry*, A. Mattar, J. Nemzek. University of Michigan, Ann Arbor, MI 48109

Studies suggest that sepsis mortality is associated with dysfunction of dendritic cells. Recently, it was found that a population of bone marrow-derived fibroblast-like cells, termed fibrocytes, has functions similar to those of dendritic cells, including cytokine production, antigen presentation, and activation of naïve T cells. Previous studies led us to hypothesize that fibrocytes traffick to the spleen and local lymphoid tissues from the septic peritoneum. In studies approved by our local IACUC, we harvested murine fibrocytes from peritoneal lavage fluid by culture and isolation using CD45+ magnetic beads in the Miltenyi system. The CD45+ cells were stained with carboxyfluorescein succinimidyl ester (CFSE). To induce sepsis, cecal ligation and puncture (CLP) was performed on anesthetized ICR mice. CLP was followed by the adoptive transfer of CFSE stained fibrocytes into the peritoneum. Control animals were subjected to the same adoptive transfer after a sham surgery, consisting of identical manipulation without puncturing the cecum. After 48 hours, the spleen and mesenteric lymph nodes were harvested for histology and flow cytometry. Image analysis of fluorescent microscopy showed that sepsis resulted in a higher area fraction of fluorescence in the mesenteric lymph nodes (CLP: 2.9 ± 1.7 vs. sham: 0.88 ± 0.52) and spleen (CLP: 4.4 ± 3.1 vs. sham: 1.0 ± 0.61). Flow cytometry showed marked evidence of CD45+/CFSE+ cells in the mesenteric lymph nodes (CLP: $2.3 \pm 1.8 \times 10^5$ cells vs. sham: $0.87 \pm 0.29 \times 10^5$ cells) while others traveled to the spleen (CLP: $1.7 \pm 1.2 \times 10^5$ cells vs. sham: $2.3 \pm 1.0 \times 10^5$ cells). The results demonstrated that fibrocytes move from the infected environment of the septic abdomen to the local lymphoid tissues, suggesting that they may play a role in antigen presentation.

P151

DIET-INDUCED OBESITY EXACERBATES CELL ADHESION IN SEPSIS.

V. Vachharajani

Wake Forest University School of Medicine, Winston-Salem, NC

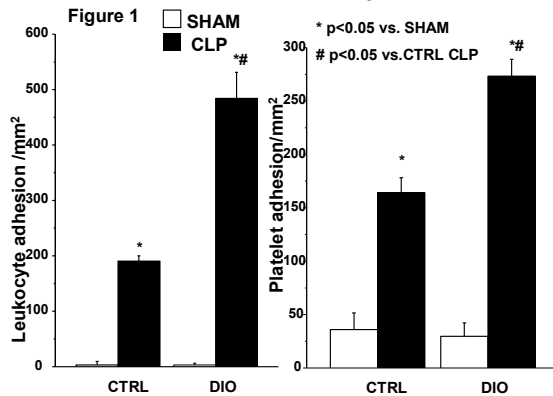
Introduction: Obesity increases morbidity in critically ill patients, including sepsis. We have shown exaggeration of inflammatory response in genetically obese (*ob/ob*) mice previously. The current study reports association of diet-induced obesity with sepsis.

Objective: To study the hypothesis that diet-induced obesity exacerbates inflammatory response in sepsis.

Methods: Diet-induced obese (DIO) C57Bl/6 and age matched control (CTRL) mice were subjected to cecal ligation and puncture (CLP) to induce sepsis or sham (SHAM) surgery. Four hours later, leukocyte (LA) and platelet (PA) adhesion in the post-capillary venules of cerebral microcirculation were studied using intravital fluorescent videomicroscopy.

Results: There was a significant increase in LA and PA of CLP mice compared to the SHAM counterparts in both, CTRL and DIO mice. Furthermore, there was a significant increase in the LA and PA in DIO compared to CTRL mice subjected to CLP. Results are depicted in Figure 1.

Conclusion: Diet-induced obesity increases leukocyte and platelet adhesion in cerebral microcirculation of mice subjected to cecal ligation and puncture.



P152

P65-STAT5 PROTEIN INTERACTIONS: A MECHANISM FOR GH RESISTANCE?.

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NF κ B mediates the inhibitory effects of TNF- α on GH-inducible gene expression. To investigate potential mechanism(s) we examined the effects of TNF on DNA-protein interactions between NF κ B and GHRE-1 (the cis-regulatory region of the IGF-I promoter); the role of "shared" transcriptional co-activator p300; and protein-protein interactions (PP) between NF κ B and STAT5 in CWSV-1 hepatocytes. EMSA was performed on nuclear extracts of TNF-treated cells and a 41 bp GHRE-1 sequence with overlapping NF- κ B and STAT5 binding sites. The data demonstrate a discrete DNA-protein interaction 2 h after TNF treatment that was not supershifted by p65 (NF- κ B subunit) antibodies. Thus, direct binding of p65 to GHRE-1 does not appear to explain GH resistance. Over expression of p300 increased TNF-inducible NF- κ B promoter activity, but when p300 was co-transfected with a GH-inducible Spi 2.1 promoter vector, it did not prevent the inhibitory effect of TNF on GH-inducible Spi 2.1 activity. Thus, p300 does not appear to contribute to GH resistance. PP interactions between NF κ B and STAT5 were examined in cells co-transfected with p65 \pm STAT5 expression vectors treated \pm GH. Nuclear extracts were immunoprecipitated with p65 antibody, then immunoblotted with STAT5 antibodies. A maximal p65-STAT5 interaction occurred 5 min after GH. p65 deletions were used to determine the p65 domain that interacted with STAT5. N- and C-terminal p65 deletion constructs were overexpressed and co-immunoprecipitation for NF- κ B and STAT5 was performed. PP were seen with both deletions. These results suggest PP between p65 and STAT5 play a role in cytokine-mediated hepatic GH resistance. (Supported by GM-55639 and T32 GM-64332).

P153

MAST CELL STABILIZATION IMPROVES SURVIVAL IN SEPSIS. L Ramos*, G Peña*, B. Cai*, C.U. Ezendokwere*, E. A. Deitch & L. Ulloa. UMDNJ-New Jersey Medical School. Newark, NJ 07101.

Sepsis, a leading cause of death in the ICU, is characterized by lethal systemic inflammatory responses leading to multiple organ failure. Here we report that mast cell stabilization restrains systemic inflammation and improves survival in sepsis. The implications of mast cell in

sepsis were studied using classical mast cell stabilizers in C57BL/6J as well as mast cell deficient Kit W-v and the counterpart wild-type Kit +/+ mice. Experimental sepsis was analyzed by using both endotoxemia and cecal ligation and puncture. Mast cell stabilizers restrained serum TNF levels during sepsis in wild-type but not in mast cell-deficient mice. Likewise, mast cell stabilizers improved survival in wild-type but not in mast cell-deficient mice. Unlike expected, depletion of mast cell in knockout mice also attenuated serum TNF levels, but it failed to improve survival. Indeed, wild-type and mast-cell deficient mice have statistically similar susceptibility to polymicrobial sepsis. Unlike early systemic TNF responses, serum HMGB1 levels correlated with the survival benefits of mast cell stabilization. Mast cell stabilizers inhibited systemic HMGB1 levels in wild-type but not in mast cell-deficient mice. Mast cell depletion in knockout mice failed to improve survival and to inhibit serum HMGB1 levels. Mast cell stabilizers failed to inhibit HMGB1 "secretion" from macrophages. However, mast cell stabilization prevented cell death and caspase-3 activation in sepsis. These results suggest that mast cells stabilization provides therapeutic benefits in sepsis by inhibiting extracellular "release" of HMGB1 from necrotic cells. In addition, mast cells can have major immunological implications regulating cell death in sepsis and may represent a pharmacological target for infectious disorders. Studies funded by USAMRMC#05308004, AHA06352230N, NIH-GM084125.

P154

DECREASED EXPRESSION OF MITOCHONDRIAL TRANSCRIPTION FACTOR A (Tfam) AND DNA POLYMERASE γ DURING CECAL LIGATION AND PUNCTURE (CLP). I. Nwaneshiudu*, L. Lee*, N. Raj, RJ Levy, C.S. Deutschman, Univ. of Penn., Phila., PA 19104.

Background: Sepsis is a common cause of death in the critically ill. This may be due to mitochondrial dysfunction. Previous work in animals made septic via CLP revealed sepsis-induced inhibition of Cytochrome C oxidase (CCO), the rate-limiting enzyme in electron transport. In part this reflects failed expression of one active mtDNA-encoded subunit of CCO, a process controlled by a single transcription factor, Tfam. mtDNA replication is required for regeneration of damaged mitochondria. This is controlled by a specific DNA polymerase, Pol γ . Both Tfam and Pol γ are nuclear-DNA encoded

Hypothesis: Failed mtDNA transcription and replication in late sepsis are associated with failed expression of Tfam and Pol γ .

Methods: Studies complied with NIH guidelines. C57Bl6 mice underwent sham (SO), single puncture CLP or double puncture CLP and were sacrificed 0, 3, 6, 16, 24, 48, and 72 hrs later. Cytoplasmic and mitochondrial fractions were isolated and immunoblotting was performed. Statistical significance was determined via ANOVA with a Bonferroni correction

Results: SO and single puncture CLP did not alter abundance of Tfam or Pol γ in either cytoplasm or mitochondria. Double puncture CLP significantly decreased abundance of both Tfam and Pol γ from 16 hrs through 72 hrs.

Conclusions: Double puncture CLP decreased both cytoplasmic and mitochondrial abundance of Tfam and Pol γ . This finding likely reflects failed transcription and/or translation of these nuclear encoded proteins. It also suggests impaired mitochondrial regeneration in severe sepsis.

P155

SEPSIS UNCOUPLES GUT EPITHELIAL PROLIFERATION AND DEATH FROM MIGRATION AND CELL DENSITY IN MICE. A. Clark*, B. Zee-Cheng*, W. Dunne*, T. Buchman, R. Hotchkiss, and C. Coopersmith Wash. U. Sch. Med. St. Louis, MO 63110

Background: The gut epithelium continuously regenerates by proliferation in crypts with migration and differentiation along villi and elimination by apoptosis or exfoliation. We have previously shown that sepsis increases gut epithelial apoptosis and decreases proliferation in a 90% lethal pneumonia model. Human sepsis mortality is estimated at 28.6% (Angus et al. 2001). Objective: To determine the effect of sepsis on jejunal proliferation, migration, apoptosis, morphology and homeostasis in a more clinically relevant 50% mortality model. Methods: FVB/N mice (n=7-16/group) were given an intratracheal injection of 2×10^6 CFU of *P. aeruginosa* or saline. Proliferation and migration were quantified by BrdU staining. Apoptosis was quantified by active caspase 3 and H and E staining. The number of jejunal epithelial cells per villus, villus length and epithelial cell density were assessed from H and E stained sections. Data were measured at 24 hours and compared by t test. Results: Septic mice had decreased proliferation (593 ± 38 vs 1390 ± 46 cells/100 crypts, $p < 0.01$) and migration (2.9 ± 0.1 vs 5.4 ± 0.2 $\mu\text{m/hr}$, $p < 0.01$) compared to shams. Septic mice had increased apoptosis by caspase 3 staining in crypts (45 ± 5 vs 14 ± 1 cells/100 crypts, $p < 0.01$) and villi (18 ± 2 vs 3 ± 1 cells/50 villi, $p < 0.01$). Similar trends were seen by H and E. Septic mice had increased numbers of cells per villus (84 ± 5 vs 67 ± 2 cells/villus, $p = 0.02$), decreased villus length (282 ± 10 vs 354 ± 15 μm , $p < 0.01$), and increased cell density (0.3 ± 0.01 vs 0.23 ± 0.01 cells/ μm , $p < 0.01$). Conclusions: Despite less epithelial cell production (decreased proliferation) and more cell loss (increased apoptosis) during sepsis, the number of cells per villus is actually *increased*. This appears to be due to slower (decreased) migration of cells and more compact cells (decreased villus length and increased cell density).

P156

ANTI-TNF PREVENTS GUT BARRIER DYSFUNCTION IN SEPTIC MICE. J. Clark, A. Samocha*, and C. Coopersmith. Wash. U. Sch. Med., St. Louis, MO 63110.

TNF has been implicated in gut barrier dysfunction due to its ability to modulate expression of tight junction components. Gut permeability is increased in sepsis; however, the mechanisms by which this occurs remain unclear. Our aim was to determine if gut barrier dysfunction in sepsis is mediated by TNF. Mice were given a 2x21-gauge cecal ligation and puncture (CLP) or sham laparotomy (n=5-16/group). At 24 hr, guts were evaluated for TNF, claudin-2, occludin, and ZO-1 by qRT-PCR. TNFR-1 was evaluated by Western blot and normalized to expression of β -actin. Systemic TNF levels were evaluated by cytometric bead array. In a separate cohort, mice were given an injection 3 hr post-operatively of monoclonal anti-TNF antibody or isotype control. Gut permeability was assayed *in vivo* by gavaging mice with 22 mg/ml FITC-Dextran 19 hr post-CLP. At 24 hr, plasma was collected and the concentration of FITC was determined. Two-way analysis was done with T test (parametric data) or Mann-Whitney test (non-parametric data). In septic mice, TNF was increased systemically (620.7 ± 551.9 vs. 15.4 ± 1.6 pg/ml; $p < 0.0001$) and locally in the gut (2.3 ± 0.3 vs. 1.0 ± 0.3 ; $p < 0.01$) compared to shams. There was a trend towards increased TNFR-1 in septic mice compared to shams (3.7 ± 0.3 vs. 1.0 ± 0.1 ; $p = \text{ns}$). Compared to shams, septic mice had increased gut permeability (467.8 ± 42.1 vs. 268.6 ± 21.7 ng/ml; $p < 0.01$). While claudin-2 was increased in septic mice compared shams (1.9 ± 0.3 vs. 1.0 ± 0.2 ; $p < 0.05$), there were no differences in occludin or ZO-1. Giving anti-TNF to septic mice normalized gut permeability to sham levels (183.2 ± 32.1 vs. 268.6 ± 21.7 ng/ml; $p = \text{ns}$). In conclusion, gut barrier dysfunction in sepsis is associated with increased systemic and local production of TNF, which may lead to parallel increases in claudin-2. Inhibiting TNF prevents sepsis-induced increases in gut permeability. Thus, TNF appears to play a central role in gut barrier modulation during sepsis.

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DECREASE OF PROAPOLIPOPROTEIN A-I WAS ASSOCIATED WITH MORTALITY OF PATIENTS WITH SEPTIC SHOCK

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Serum high density lipoprotein (HDL) cholesterol has been reported as a prognostic factor for severe sepsis and septic shock. Proapolipoprotein A-I (proapo A-I) is the only secretory form of apolipoprotein A-I (apo A-I), the major protein constituent of HDL cholesterol. Our aim was to examine the changes in serum proapo A-I, apo A-I, and HDL cholesterol of patients with septic shock during the first 24-h period of therapy and to determine whether these changes are associated with the prognosis. August 2007 to January 2008, consecutive patients admitted to the emergency intensive care unit (ICU) with septic shock were enrolled. We obtained serum samples from the patients at admission (0 h) and 24 h after admission (24 h). We identified serum proapo A-I and apo A-I using 2-dimensional gel electrophoresis followed by Western blotting and Mass spectrometry. Then, we compared the changes in serum expression intensities (EIs) of proapo A-I and apo A-I during the first 24-h period of therapy between the survivors (SURV) and the non-survivors on day 30 (NON-SURV). We also compared the serum concentration of HDL cholesterol between the two groups. Thirteen patients were grouped into SURV and fourteen into NON-SURV. The EI of proapo A-I remained unchanged in SURV but decreased in NON-SURV ($p = 0.020$). The serum concentrations of HDL cholesterol also decreased in NON-SURV ($p = 0.044$). However, the EI of apo A-I remained unchanged in both SURV and NON-SURV. In conclusion, the decrease of serum proapo A-I during the first 24-h period of therapy was responsible for the decrease of serum HDL cholesterol and associated with the mortality of patients with septic shock.

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SCAVENGER RECEPTOR-A (SR-A) CONTRIBUTES TO MORTALITY AND THE DEVELOPMENT OF A PRO-INFLAMMATORY PHENOTYPE IN FULMINATING SEPSIS.

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The critically ill patient frequently develops a complex disease spectrum that may include septic shock. The mechanisms that lead to the development of shock are not fully understood. Macrophage scavenger receptors (SR-A) bind to and internalize polyanions, notably oxidized LDL, but SR-A has also been shown to bind multiple PAMPs, including LPS. The present study sought to determine the role of SR-A in polymicrobial sepsis. Sepsis was induced in SR-A deficient (SR-A^{-/-}) and wild type (WT) mice by cecal ligation and puncture (CLP). SR-A^{-/-} septic mice showed an increased median survival time (300 h vs. 43 h) and an increased long term survival rate (41.0% vs. 7.9%, p<0.05) when compared to WT mice. Analysis of serum cytokines revealed that septic SR-A^{-/-} mice showed attenuation (p<0.05) of serum IL-5, IL-6, IL-10 and MCP-1, when compared to septic WT mice. However, serum cytokine levels were increased (p<0.05) in septic SR-A^{-/-} compared to non-infected control SR-A^{-/-} mice. Analysis of MPO activity revealed that sepsis increased lung neutrophil infiltration, however, there was no significant difference in MPO activity in the presence or absence of SR-A. In a similar fashion, SR-A deficiency did not alter sepsis induced splenocyte apoptosis. The data indicate that SR-A plays an important role in the pathophysiology of polymicrobial sepsis and septic shock. Specifically, SR-A contributes to mortality and the development of a pro-inflammatory phenotype in sepsis. However, SR-A had no effect on lung neutrophil infiltration or splenocyte apoptosis in response to septic shock. We speculate that modulation of SR-A may be an effective means of altering inflammatory responses and outcome in septic shock.

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SURVIVAL IN A TWO HIT MODEL OF SEPSIS IS DEPENDENT ON TIMING OF SECONDARY INJURY.

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Background: Secondary infection is a significant cause of increased morbidity and mortality in patients with sepsis. Although it is clear there is a period of immunoparalysis associated with the septic condition, the extent and duration of this immune suppression is unknown. **Objective:** The purpose of this study was to evaluate the host immune status and its susceptibility or resistance to secondary infection at different time points following CLP. **Methods:** The CLP model was employed as the primary injury. As a "second hit," *Pseudomonas aeruginosa* was given intranasally to mice 4 or 7 days following CLP. Survival studies evaluated differences over 7 days following pneumonia (n=14/group). For acute studies samples were harvested 18 hours after pneumonia and assessed for lymphocyte apoptosis (TUNEL), serum cytokines, cultures of BAL fluid and blood (n=5-6/group). **Results:** Single injury animals (CLP or pneumonia), had a ~15% mortality rate. Double injury animals, CLP followed by pneumonia at 4 days, had 80% mortality. Double injury animals at 7 days had only 15% mortality (p<0.01). Animals receiving pneumonia 4 days after CLP had significantly increased apoptosis of T and B splenocytes compared to the 7 day group (19.2 vs. 8.3pg/ml, p<0.01, 9.0 vs. 3.9, p<0.01, respectively). Four of five animals in the 4 day group were bacteremic compared to one of six in the 7 day group. Finally, IL-1 α , IL-6 and G-CSF serum levels were significantly lower in the 4 day group (84.3 vs. 880pg/ml, p<0.01, 103 vs. 903, p<0.01, and 3121 vs. 5139, p=0.015, respectively). **Conclusion:** Following CLP, an immunosuppressive state persists for at least 4 days as evidenced by decreased inflammatory cytokine production and increases in mortality, apoptosis, and bacterial load. By 7 days the immune system, although not returned to baseline, is able to mount an effective response.

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FIBROCYTE IMPACT ON T-CELL PROLIFERATION AND CYTOKINE PRODUCTION.
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Mortality seen during the course of septic injury is associated with the depletion of T-cells and dendritic cells (DCs). The known ability of fibrocytes (fb) to present antigen may serve as an alternative for DC functions during sepsis. Thus, the ability of fb to present bacterial antigen to T-cells throughout the course of sepsis may reconstitute depleted T-cells resulting in less mortality. However, fb have only been studied using viral antigen. The purpose of this study was to assess the ability of fb to present bacterial antigens to T-cells and the cytokine response to bacterial products. Therefore, purified T-cells were cultured with purified fb with or without heat inactivated E. Coli (HIE) and assessed for T-cell proliferation (CFSE) and activation (CD69) by flow cytometry; cytokine production by ELISA. Fibrocytes from mouse peritoneal lavage fluid were cultured for two weeks and separated using anti-CD45 antibody-coupled magnetic beads (Miltenyi). Fibrocytes were loaded with HIE at a ratio of 5 to 1. T-cells were purified from mouse spleens using a Dynal negative isolation kit, then CFSE stained. Then T-cells were added at a 10 to 1 ratio to loaded (HIE) or unloaded fb. T-cells cultured with unloaded or loaded fb for 3 days resulted in a 2 and 9 fold increase respectively for T-cell proliferation and activation when compared to T-cells alone or T-cells + HIE. At 1wk, T-cells incubated with loaded fb resulted in a further 15% increase for proliferation and 7% increase for activation when compared to T cells + unloaded Fb. Fb cultured with T-cells in the presence of LPS resulted in increased levels of IFN-g production by 72hrs. T-cells incubated with loaded or unloaded fb results in increased T-cell proliferation, activation, and T_H1 cytokines. Fb impact on T-cell proliferation may be useful in reconstituting T-cell numbers in septic animals.

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ANTI-IL10 THERAPY INCREASES SURVIVAL IN A TWO-HIT MODEL OF SEPSIS. J. Muenzer*, C. Davis, K. Chang*, D. Osborne*, M. Dunne*, C. Coopersmith, R. Hotchkiss. Wash. Univ. Sch. of Med., St. Louis, MO 63110.

Background: The initial hyperinflammatory phase of sepsis is followed by a state of immunoparalysis and is associated with significant risk of secondary infection. IL-10 is a mediator of immune suppression and its elevation has been associated with poor outcome in sepsis. The immune modulator AS101 is known to down regulate IL-10.

Objective: The purpose of this study was to evaluate the effect of IL-10 down regulation using AS101 in a two hit model of sepsis. **Methods:** CLP was employed as the primary injury followed by a "second hit," *P. aeruginosa* (Pa). Pa was given intranasally in mice 4 days following CLP. Survival studies evaluated differences for 7 days after pneumonia (n=17/group). Animals were treated with 10ug of AS101 or vehicle SC at 24 hours post CLP and then q24h. For acute studies samples were harvested 18 hours after pneumonia and assessed for lymphocyte apoptosis (Caspase-3), serum cytokines, cultures of BAL and blood (n=6/group). Groups were compared using the Mann Whitney U test. Chi square was used for survival. **Results:** AS101 treated animals had improved survival compared to controls (88% vs. 53%, p<0.02). AS101 animals showed decreased serum IL-10 (229 vs. 31pg/ml p=0.002). Serum IL-12p40 was significantly increased in treated animals (291 vs 77pg/ml, p=0.02). There was no difference in IL-6, IL-1 α , IL-1 β , TNF α , or IFN γ . BAL cultures in AS101 animals showed significantly lower growth (83 vs 6 x10³ CFU, p<0.03). None of the AS101 animals had bacteremia compared to three of six controls. Caspase-3 revealed significant decreases in T and B lymphocyte apoptosis in treated animals (5.4 vs 9.2, p<0.01 and 7.5 vs 5.1, p<0.01). **Conclusions:** AS101 down regulates IL-10, improves survival, increases IL12, and decreases bacterial load in a two hit model of sepsis. Immune modulation following a septic insult may provide a strategy for decreasing secondary infections.

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MECHANISMS OF MRSA PNEUMONIA-INDUCED GUT EPITHELIAL APOPTOSIS.
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Methicillin-resistant *S. aureus* (MRSA) pneumonia is common in intensive care units and causes increased morbidity and mortality. We have previously shown that *P. aeruginosa* pneumonia-induced sepsis causes increased gut epithelial apoptosis. We wanted to determine 1) if MRSA pneumonia causes increased gut epithelial apoptosis and 2) if so, through which signaling pathway apoptosis occurs. FVB/N mice were given 2×10^7 CFU of MRSA (septic) or saline (sham) intra-tracheally. They were sacrificed 1 day later to quantify gut apoptosis by H&E and caspase-3 staining (apoptotic cells/100 crypts; n=10-13). Western blots were performed on whole-bowel lysates. Protein densities were normalized to β -actin expression, and results reported as fold difference from sham (n=5). Data were analyzed with the Mann-Whitney-U test. Septic mice had increased gut apoptosis compared to sham (H&E: 74 ± 30 vs 16 ± 5 , $p < 0.02$; caspase-3: 52 ± 16 vs 10 ± 2 , $p < 0.001$). In the extrinsic pathway, expression of soluble Fas ligand (Fas-L) was increased in septic mice (15 ± 4 vs 1 ± 0.5 ; $p < 0.01$). Pro-apoptotic Bid, considered to be a crosstalk mediator between the intrinsic and extrinsic pathways, was increased in septic mice (6 ± 0.9 vs 1 ± 0.2 ; $p < 0.01$). In the intrinsic pathway, pro-apoptotic Bax and anti-apoptotic Bcl-xL were elevated in septic mice (Bax: 2 ± 0.1 vs 1 ± 0.1 , $p < 0.02$; Bcl-xL: 7 ± 0.6 vs 1 ± 0.2 , $p < 0.01$), however, the Bax/Bcl-xL ratio, often used as a cellular rheostat to determine if a cell undergoes apoptosis was reduced in the septic group (0.2 ± 0.02 vs 1 ± 0.3 ; $p < 0.01$). In conclusion, MRSA pneumonia increases gut epithelial apoptosis. Increased expression of pro-apoptotic, extrinsic pathway mediator Fas-L, crosstalk mediator Bid and decreased anti-apoptotic ratio of Bax/Bcl-xL in the intrinsic pathway suggest that extrinsic pathway may predominate in MRSA-pneumonia induced gut epithelial apoptosis.

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JAK2 AS A PHARMACOLOGICAL TARGET IN SEPSIS.

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Sepsis, a leading cause of death in the ICU, is characterized by lethal systemic inflammatory responses leading to multiple organ failure. We previously reported that nicotine attenuates systemic inflammation and improves survival in experimental sepsis^{1,2}. It was proposed that nicotine controls systemic inflammation via the alpha7-nicotinic acetylcholine receptor(nAChR)^{1,2}-JAK2^{3,4} pathway. Here, we report that JAK2 inhibition attenuates systemic inflammation and improves survival in experimental sepsis. JAK2 inhibition with AG490⁵ was confirmed by analyzing STAT3 phosphorylation. Unlike expected, JAK2 was not required for the anti-inflammatory potential of alpha7nAChR in macrophages. Nicotinic stimulation inhibited TNF production and HMGB1 secretion in JAK2-inhibited cells. Treatment with AG490 itself, inhibited JAK2, prevented STAT3 phosphorylation, and blunted TNF production and HMGB1 secretion in macrophages. *In vivo*, treatment with AG490 prevented systemic inflammation and attenuated serum TNF and HMGB1 levels during endotoxemia. JAK2 inhibition prevented lethal endotoxemia and improved survival in a concentration dependent manner. Delayed treatment with AG490 started at 12 hours after the onset of sepsis rescued the mice and improved survival in polymicrobial sepsis induced by cecal ligation and puncture (CLP). These results suggest that JAK2 can be a potential pharmacological target for the treatment of sepsis. Studies funded by USAMRMC#05308004, AHA06352230N, NIH-GM084125.

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THERAPEUTIC EFFECTS OF CHLOROGENIC ACID IN EXPERIMENTAL SEPSIS.

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Sepsis is an serious clinical condition characterized by complex immunologic response to infectious stimuli, such as overwhelming activation of the inflammatory system and countervailing response from the anti-inflammatory system. However, the cause of this perturbation is yet to be elucidated. Chlorogenic acid, the ester of caffeic acid with quinic acid, is one of the phenolic compounds present in *Lonicerae flos*. In this study, we report that chlorogenic acid therapeutically reverses the lethality induced by cecal ligation and puncture (CLP), a clinically relevant model of sepsis, or the lethality of endotoxemia induced by injection of LPS. The administration of chlorogenic acid ameliorated the multiple organ dysfunction syndrome (MODS). The increases observed in the levels of tumor necrosis factor (TNF)- α and interleukin (IL)-1 β induced by CLP were inhibited, and yet, the levels of interferon (IFN)- γ , IL-2, and IL-12 were augmented by chlorogenic acid. The chlorogenic acid treatment suppressed the serum level of high mobility group box 1 protein, the late mediator of lethal systemic inflammation in sepsis. The apoptotic loss of immune cells, responsible for the substantially impaired immune response observed in sepsis, is an entirely novel and potentially important therapeutic target in the treatment of sepsis. Results obtained by flow cytometric analysis indicated that chlorogenic acid prevented the apoptosis of immune cells. In support of this, administration of chlorogenic acid to mice undergoing sepsis caused by CLP markedly enhanced bacterial clearance. Furthermore, chlorogenic acid activated the phagocytic and bactericidal activities of macrophages *in vitro*. Consequently, chlorogenic acid may serve as a potential multistep therapeutic agent useful in the clinical treatment of sepsis.

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CHLOROGENIC ACID DECREASES EXPRESSION OF TOLL-LIKE RECEPTOR-2 AND 4 AND PREVENTS ACTIVATION OF MAPKS AND NF-KB IN LIVER DURING POLYMICROBIAL SEPSIS.

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Toll-like receptors (TLRs) play a crucial role in the host responses to pathogens in the innate immune system and are known to be involved in the development of inflammatory diseases like sepsis. Recently, TLRs were identified in many organs including the liver and mitogen-activated protein kinases (MAPK) are recognized as the key elements in the TLR signaling pathway. Chlorogenic acid is one of the most abundant polyphenols in the human diet and its antioxidant and anti-inflammatory properties of chlorogenic acid have been established in human and animal studies. We investigated the effects of chlorogenic acid on TLRs expression and its signaling pathway during sepsis. ICR mice were subjected to sepsis by cecal-ligation and puncture (CLP). The mice were received vehicle or chlorogenic acid (20 mg/kg body weight) intravenously. CLP increased TLR2 and 4 mRNA levels on liver, heart, lung and kidney 0, 1, 3, 6, 12 and 24 h after CLP, which showed distinct differences especially in liver and its peak was 6 hr after CLP. These increases were attenuated by chlorogenic acid treatment. TLR4 expression, NF-kappaB and MAPKs activities were measured by Western blot. We found that TLR4, NF-kappaB and MAPKs activities in liver, including extracellular regulated kinases (ERK), c-jun N-terminal kinase (JNK) and p38 MAPK increased 6 hr after CLP. Chlorogenic acid attenuated TLR4, NF-kappaB, JNK and p38 MAPK activities, but not on ERK activities. These data demonstrate that chlorogenic acid has significant inhibitory effects on the activation of TLR4, NF-kappaB and MAPKs in liver during polymicrobial sepsis in mice.

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CELL SURFACE RECEPTORS EXPRESSION AND PRODUCTION OF INFLAMMATORY CYTOKINES BY MONOCYTES OF PATIENTS WITH LEPTOSPIROSIS. R. Salomao, T. Pavanelli*, M. Brunialti*, A. Ko*. Escola Paulista de Medicina, Federal University of Sao Paulo, UNIFESP, São Paulo, SP, Brazil, Postal code 04039-032.

Leptospirosis is one of the world's most important zoonoses. Toxins present in the cells of the spirochete and released in affected tissues play a major role in its pathogenesis. Objectives: To evaluate the expression of TLR2, TLR4 and CD14 receptors on the monocytes surface and the in vitro cellular response to TLRs ligands, assessed by production of monocytes-derived cytokines in patients with leptospirosis. Methods: 07 patients with clinical and laboratory diagnosis of leptospirosis and 07 healthy volunteers, used as controls, were included. The peripheral blood mononuclear cells (PBMC) were separated using ficoll-paque, frozen and stored in liquid nitrogen. TLR2, TLR4 and CD14 expression on the monocytes surface was measured by flow cytometry. For cytokine induction, PBMC was incubated for 6 hours with LipL32 (1000ng/mL), MALP-2 (0.4 U / mL), or LPS (100ng/mL). Intracellular TNF- α and IL-6 was detected in monocytes by flow cytometry. Results: There was no significant difference in the production of IL-6 and TNF- α in monocytes between patients with leptospirosis and healthy volunteers when PBMC were stimulated in vitro with MALP-2 and LipL32. Following LPS stimulation, detection of IL-6 was similar to controls, and TNF- α was lower in patients ($p = 0.035$). Regarding the expression of cell surface receptors, an increased expression of TLR2 ($p = 0.025$) was observed on the monocytes of patients, while no difference was found in the expression of TLR4 and CD14. Conclusions: Monocytes of peripheral blood of patients with leptospirosis retain the ability for antigen recognition and cellular signaling, assessed by the expression of cell surface receptors and the detection of intracellular IL-6 and TNF- α by peripheral blood monocytes.

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THE PROTECTIVE ROLE OF SPLACNIC SYMPATHETIC BLOCKAGE BY THORACIC EPIDURAL ANALGESIA (EA) IN HEMODYNAMIC DYSFUNCTION OF SEPTIC PIGS. A.Yamamura*, (I.Koh*), F.Sotelo*, J.Menchaca-Diaz*, T.Koh*, A.Liberatore*, A.Soubhie*, C.Margarido*, E.Silva*. IEP-HSL/UNIFESP. São Paulo, S.P., Brazil. CEP:04039-032.

White Large pigs were distributed in Epidural (EG), Sepsis (SG), Sepsis plus epidural (SEG) and Sham (ShG) groups (G). (n=6/G). Severe sepsis was induced by *E.coli*, i.v. inoculation (10^8 CFU/mL/kg soon after hemodynamic stabilization (Time zero=T0) and EA (0.05% bupivacaine) was realized soon after sepsis. Femoral and pulmonary artery catheters and Transonic flowmeter probes at superior mesenteric artery (SMA) and portal vein (PV) were placed for macro-hemodynamic and regional flow monitoring, respectively, at T0, T30, T90, T150 and T210 min. by laparotomy. Unlike epidural anesthesia, the EA effect was CI increase (18%), transient systemic vascular resistance index (SVRI) and medium arterial pressure (MAP) increase with mild or non changes in other hemodynamic parameters. In SG, hyperdynamic state was observed from T30 to 90 with trend to hypodynamic state from T150 to T210; Cardiac index (CI) (*from T90-210), mixed venous oxygen saturation (*from T150), pulmonary compliance (PC) (*from T30), PaO₂/FiO₂ ratio (from T90) were decreased (*significant); and increased SVRI (T210) were observed. Besides, SMA and PV flow were highly decreased from T90 (*T150-210, around 26%). In SEG, there was an increased pulmonary vascular resistance with mild pulmonary compliance reduction suggesting the lung tissue preservation by EA. Besides, SMA and PV flow plus MAP were preserved though CI reduction and SVRI increase, demonstrating EA protective role even in severe sepsis possibly by the splacnic sympathetic blockage. In conclusion, the autonomic neuromodulation by EA demonstrated to have a protective role in preventing sepsis related splacnic hemodynamic dysfunction and subsequent macro hemodynamic alterations.

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PRESERVATION OF THE GUT MICROCIRCULATION IN SEVERE SEPSIS BY SPLACNIC SYMPATHETIC BLOCKAGE INDUCED THROUGH THORACIC EPIDURAL ANALGESIA (EA) IN PIGS. (I.Koh*), J.Menchaca-Diaz*, A.Yamamura*, F.Sotelo*, T.Koh*, A.Liberatore*, A.Soubhie*, C.Margarido*, E.Silva*. UNIFESP/IEP-HSL-SMA. São Paulo, S.P., Brazil. CEP:04039-032.

Pigs were distributed in Epidural (EG), Sepsis (SG), Sepsis plus epidural (SEG) and Sham (ShG) groups (G). (n=6/G). Severe sepsis was induced by *E.coli*, i.v. inoculation (10^8 CFU/mL/kg) at time zero (T0) and EA (4mL of 0.05% bupivacaine) was realized after sepsis. Femoral and Swan Ganz catheters and Transonic flowmeter probes at superior mesenteric artery (SMA) and portal vein (PV) were placed for macrohemodynamic and regional flow monitoring, respectively at T0, T30, T90, T150 and T210 min. by laparotomy. Besides, ileal mucosa (IM) and seromuscular (IS) tissue perfusion were also monitored by Laser Doppler. The EA effect were: mild increase of SMA flow (6 to 14%), significant increase in IM and IS flow (30% at T150 and T210) and unchanged PV flow and PV pressure. In SG, SMA and PV flow were highly decreased (SMA: 26% from T150 to 210; PV: 16% at T90 and 40% at T150-210), besides a significant PV pressure increase (T90=20% and 40% at T150 to T210). Also, a progressive decrease in IM and IS flow was seen (20% at T30 up to 40% at T210). In contrast, in SEG were observed an increased blood flow at MSA (6-19%), IM and IS (30% at T150 and T210), and only PV flow was slightly decreased (4-8%) at all periods. The PV pressure was only increased at T30 (20%), returning to basal level at other periods. The findings in SEG associated to the preservation of the macrohemodynamic (data shown elsewhere) suggest that splacnic sympathetic blockage was able to diminish the effect of selective splacnic hypoperfusion mechanism seen in sepsis, thus preventing tissue damage and gut origin inflammatory response which is believed to crosstalk with the systemic immunity resulting in MODS.

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EXTRACELLULAR HEAT SHOCK COGNATE PROTEIN 70 INDUCES CARDIAC FUNCTIONAL TOLERANCE TO ENDOTOXIN: DIFFERENTIAL EFFECT ON MYOCARDIAL TNF- α AND ICAM-1 LEVELS X Su, JB Sykes, L Ao, JC. Cleveland, N Zou, DA. Fullerton and X Meng Department of Surgery, University of Colorado Denver, Denver, CO 80045, USA

Background: Induction of cardiac tolerance to endotoxin by non-noxious agents may have a therapeutic potential. Heat shock protein 70 (Hsp70) has been shown to induce the inflammatory response through Toll-like receptor 4 (TLR4) in several cell types, and a recent study demonstrates that HSP70 induces macrophage tolerance to endotoxin. Our previous study has found that heat shock cognate protein 70 (HSC70) is capable of activating TLR4 in the heart to induce pro-inflammatory cytokine expression. This study tested the **hypothesis** that HSC70 preconditioning induces cardiac tolerance to endotoxin. We examined whether in vivo HSC70 preconditioning in mice preserves cardiac function during subsequent endotoxemia, and whether the cardiac effect of HSC70 preconditioning is associated with an influence on the expression of TNF- α and ICAM-1 during endotoxemia. **Methods and Results:** We treated mice with HSC70 (5 μ g/ml of blood, iv) 24 h prior to an exposure to endotoxin (0.5 mg/kg, iv). HSC70 preconditioning abrogated endotoxemic cardiac dysfunction, and this effect was comparable to that of endotoxin preconditioning. However, HSC70 preconditioning had no significant effect on circulating and myocardial TNF- α levels, while endotoxin preconditioning reduced the production of this pro-inflammatory cytokine. However, preconditioning with either HSC70 or endotoxin significantly reduced myocardial ICAM-1 expression during subsequent endotoxemia. **Conclusions:** This study demonstrates that HSC70 preconditioning induces cardiac functional tolerance to endotoxin that is associated with a significant down-regulation of myocardial ICAM-1 expression. Thus, the mechanism by which HSC70 preconditioning preserves cardiac function appears to involve down-regulation of myocardial ICAM-1 expression.

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SURGICAL SEPSIS. L Moore*, F Moore, S Todd*, K Turner*, J Sucher, A Valdivia*, M Sailors*, B McKinley. The Methodist Hospital, Dept of Surgery, Houston TX 77030

Objective: We describe current epidemiology of sepsis in a general surgical ICU.

Methods: Sepsis (S) and severe sepsis/septic shock (S/S) were designated at diagnosis using guideline criteria: MAP<65 mmHg, [lactate]>4 mM, or UO<0.5 mL/hr-kg. Case groups were compared (t, X² tests; p≤0.05). **Results:** In 12 mo ending Sep 2008, 127 patients had 73 cases of S and 66 of S/S. S and S/S ages were similar. More females had S/S. Day 1 SOFA and APACHE II scores were greater for S/S. Infection sources were: abdomen [S=45 (62%), S/S=35 (53%)], soft tissue [S=7 (10%), S/S=12 (18%)], lung [S=7 (10%), S/S=5 (8%)], invasive line [S=2 (3%), S/S=4 (6%)], urinary tract [S=2 (3%), S/S=4 (6%)]. Negative culture: 16 (12%) cases.

mean±sem	Cases of S (n=73)	Cases of S/S (n=66)	p
age (yr)	57±2 (n patients=65)	61±2 (n patients=62)	0.13
female (%)	35	60	0.13
SOFA day1	8.7 ± 0.5	10.3 ± 0.5	0.02
APACHE II day1	22 ± 1	29 ± 1	0.00
MAP (mmHg)	86 ± 3	69 ± 3	0.00
[lactate] (mM)	2.1 ± 0.03	4.1 ± 0.4	0.00
INR	1.4 ± 0.03	1.8 ± 0.1	0.00
[platelet] (k/mm ³)	262 ± 15	209 ± 18	0.03
IV fluid 1 st 6 hr (L)	1.2 ± 0.9	1.9 ± 0.2	0.00
vasopres>12hr (%)	0	52	0.00
ICU free (dy)	20 ± 1 (n patients=65)	19 ± 1 (n patients=62)	0.81
hosp survival (%)	91 (n patients=65)	76 (n patients=62)	0.05

Conclusion: Abdominal and soft tissue sources were predominant. Compared to S, S/S patients were of similar advanced age, more likely female, had worse SOFA and APACHE II scores, more deranged physiology, and required more aggressive intervention. S/S had worse survival rate, but S and S/S had similar ICU stays, which may reflect organ dysfunction undistinguished by guideline criteria. Early recognition and aggressive intervention is needed for sepsis in surgical ICU patients.

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MMP EXPRESSION IN CHILDREN WITH INFLAMMATORY CARDIOMYOPATHY.

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Introduction: Inflammatory cardiomyopathy, IC, is a complex syndrome resulting from the acute invasion of cardiotropic viruses and immunologic mediators with potential for extensive cardiomyocyte destruction and heart failure. Murine models suggest cardiac MMP interactions may regulate IC. Circulating MMP's may also eliminate infection and remodel damaged cardiac muscle. MMP profiles have not been characterized in pediatric IC.

Methods: 15 children (± 8.3 years) admitted with IC and left ventricular dysfunction were enrolled. 20 children (± 4.7 years) undergoing general anesthesia were used as controls. Serum was collected at enrollment, 24, 72 hrs after enrollment and at discharge. The hospital course and echocardiography data were reviewed.

Results: Children with IC had significantly higher levels of MMP-1 compared to controls at enrollment (32.4 ± 16.8 ng/ml vs 10.6 ± 6.6 , $p < 0.05$), 24 hr (28 ± 13.5 ng/ml, $p < 0.05$) and discharge (20.9 ± 7.1 ng/ml, $p < 0.05$). MMP-3 levels at 72 hrs were also statistically greater than control (8.5 ± 1.9 ng/ml vs 5.9 ± 1 ng/ml, $p < 0.05$). Two patients died and 4 underwent cardiac transplantation. The remaining patients experienced ~ 1.3 ventilator days, 6 ICU days and 15 hospital days. Patients who died or received cardiac transplant had significantly depressed admission shortening fraction compared to those discharged (8.3 ± 3.6 vs 16.3 ± 6.5 , $p < 0.05$).

Discussion: Circulating MMP-1,-3 are elevated during the course of pediatric IC and return to baseline with disease progression. TIMP-2 tends to decrease in a temporal fashion over disease course, and no changes in MMP-9 were detected. These results suggest the interaction of specific serum MMP and TIMP may have a role in the pathogenesis of pediatric IC may be a therapeutic target for decreasing its morbidity and mortality.

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CARDIAC RESPONSE AND MOLECULAR MECHANISMS OF CARDIAC DYSFUNCTION AS RELATED TO AGE.

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Purpose: To characterize and identify gene differences in cardiac response to septic challenge in different age groups.

Experimental Design: Myocardial response following LPS was determined by fractional shortening (FS) and heart rate (HR) in **w**eaned (3 week old), **a**dolescent (9 week old) and **a**dult (4 month old) rats. ECHO was done at 0, 2, 4, 8, 12, 24, and 36h post LPS. Hearts were collected and protein harvested at 4h. Cardiac protein was analyzed by protein kinase and apoptotic proteomic microarray.

Results: HR of LPS challenged **a**, and **ad** rats elevated at 4h. In comparison, **w** rats demonstrated a HR drop of 18% at 2h, which then recovered and elevated at 8 and 24h. **W** animals continued to differ in HR from other age groups, maintaining a normal HR. In difference to HR, the shortening fraction of all LPS challenged groups increased approximately 29% 2h post LPS. **W** rats showed no significant change from controls, compared to **a** and **ad** rats, which exhibited significant drops in fractional shortening (24% at 4h until recovery at 36h for **a**, and 18% for **ad**). Protein expression in the age groups was also examined. Sixty different proteins were screened, and 37 were altered in the **w** as compared to the older groups. One of the strongest regulated was Suppressor of Cytokine Signaling 4 (SOC4). Continuing work is examining the role of SOC4 in age related cardiac differences.

Conclusions: Our results suggest there are differences in cardiac function and patterns of cardiac protein expression in different age groups of rats, suggesting there may be differing mechanisms for the physiologic differences in cardiac dysfunction seen in adults and children during a septic event.

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HUMAN ADRENOMEDULLIN (AM) AND ITS BINDING PROTEIN (AMBP-1) AMELIORATE SEPSIS-INDUCED ORGAN INJURY AND MORTALITY IN JAUNDICED RATS. J. Yang*, R. Wu, M. Zhou, P. Wang. North Shore-LIJ Medical Center, Manhasset, NY 11030.

Sepsis is a serious complication for patients with obstructive jaundice. We have shown that AM/AMBP-1 is protective in sepsis. However, it remains unknown whether AM/AMBP-1 ameliorates sepsis-induced organ injury and mortality in the setting of biliary obstruction. To study this, obstructive jaundice was induced in male adult rats by common bile duct ligation (BDL). One week after BDL, the rats were subjected to sepsis by cecal ligation and puncture (CLP). Plasma levels of AM and AMBP-1 were measured at 20 h after CLP. In additional groups of BDL+CLP rats, human AM/AMBP-1 (24/80 µg/kg BW) or vehicle (i.e., human albumin) was administered intra-venously at 5 h after CLP. Blood and tissue samples were collected at 20 h after CLP for various measurements. To determine the long-term effect of human AM/AMBP-1 after BDL+CLP, the gangrenous cecum was removed at 20 h after CLP and 7-day survival was recorded. Our results showed that plasma levels of AM were significantly increased while AMBP-1 levels were markedly decreased after BDL+CLP (n=8, P<.05). As shown in the table below, administration of human AM/AMBP-1 attenuated tissue injury and inflammatory responses after BDL+CLP.

	Sham	BDL+CLP-Vehicle	BDL+CLP-AM/AMBP-1
ALT (IU/L)	37±7.2	197±12.4*	116±10.1*#
AST (IU/L)	35±1.4	281±13.8*	206±10.7*#
Lactate (mmol/L)	2.1±0.1	5.6±0.4*	3.6±0.15*#
Creatinine (mmol/L)	0.5±0.1	1.3±0.1*	0.8±0.1*#
TNF-α (pg/ml)	6.7±1.2	60.4±4.4*	37.1±4.5*#
IL-6 (pg/ml)	107±6.0	780±61.5*	541±36.5*#
Hepatic MPO (U/g)	0.3±0.1	2.4±0.4*	1.6±0.1*#
Intestinal MPO (U/g)	0.1±0.01	6.8±1.3*	3.4±0.7*#
Pulmonary MPO (U/g)	9.5±0.6	72.1±10.5*	50.5±4.9*#

(Mean±SE, n=8, one-way ANOVA: *P<.05 vs. Sham; # P<.05 vs. Vehicle)

Moreover, human AM/AMBP-1 significantly increased the survival rate from 21% (n=14) to 53% (n=15). Thus, human AM/AMBP-1 can be developed as a novel treatment for sepsis in jaundiced patients.

P174

ANTAGONISM OF ALPHA_{2A}-ADRENOCEPTOR: A NOVEL APPROACH TO INHIBIT INFLAMMATORY RESPONSES IN SEPSIS. F. Zhang*, R. Wu, X. Qiang*, M. Zhou, P. Wang. North Shore-LIJ Medical Center, Manhasset, NY 11030.

Our previous studies have indicated that the release of the sympathetic neurotransmitter, norepinephrine (NE), from the gut is increased in sepsis, and that NE potentiates endotoxin-induced TNF- α production via the A subtype of α_2 -adrenoceptors (i.e., α_{2A} -AR) on the surface of Kupffer cells. A specific antagonist for α_{2A} -AR, BRL-44408 maleate, reduces TNF- α secretion in cultured Kupffer cells. We, therefore, hypothesized that BRL-44408 maleate inhibits inflammatory responses and reduces organ injury in sepsis. To study this, sepsis was induced in male rats by cecal ligation and puncture (CLP). At 5 h after CLP, BRL-44408 maleate (1.25, 2.5, or 5.0 mg/kg BW) or vehicle (i.e., normal saline) were administered intravenously. Blood and intestinal samples were collected at 20 h after CLP. Serum levels of TNF- α , IL-6, keratinocyte-derived chemokine (KC), macrophage inflammatory protein-2 (MIP-2), liver enzymes (i.e., AST and ALT), and lactate were measured. The intestinal levels of TNF- α , IL-6 and myeloperoxidase (MPO) activities were also analyzed. In additional groups of animals, the necrotic cecum was excised at 20 h post-CLP and the 10-day survival was recorded. Our results showed that serum levels of pro-inflammatory cytokines, chemokines, liver enzymes, lactate and intestinal levels of TNF- α , IL-6 and MPO were significantly elevated at 20 h after CLP. BRL-44408 maleate treatment significantly reduced serum levels of pro-inflammatory cytokines, chemokines, liver enzymes and lactate, and dramatically decreased TNF- α , IL-6 and MPO levels in the gut. Moreover, BRL-44408 maleate at the doses of 2.5 or 5.0 mg/kg BW significantly increased the survival rate. In conclusion, modulation of the sympathetic nervous system by blocking α_{2A} -AR appears to be a novel treatment for sepsis.

P175

PPAR δ REGULATES SYSTEMIC INFLAMMATION IN POLYMICROBIAL SEPSIS IN RODENTS.

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The nuclear receptor PPAR δ is an important regulator of lipid metabolism. Recent *in vitro* studies have shown that PPAR δ exerts potent anti-inflammatory effects. Here, we investigated whether PPAR δ modulates the inflammatory response during polymicrobial sepsis *in vivo*. In a pharmacological study, male Wistar rats were subjected to cecal ligation and puncture (CLP). Treatment with GW07472, a specific PPAR δ ligand (1 mg/kg i.p.) reduced tissue injury and neutrophil infiltration (as evaluated by myeloperoxidase) in lung, liver and cecum (358 \pm 56, 0.96 \pm 0.55 and 223 \pm 37 U/100 mg tissue, respectively) when compared with vehicle treated rats (478 \pm 63, 4.31 \pm 1.23 and 365 \pm 78 U/100 mg tissue, p<0.05). Treatment with GW07472 also reduced elevation of plasma levels of TNF α , IL-6, IL-1 β , leptin and MCP-1 (as evaluated by multiplex array) when compared with vehicle treatment. In comparative experiments, heterozygous PPAR δ -deficient (PPAR $\delta^{+/-}$) mice suffered extensive lung injury and exaggerated lethality (69% within 24 h) when compared to wild-type animals subjected to CLP (22% lethality, p<0.05). PPAR $\delta^{+/-}$ mice also exhibited high plasma levels of KC chemokine (9878 \pm 786 pg/ml) when compared to wild-type mice (5638 \pm 2243 pg/ml, p<0.05). The increased inflammatory response in PPAR $\delta^{+/-}$ mice was associated with elevated DNA binding of NF- κ B in the lung when compared to wild-type animals (2.2 \pm 0.7 versus 1.4 \pm 0.1 fold increase p<0.05). Therefore, our data suggest that PPAR δ is a crucial anti-inflammatory nuclear regulator and may serve as a therapeutic target for the treatment of sepsis. (Supported by NIH R01GM067202).

P176

PROTECTIVE EFFECTS OF MITOCHONDRIA-TARGETED VITAMIN E IN PNEUMONIA-RELATED SEPSIS. Q. S. Zang, D. Maass, H. Sadek, N. Williams, and D. Frantz. UTSW, TX 75390-9160

Objective: This study examined if increasing oxidative defense specifically in mitochondria protect heart from sepsis-related injuries. **Methods:** Mitochondria-targeted vitamin E (Mito-Vit-E) was synthesized according to published methods. Mito-Vit-E (25 mg/kg/day by oral gavage) was given to SD rats for the 7 days prior to septic challenge by *S. pneumoniae* (vehicle for shams). Blood and heart tissue were collected 24 hours after the infection. Cardiac mitochondrial damage was evaluated in the heart tissue. TNF- α levels were measured in the blood. Drug accumulation in the heart was quantified by mass spectrometry. In parallel, cardiac function was analyzed in Mito-Vit-E or vehicle treated rats by echocardiography before and after (5 days) the septic challenge. **Results:** In the rat pneumonia-related sepsis model, pretreatment with Mito-Vit-E suppressed circulating TNF- α production and protected mitochondrial integrity in the heart. Furthermore, this treatment provided cardiac protection after septic challenge. *In vivo* drug accumulation was also confirmed. **Conclusion:** Mitochondrial oxidants contribute to inflammatory response and cardiac dysfunction in sepsis. Mitochondria-targeted antioxidants may provide positive outcomes for severe sepsis. **Supported by NIH 5 P50 GM21681-42 and Surgery Departmental Funding.**

	Vehicle		Mito-Vit-E	
	Sham	Sepsis	Sham	Sepsis
TNF- α (pg/ml)	3 \pm 1	17 \pm 2*	2 \pm 1	5 \pm 2*¶
Mitochondrial membrane damage	20 \pm 5 %	40 \pm 8%*	18 \pm 2 %	25 \pm 6 % ¶
Cardiac Function (left ventricular fractional shortening)				
Vehicle		Mito-Vit-E		
Before Sepsis	After Sepsis	Before Sepsis	After Sepsis	
94 \pm 1 %	81 \pm 4 %*	94 \pm 1 %	93 \pm 1 % ¶	

*indicates significant difference between sham and sepsis groups. ¶ indicates significant difference between vehicle treated and drug treated groups.

P177

RHAPC IMPROVES CEREBRAL MICROCIRCULATION IN OVINE ACUTE LUNG INJURY AND SEPTIC SHOCK.

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Objective: Aim was to investigate recombinant human activated protein C (rhAPC) on cerebral microvascular blood flow (CMBF) in ovine septic shock (1). **Methods:** Fifteen sheep were chronically instrumented, and randomly allocated to either the sham, control or treatment group (n=5 each). ALI and septic shock was produced in control and treatment groups according to an established protocol (1). Shams received the vehicle. Sheep were ventilated (FiO₂ 1.0) for 24h and rhAPC (24µg/kg/h) was given i.v., starting 1 h post injury in the treatment group (1). Animals were fluid resuscitated, and colored microspheres were injected at baseline (BL) and 24h. Brain tissues were obtained during necropsy. Statistics: two-way ANOVA and Student-Newman-Keuls test. Data: Mean±SEM (P<0.05). **Results:** Cardiovascular variables and overall CMBF (mL/ min/g tissue) remained stable in sham animals. In control group, cardiac index (CI in L/min/m²) increased significant-ly after 24h vs. BL (BL: 4.9 ±0.4 vs. 24h: 7.8±0.4), associ-ated with a significant drop in systemic vascular resistance index (SVRI in dynes/cm⁵/m², BL: 1440±69 vs. 24h: 535 ±52, each p<0.05). RhAPC stabilized CI (BL: 5.0±0.3 vs. 24h: 6.1±0.3) and SVRI (BL: 1450 ±79 vs. 24h: 925±80, p<0.05 each). After 24 hours, in control sheep, the CMBF significantly increased over time in Cerebellum (190±25%), Thalamus (150±27%), and Pons (170±20% of BL) respectively, and was significantly attenuated for rhAPC treated animals in Cerebellum (120±25%), Thalamus (110±10%), and Pons (105±11% of BL), each p<0.05. **Conclusion:** RhAPC significantly improved hemodynamic variables and stabilized cerebral microvascular blood flow in this model, adding to the multiple useful effects of rhAPC in the treatment of septic shock. **Reference:** (1) Maybauer MO et al., Crit. Care Med. 2006; 34(9):2432-38

P178

CEFTAZIDIME REDUCES CARDIAC 3-NITROTYROSINE AND MALONDIALDEHYDE LEVELS IN OVINE ARDS AND SEPTIC SHOCK.

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Objective: We have recently shown that ceftazidime (CEF) improved cardiopulmonary performance in ovine septic shock (1) and aimed to further investigate these effects measuring cellular enzymes involved with the peroxynitrite pathway. **Methods:** Fifteen sheep were chronically instrumented, and randomly allocated to either the sham, control or CEF group (n=6 each). ALI and septic shock was produced in control and CEF groups according to an established protocol (1). Shams received the vehicle. Sheep were ventilated (FiO₂ 1.0) for 24h and CEF was given as 3g IV bolus at 1h and 13h post injury in the CEF group (1). Heart tissue was obtained during necropsy and analyzed using ELISA. Statistics: two-way ANOVA and Student-Newman-Keuls test. Data: Mean±SEM (*P<0.05). **Results:** After 24h 3-NT levels (nM/mL/mg) were 19±3 in sham and significantly increased in the control group (101±11*). The CEF group (25±15*) showed significantly lower 3-NT tissue levels than controls. MDA levels (mcM/mL/mg) were 48±5 in sham and significantly increased in the control group (85±3*). The CEF group (60±10*) showed significantly lower MDA tissue levels than controls. The MPO activity (mU/mg) showed no differences between groups, sham (180±11), control (200±15), and rhAPC (185±20), respectively. **Conclusion:** Ceftazidime has no influence on cardiac MPO levels, but significantly reduced heart tissue 3-NT and MDA levels in ovine ARDS and septic shock, thereby improved cardiac performance. These findings may lead to further investigations of ceftazidime and cardiovascular function.

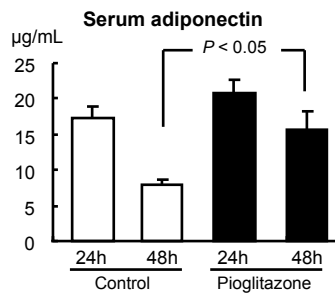
Reference: (1) Maybauer MO et al., Intensive Care Med. 2007 Jul;33(7):1219-27

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PPAR γ AGONIST ATTENUATES INFLAMMATORY RESPONSE THROUGH THE ACTIVATION OF ADIPOCYTE IN MICE POLYMICROBIAL SEPSIS

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Objective: To clarify the association between the salutary effect of peroxisome proliferator-activated receptor (PPAR) γ agonist and the alteration of adiponectin production during polymicrobial sepsis. **Methods:** Male C57BL/6 mice (8 weeks old) were subjected to sepsis by cecal ligation and puncture (CLP). The mice were divided into two groups. Pioglitazone group was administrated PPAR- γ agonist (pioglitazone) 10mg/kg/day i.p. for 7 days before CLP, and control group was not. Serum adiponectin levels were measured by ELISA, and mRNA levels of IL-6 on visceral fat were assessed by real-time PCR on 24 and 48 h after CLP. **Results:** The administration of pioglitazone significantly improved survival rate after CLP compared to control group. Serum adiponectin levels of pioglitazone group on 48 h after CLP significantly increased compared with those of control group. The IL-6 mRNA levels of control group significantly increased on 24 h after CLP, but not those of pioglitazone group. **Conclusions:** These data suggest that pioglitazone administration on polymicrobial sepsis improves survival rate and might attenuate the inflammatory response through the activation of adipocyte function. (supported by Grant for Japanese Scientific Research (C) 19591518)



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THE CANNABINOID RECEPTOR 2 AGONIST, GP1A, ENHANCES THE HOST RESPONSE TO SEPSIS.

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Previously, we have shown that septic mice deficient in the cannabinoid receptor 2 (CB2R) exhibited increased mortality, tissue damage, and neutrophil dysfunction. Here, we hypothesized that during sepsis, CB2R gain-of-function would improve the murine response to sepsis. To induce sepsis, mice were subjected to a cecal ligation and puncture (CLP) using a 23-gauge needle (single punch) followed by an 80% ligation of the cecum. Prior to and during the first 24 hours of sepsis, the CB2R is expressed on peritoneal leukocytes. Further, ligation of the CB2R is pro-inflammatory, as evidenced by increased TNF- α production by peritoneal neutrophils. Consistent with a novel role for CB2R in sepsis, CB2R-agonist treatment in wild-type mice increased the mean survival time in response to CLP. Treatment with CB2R-agonist decreased serum IL-6 levels, bacteremia, and damage to the lungs as compared to vehicle-treated mice. Finally, the CB2R agonist decreased neutrophil recruitment, while increasing neutrophil activation, function and p38 activation at the site of infection compared to vehicle-treated mice. Altogether, we show that the CB2R plays a novel role in neutrophil recruitment and function, thereby acting upon a major regulatory pathway of mortality in sepsis. Therefore, the utilization of CB2R agonists represents a novel therapeutic strategy.

Support: The project described was supported by Award Number R01GM072760 (CCC) from the National Institute of General Medical Sciences.

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THE ROLE OF INTERFERON GAMMA (IFN γ) IN THE PATHOGENESIS OF CLP-INDUCED SEPTIC SHOCK. E. Sherwood, C. Romero, A. Etogo, J. Nunez, R. Mamoudizad. University of Texas Medical Branch, Galveston, TX

Introduction: IFN γ is known to be a mediator of endotoxin-induced shock. However, the importance of IFN γ in clinically relevant models of sepsis, such as CLP, is not well defined. **Objective:** To examine the pathobiology of IFN γ during CLP-induced septic shock. **Experimental design and methods:** Wild type (WT) and IFN γ -deficient (IFN γ KO) mice were subjected to CLP. Survival, bacterial clearance and inflammation were evaluated. Further studies examined cellular/tissue sources of IFN γ and the pro-inflammatory functions of myeloid/lymphoid cells in WT and IFN γ KO mice. **Results:** Post-CLP survival was significantly ($p < 0.05$) higher in IFN γ KO mice compared to wild type mice (50% vs 0%). Bacterial count were not significantly different between groups but plasma MIP-2 (1219 vs 2360), IL-6 (611 vs 2655) and IFN γ (26 vs 0) concentrations (pg/ml) were significantly lower in IFN γ KO mice. IFN γ mRNA expression was not detectable after CLP in heart and lung from WT mice and was weakly expressed in spleen and liver. Intracellular IFN γ was not detectable in splenocytes after CLP but was produced by a large proportion of peritoneal leukocytes. Analysis of intraperitoneal leukocytes showed that IFN γ was produced by NK (NK1.1⁺CD3⁻) cells and 2 populations of myeloid cells (F4-80⁺Gr-1^{lo}Ly6G⁻Ly6C⁺ and F4-80⁺Gr-1^{hi}Ly6G⁺Ly6C⁺). The myeloid cell phenotypes correspond with inflammatory monocytes and immature myeloid cells. Surprisingly, increased numbers of NK cells were recruited in the peritoneal cavity of IFN γ KO mice compared to wild type controls. However, peritoneal NK cells from IFN γ KO mice expressed lower levels of the activation marker CD69. **Conclusions:** IFN γ facilitates systemic inflammation during CLP-induced septic shock. The IFN γ -mediated inflammatory response appears to arise from intraperitoneal interactions between NK and myeloid cells.

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GENETIC ABLATION OF NADPH OXIDASE ATTENUATES SEPSIS INDUCED INFLAMMATION AND MORTALITY IN NRF2-DISRUPTED MICE. R. Thimmulappa*, X. Kong* and S. Biswal. Johns Hopkins University, Baltimore, MD.

Rationale: Nrf2 is a primary transcription factor that regulates cellular antioxidant defenses. We previously reported Nrf2-disruption enhanced mortality in response to polymicrobial sepsis and endotoxin shock due to excessive systemic inflammation. In vivo and in vitro studies demonstrated that lethal systemic inflammatory response was due to augmented innate immune-inflammatory response in Nrf2-deficient mice mediated by excessive reactive oxygen species (ROS) generation. The present study was designed to investigate (a) if ablation of NADPH oxidase, major source of ROS, limits sepsis induced lethal systemic inflammation and mortality in Nrf2-deficient mice; (b) role of cellular antioxidant defenses in regulating NADPH oxidase dependent TLR4 signaling and sepsis immunopathogenesis. **Method:** (A) Wild type (Nrf2+/+), Nrf2-deficient (Nrf2-/-), Gp91phox-deficient (gp91phox-/-) and Nrf2 and gp91phox double knockout mice (Nrf2-//gp91phox-/-) mice were subjected to CLP. Blood bacteremia, serum cytokines and mortality were assessed. TLR4 signaling was investigated in macrophages. **Results:** Ablation of NADPH oxidase component, gp91phox in Nrf2-/- mice significantly improved survival. Blood bacteremia and serum IL-6 were significantly low in Nrf2-//gp91phox-/- compared to Nrf2-/- mice. Compare to Nrf2-/-, macrophages from Nrf2-//gp91phox-/- showed significantly reduced ROS levels, surface trafficking of TLR4 and recruitment of MYD88 and TRIF, phosphorylation of IKB and IRF3 and expression of cytokine in response to LPS stimulation. **Conclusion:** Redox homeostasis in innate immune cells is critical in sepsis pathogenesis. Nrf2 modulates immunopathogenesis of sepsis by redox regulation of innate immune response. (Supported by NIGMS R01GM079239)

P183

HEPATOCYTE-SPECIFIC DELETION OF NRF2 AUGMENTS SEPSIS INDUCED MORTALITY.

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Rationale: Nrf2 is a primary transcription factor that regulates stress response transcriptional program that includes antioxidant defenses (GSH biosynthesizing enzymes, NQO1, HO-1), xenobiotic detoxification enzymes (GSTs), chaperones, and proteasomal pathway. Deletion of Nrf2 enhances susceptibility to immune- and prooxidant-mediated acute liver injury. Previously, we demonstrated that global disruption of Nrf2 in mice enhances sepsis-induced mortality. Present study was designed to determine the role of Nrf2-dependent stress response program in liver in mediating sepsis pathogenesis. **Methods:** Mice with conditional deletion of Nrf2 in hepatocytes (Alb-Nrf2^{-/-}) and Nrf2 flox/flox was subjected to CLP. Mortality, liver injury and systemic inflammation were assessed. Liver antioxidants and reactive oxygen species (ROS) were measured. **Results:** CLP induced greater mortality in Alb-Nrf2^{-/-} mice compared to Nrf2 flox/flox mice. Serum markers of liver injury (ALT), and cytokines (IL-6 and HMGB1) were significantly higher in Alb-Nrf2^{-/-} mice compared to Nrf2 flox/flox. Antioxidant defenses were significantly lower while ROS levels were higher in Alb-Nrf2^{-/-} mice compared to Nrf2 flox/flox mice. **Conclusion:** Nrf2-dependent stress response in hepatocytes is critical in modulating sepsis pathogenesis. (Supported by NIGMS R01GM079239)

P184

EFFECT OF SERUM AND FIBRONECTIN-BINDING PROTEINS ON *STAPHYLOCOCCUS AUREUS* ADHERENCE TO SILASTIC CATHETERS.

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Infection of intravascular catheters is a major source of morbidity and mortality in critically ill patients, and *S. aureus* is a frequent pathogen. The mechanisms by which microbes adhere to catheters, and persist, remain poorly defined. *S. aureus* fibronectin-binding proteins are one mechanism by which this organism may adhere to cells or surfaces. We designed experiments to clarify if fibronectin-binding proteins played a role in *S. aureus* adherence to silastic catheters exposed to serum or heparin. Catheter segments were pretreated 15 min with Hank's Balanced Salt Solution (HBSS), fetal bovine serum (FBS), or heparin (1000 U/mL). Catheters were then flushed with HBSS and defined inocula (10^7 /mL) of *S. aureus* 8325-4 (wild-type) or DU5883 (fibronectin-binding protein deficient) added for 15 min, and then flushed with HBSS. Catheter segments (1 cm) were removed, sonicated, and supernatants were quantitatively cultured. Data were analyzed by ANOVA with Fisher's post hoc, and $P < 0.05$ was significant. FBS-treated catheters had fewer ($p < 0.05$) numbers (avg \pm SE \log_{10} /cm) of adherent *S. aureus* 8325-4 (4.3 ± 0.1) versus catheters pretreated with HBSS (4.8 ± 0.1) or heparin (4.6 ± 0.1). FBS pretreated catheters had increased ($p < 0.05$) adherence of *S. aureus* DU5883 (5.1 ± 0.1) versus catheters pretreated with HBSS (4.9 ± 0.1) or heparin (5.0 ± 0.1). These data suggest that (a) fibronectin-binding proteins may play a role in *S. aureus* adherence to silastic catheters; (b) heparin may not affect *S. aureus* binding to silastic catheters, irrespective of the presence of bacterial fibronectin-binding proteins; and (c) in the presence of bacterial fibronectin-binding proteins, serum components may inhibit *S. aureus* adherence to silastic catheters. These unexpected findings provide a basis for future experiments designed to clarify the molecular mechanisms involved in *S. aureus* binding to silastic catheters.

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ACTIVATION OF ENDOTHELIAL INTRINSIC NF- κ B PATHWAY MEDIATES VASCULAR DYSFUNCTION IN ENDOTOXEMIC MICE. D. Song, X. Ye, S. Liu (E. Miller). Feinstein Institute for Medical Research, NHP, NY 11040.

Little is known about the causative contribution of endothelial intrinsic NF- κ B signaling to the pathogenesis of septic vascular dysfunction. This study defined the causal role of endothelial intrinsic NF- κ B in endotoxemic vascular dysfunction. Wild type (WT) and transgenic mice (TG) that conditionally overexpress a mutant I- κ B α selectively on endothelium were injected with saline (1 ml/kg, i.p.) or *E Coli* LPS (10 mg/kg, i.p.). Vasoconstrictor response to norepinephrine (NE, 30, 100 and 300 ng, bolus injection), vasodilator response to endothelium-dependent vasodilator, acetylcholine (Ach) and to endothelium-independent vasodilator, sodium nitroprusside (SNP, both at 1, 10 and 100 ng, bolus injection) were studied in isolated, physiological salt perfused mesenteric vascular bed. The increase in perfusion pressure elicited by the 3 doses of NE was 17 \pm 2, 28 \pm 3, and 46 \pm 3 mmHg for WT-con, 6 \pm 2, 11 \pm 2, and 26 \pm 3 mmHg for WT-LPS, 18 \pm 2, 29 \pm 2, and 44 \pm 3 mmHg for TG-con, and 18 \pm 2, 26 \pm 3, and 44 \pm 6 mmHg for TG-LPS group (P < 0.05 between WT-LPS and other 3 groups at all doses). The drop in perfusion pressure elicited by the 3 doses of Ach was 37 \pm 2, 47 \pm 2, and 56 \pm 2 mmHg for WT-con, 29 \pm 2, 34 \pm 2, and 41 \pm 2 mmHg for WT-LPS, 32 \pm 2, 44 \pm 3, and 55 \pm 3 mmHg for TG-con, and 33 \pm 2, 42 \pm 1, and 52 \pm 1 mmHg for TG-LPS group (P < 0.05 between WT-LPS and other 3 groups). Drop in perfusion pressure elicited by the 3 doses of SNP was comparable among the 4 groups of mice. Our data demonstrate that blockade of endothelial specific NF- κ B signaling alone is sufficient to restore vasoconstrictor response to NE and to prevent the impairment of endothelium-dependent vasodilator response to Ach in endotoxemic mice, indicating a pivotal role of endothelial intrinsic NF- κ B pathway in the pathogenesis of endotoxemic vascular dysfunction. (Supported by NIH R01GM063907).

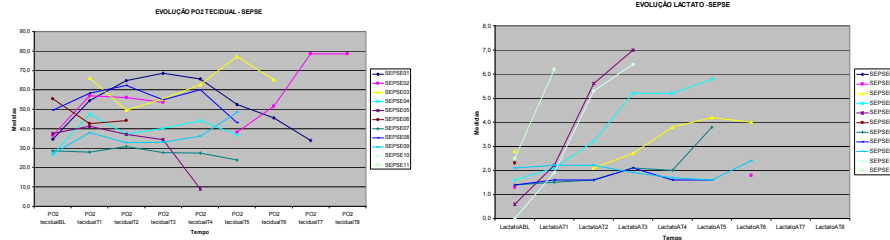
P186

LACTATE GENERATION IS NOT RELATED TO TISSUE PO₂ LEVELS IN SEPSIS

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Objectives: Analyze behavior of tissue pO₂ (tissue partial pressure of oxygen) measured in liver during sepsis and correlates its reduction with lactate levels. **Methods:** Eleven large white pigs, weight 35 Kg, in general anesthesia (Isoflurane, Fentanyl, Pancuronium), fully monitored (electrocardiography, etCO₂, invasive pressure, pulmonary artery catheter, portal vein Doppler ultrasound flow, small bowel tonometry) were submitted to fecal peritonitis sepsis (1g/Kg of feces plus 150 ml warm saline) after pO₂ and LDF (Laser Doppler Fluxometry) probes were placed inside liver parenchyma. Laboratory and hemodynamic data were registered hourly. After experiments, pigs were sacrificed with sedative overdose and KCl19,1% injection. **Results:** The model is well studied and very consistent. Hypotension occurs only in late phases (8th hour). Lactate generation seems occur earlier (1st hour) than tissue pO₂ levels reduction (4th hour), in septic pigs.



Conclusions/perspectives: Lactate generation not seems to be related only to tissue hypoxia in septic pigs. Inflammation and mitochondrial dysfunction probably may play a role in this pathological process. Further studies are needed to clarify these mechanisms. Maybe other interventions, not only oxygen uptake optimization, ought to be necessary to early reversal of septic cascade.

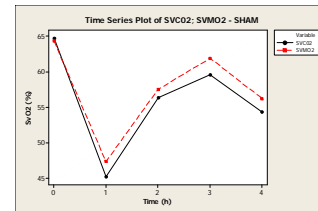
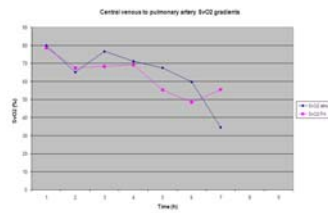
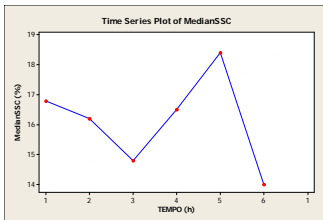
P187

DOES RIGHT ATRIUM TO MIXED VENOUS SvO₂ GRADIENTS MIRROR HEART OXYGEN UPTAKE?

A. Pereira*; P. Rehder*; C. Dias*; L. Figueiredo; G. Gutierrez; E. Silva. "Instituto do Coração-InCor" - Applied Physiology Laboratory from "Universidade de São Paulo-USP" - 05403-900 - São Paulo/SP – Brazil

Objectives: Analyze behavior of SvO₂ (venous oxygen saturation) measured in coronary sinus and correlates it with central to mixed venous SvO₂ gradients. **Methods:** 16 large white pigs, weight 35 Kg, in general anesthesia (Isoflurane, Fentanyl, Pancuronium), fully monitored (electrocardiography, etCO₂, invasive pressure, pulmonary artery catheter, portal vein Doppler ultrasound flow, small bowel tonometry) were studied. 15 were submitted to fecal peritonitis sepsis (1g/Kg of feces plus 150 ml warm saline) after fluoroscopy-guided coronary sinus catheterization and the last one was *sham*. Laboratory (blood samples collected from coronary sinus, right atrium, pulmonary artery) and hemodynamic data were registered hourly. After experiments, pigs were sacrificed with sedative overdose and KCl 19,1% injection. **Results:** Central to mixed venous SvO₂ curve distances vary along the time (h) - *graph.1* - more in septic pigs than in sham (*graph2*). Measurements of SvO₂ from coronary sinus reach extremely low values (*graph3*).

Conclusions: Absolute SvO₂ gradients variations along time, in sepsis, may be consequence of coronary sinus contribution, considering its extremely low values observed. Further studies should explore if these gradients variations may be an indicator of myocardial oxygenation status.



P188

C5A ABNORMALITIES IN ED PATIENTS WITH SEVERE SEPSIS. D. Bracho*, A. Jones, J. Younger, U Michigan, Ann Arbor, MI 48109.

C5a dysregulation during rodent sepsis worsens organ dysfunction and mortality, but has been measured in very few septic humans. METHODS: We prospectively studied 20 emergency department (ED) patients presenting with severe sepsis (2+ SIRS criteria and hypoperfusion or lactate > 4 mM) and 10 healthy adults for serum C5a abnormalities. C3, C3a, C5a, C4d (a marker of MBL/classical pathway activation) and Bb (a marker of alternative pathway activation) were measured at ED presentation and after 24 hours of goal directed therapy. RESULTS: C3 levels were depressed in septic patients (0.8-fold the value for volunteers); all other mediators were increased – C3a (11.6x), C5a(1.8x), Bb(6.1x), and C4d (3.5x) – compared to healthy controls ($p < 0.01$ for each). Although both activation pathways were statistically associated with elevated C5a levels, the alternative pathway was most strongly correlated (0.8 ng/ml C5a per 1 ug/ml Factor B, $r^2 = 0.24$, $p < 0.05$). Remarkably, neither C5a nor any of the other complement mediators were significantly improved 24 hours after the onset of goal directed therapy with crystalloid, blood, pressors, mechanical ventilation, and antibiotics. Additionally, C5a levels correlated inversely with initial and 24-hr SOFA illness severity score (-1.0 ng/ml per SOFA point, $p < 0.05$). A trend towards negative correlation to blood lactate concentration was seen but was not statistically significant. CONCLUSIONS: C5a is significantly increased at the time of ED presentation in outpatients with severe sepsis. Activation occurs through both pathways, although the alternative pathway may dominate. Evidence suggests C5a abnormalities are not easily correlated to illness severity, and 24 hours of aggressive, contemporary resuscitation does not reverse these abnormalities.

P189

A REAL-TIME ASSAY FOR IDENTIFYING COMPLEMENT-MEDIATED BACTERIAL KILLING DEFECTS IN SEPTIC PATIENTS. S. Yin*, C. Nypaver*, D. Bracho*, M. Lee*, D. Bortz*, A. Jones, J. Younger*, U Michigan, Ann Arbor, MI 48109.

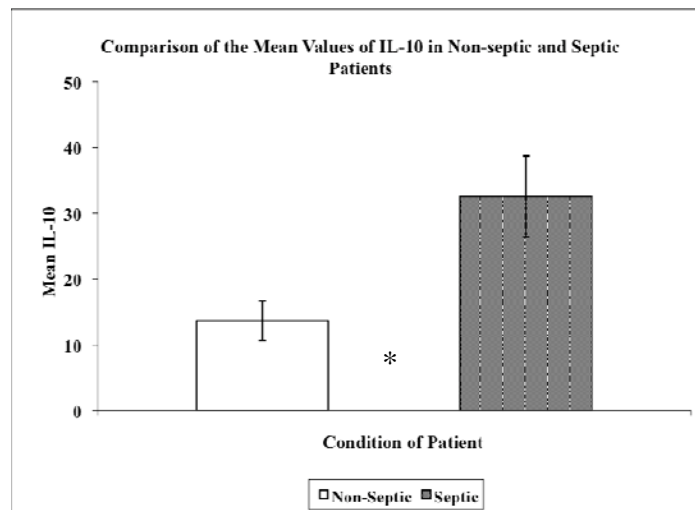
Functional measurement of complement activity in patients has traditionally been limited to erythrocyte lysis assays that, while convenient, only indirectly assess the key issue of bactericidal capacity. We developed a real-time assay of complement mediated killing using a luminescent strain of *K. pneumoniae*. METHODS: Luminescent *K. pneumoniae* was incubated with serum from 20 patients with severe sepsis and 10 healthy controls. Light output was measured every 8 minutes for 2 hours, and results were analyzed using a nonlinear statistical model incorporating estimates of the rate of complement killing and the delay between inoculation and the onset of effective complement-mediated attack. RESULTS: The assay was highly reproducible and was not affected by commonly used antibiotics except for levafloxacin. Heat inactivation completely abrogated loss of luminescence, suggesting complement specificity. Compared to healthy controls, patients with severe sepsis had, on average, slightly faster but not statistically different killing rates ($4.8 \times 10^{-3} \pm 1.5 \times 10^{-3} \text{ sec}^{-1}$ in controls versus $9.6 \times 10^{-3} \pm 2.7 \times 10^{-3} \text{ sec}^{-1}$ in septic patients, 95% CI for difference = -1.3×10^{-3} , 1.06×10^{-2}). However, septic patients demonstrated a substantial delay in the onset of killing (5.5 ± 1.8 minutes in healthy controls versus 13.8 ± 2.7 minutes in septic patients, $p < 0.01$). Further analysis indicated that within septic patients, subsets with greater than normal and worse than normal bactericidal activity existed. CONCLUSIONS: We report a new assay for a clinically relevant functional assessment of complement activity, which demonstrates a novel defect in the time to effective complement activation in patients with severe sepsis.

P190

ELEVATED ADMISSION IL-10 LEVEL IS ASSOCIATED WITH DEVELOPMENT OF SEPSIS IN CRITICALLY ILL ADULTS M. Ariefdjohan*, K. Queensland*, L. Weitzel*, D. Heyland*, P. Wischmeyer University of Colorado, Denver, 80045

BACKGROUND: Elevated IL-10 levels at ICU admission are associated with the development of sepsis in critically ill neonates and trauma patients. The potential relationship of an elevated admission IL-10 and the subsequent occurrence of sepsis in a general adult ICU population is unclear. **METHODS:** 123 patients had blood collected for IL-10 on day 1 of ICU admission. Patients were from a non-selected subgroup obtained from a total of 597 patients enrolled in a prospective observational ICU trial. In our subgroup, 78 (63%) patients were diagnosed with sepsis (defined as SIRS + infection) during ICU stay. Subgroup patients mortality was 25% and all were mechanically ventilated at admission. IL-10 measured via Mesoscale. **RESULTS:** IL-10 levels on ICU day 1 were significantly elevated in patients who ultimately developed sepsis versus patients not diagnosed with sepsis (*-p=0.0067 via t-test).

CONCLUSIONS: Elevated IL-10 levels at admission to the ICU may have diagnostic value in predicting the development of sepsis in a general ICU population.



P191

PROGENITOR CELL THERAPY FOR TRAUMATIC BRAIN INJURY: EFFECT OF SERUM OSMOLARITY ON VIABILITY AND CYTOKINE PRODUCTION.

P. Walker,* F. Jimenez,* C. Cox. University of Texas Medical School-Houston, Houston, TX 77030

Introduction: The potential translation of mesenchymal stem cell (MSC) therapy into a multimodal protocol for traumatic brain injury (TBI) requires evaluation of viability and cytokine production in a hyperosmolar environment. Optimization of MSC therapy requires delivery to the target area without significant loss of cellular function or viability. No model evaluating the potential efficacy of MSC therapy at varying osmolarities currently exists. **Methods:** Rat MSCs were characterized with flow cytometric immunophenotyping. The osmolarity of growth media was measured and adjusted by the addition of sodium chloride or distilled water. MSCs were cultured in media of different osmolarities for 24 hours with and without 20% brain supernatant from rats 6 hours after cortical injury. Viability and pro inflammatory cytokine levels were measured. **Results:** MSCs showed no difference in viability at 24 hours.

Osmolarity	250	270	290	310	330	350	370
Viability (%)	92.1	91.6	90.0	91.5	92.7	93.2	93.9

MSCs cultured in 20% brain supernatant showed no significant difference in proinflammatory cytokine production.

Osmolarity	Cytokine production (picograms)			
	IL - 1 α	IL - 1 β	IL - 6	TNF α
250	103.0	382.4	328.4	12.6
270	103.2	404.9	344.4	12.6
290	130.7	454.4	324.4	13.4
310	130.0	374.0	296.8	11.6
330	115.0	399.8	286.4	11.4
350	117.0	398.2	296.4	10.8
370	102.0	378.9	315.2	11.0

Conclusion: Progenitor cell therapy for TBI may require survival and activity in a hyperosmolar environment. Culture of MSCs at such conditions shows no significant effect on cell viability or cytokine production, indicating cellular tolerance of varying osmolarities.

P192

ROLE OF SDF-1 IN HOMING OF PROGENITOR CELLS TO INJURED TISSUE.

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Background: Previously we have shown that following trauma, hematopoietic progenitor cells (HPCs) home to injured tissue. Stromal cell-derived factor-1 (SDF-1) is a chemokine known to play an important role in the mobilization and homing of HPCs from bone marrow (BM) to the periphery. This study examines the role of SDF-1 in HPC mobilization and homing following traumatic injury. **Methods:** Male Sprague-Dawley rats were subjected to unilateral lung contusion (LC) or no intervention (control). Following contusion, peripheral blood (PB), BM and lung tissue were harvested. SDF-1 was measured from supernatant in duplicate samples by ELISA. In addition, lung tissue was harvested for growth of HPCs (BFU-E, CFU-E, and CFU-GEMM). **Results:** Following lung contusion, there was a significant rise in SDF-1 levels in lung tissue and PB as compared to control (table). Conversely, SDF-1 levels in BM were found to drop after LC. Presence of HPCs in the injured lung was evident by growth of colonies in cultures (BFU-E: 23±6, CFU-E: 33±10, CFU-GEMM: 25±7), compared to no colonies in control group.

Sample	control (pg/ml)	LC (pg/ml)	P-value
Lung	480 ±40	650 ±110	0.02*
PB	950 ±110	1240 ± 210	0.04*
BM	2820 ± 230	1990 ± 870	0.35

SDF-1 levels are expressed as mean ± SD.

*p values <0.05 by Mann Whitney test (n=4-6/group)

Conclusion: Following lung contusion, there is significant mobilization of HPCs to site of injury. Also SDF-1 levels are increased in both the contused lung and peripheral blood but not the bone marrow. This implies that SDF-1 appears to be a mediator in the mobilization and homing of HPCs to injured tissue.

P193

HUMAN MESENCHYMAL STEM CELLS INHIBIT VASCULAR PERMEABILITY THROUGH CONTACT WITH ENDOTHELIUM.

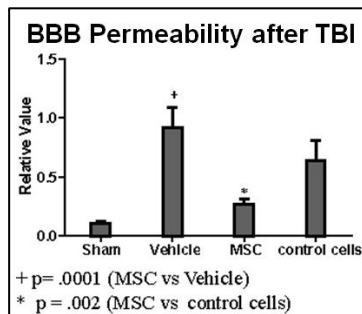
S. Pati*, M. Harting*, F. Jimenez*, C. Cox*, M. Gerber*, M. Reitz*, P. Dash* and J. Holcomb. University of Texas Health Sciences Center-Houston, TX, 77030

Stem cell therapy using mesenchymal stem cells (MSCs) has shown promise in the treatment of several disorders characterized by vascular instability including stroke, MI, limb ischemia, and traumatic brain injury (TBI). The mechanism of action of MSCs is largely unknown. **Hypothesis:** IV MSC administration has stabilizing effects on the endothelium that counteracts the effects of traumatic injury on vascular permeability

Methods: *In vitro*, MSCs were co-cultured with HUVECs (ECs) and then separated by MACS cell sorting. EC's were seeded on to collagen coated transwells and EC monolayers were allowed to form. VEGFA (10 ng/ml) was used to induce permeability to 40kd Dextran. *In vivo*, permeability was studied using an established mouse model (CCI) of traumatic brain injury (TBI). 2×10^6 MSCs were administered IV after TBI. Blood-brain-barrier (BBB) permeability to Evans Blue dye was assessed.

Results: *In vitro*, MSCs-EC co-culture inhibited endothelial permeability of monolayers by 30% ($p < .05$ all studies). This effect was dependent on co-culture with contact between the 2 cell types. *In vivo*, (See **Figure**) IV MSCs inhibited BBB permeability by 65% $p < .05$ $n = 7$ animals. Control cells (HUVECs) administered IV did not have a protective effect on BBB permeability after TBI.

Conclusion: IV MSCs in vitro and in vivo after vitro studies suggest that by contact between ECs



inhibit vascular permeability traumatic brain injury. In these effects are mediated and MSCs.

P194

MOLECULAR MECHANISMS BY WHICH THE ANGIOTENSION II-RECEPTOR BLOCKER, LOSARTAN, IMPROVES INSULIN SIGNALING IN THE LIVER FOLLOWING TRAUMA AND HEMORRHAGE. L. Li*, L. Zhao* and J. Messina. Department of Pathology, University of Alabama at Birmingham, Birmingham, AL, 35294.

Hyperglycemia and insulin resistance develop rapidly following injury, hemorrhage, infection and critical illness. However, the mechanisms of this acute insulin resistant state are not well understood. Although blocking the renin-angiotensin system has been shown to chronically improve insulin sensitivity and decrease the risk for type 2 diabetes, whether and how the renin-angiotensin system contributes to the development of acute insulin resistance is not known. In the current study we found that losartan, an antagonist of angiotension II type 1 receptors, can rescue the impaired hepatic insulin signaling in hemorrhaged male rats. To explore potential mechanisms for the improvement of acute insulin resistance by losartan, the effects of losartan on the generation of reactive oxygen species (ROS) was investigated since ROS have been implicated to play a role in chronic insulin resistance. Two components of ROS, superoxide and hydrogen peroxide, were measured and shown to be significantly increased following trauma and hemorrhage. However, their levels were dramatically reduced in liver by pretreatment of rats with losartan prior to hemorrhage. The involvement of ROS in the development of acute hepatic insulin resistance was further demonstrated by the administration of N-Acetyl cysteine (NAC), an antioxidant agent. NAC pretreatment improved hepatic insulin signaling following trauma and hemorrhage. Co-administration of losartan and NAC showed additive effects in reducing the levels of ROS. Moreover, the combination of losartan and NAC further improved hepatic insulin signaling following trauma and hemorrhage. Our study indicates that ROS contribute to the development of acute hepatic insulin resistance following hemorrhage, and the reversal of acute hepatic insulin resistance by losartan is, at least in part, to suppress the generation of ROS.

P195

ANALYSIS OF INTESTINAL SMOOTH MUSCLE GENES INDUCED BY INTESTINAL EDEMA FOR COMMON REGULATORY ELEMENTS.

K. Uray*, C. Cox, G. Laine*, Department of Surgery, University of Texas Medical School at Houston, Houston, TX 77030

Intestinal interstitial edema that often develops after fluid resuscitation in trauma patients has been shown to decrease intestinal contractile activity; however, the signaling pathways involved in edema induced intestinal dysfunction are poorly understood. The purpose of this study was to understand the multi-factorial mechanisms leading to edema-induced intestinal dysfunction, by way of gene expression analysis with subsequent examination for common function specific regulatory elements. Intestinal edema was induced with a combination of resuscitation fluid administration and mesenteric venous hypertension (RESUS+VH). Control animals were subjected to laparotomy only (CONT). The combination of RESUS+VH induced significant tissue fluid accumulation (3.51 ± 0.03 vs. 4.46 ± 0.24 , $p < 0.01$), indicating intestinal edema development compared to CONTROL. Analysis of the microarray data showed the combination of RESUS+VH altered (>2-fold change) the expression of 752 genes compared to CONTROL. NF-kappaB transcription factors were found to be the most important binding elements in the RESUS+VH group according to both oPOSSUM and DiRE analysis. Subsequent analysis of intestinal smooth muscle tissue showed significantly increased NF-kappaB activation in the RESUS+VH group compared to CONTROL (7.07 ± 0.33 vs. 10.72 ± 2.26 , $p < 0.05$). Inhibition of NF-kappaB with pyrrolidinedithiocarbamate (PDTC) attenuated edema-induced decreases in intestinal contractile dysfunction and myosin light chain phosphorylation. We conclude that NF-kappaB plays a role in the development of edema-induced intestinal contractile dysfunction.

P197

ANDROSTENEDIOL MODULATES SYSTEMIC CHEMOKINE EXPRESSION IN A MURINE SHOCK/SEPSIS MODEL

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Objective: Sepsis and multiple organ failure are common complications following a traumatic insult. They are based, among others, on an overwhelming inflammatory reaction. Additionally, gender seems to play a role as women are more protected against posttraumatic complications than men. Sex steroid precursor such as androstenediol has shown to lower mortality in trauma models. Hence, we determined the influence of androstenediol on systemic chemokine levels in a shock and sepsis model. Methods: Volume-controlled hemorrhagic shock was induced with subsequent resuscitation after one hour in C57Bl/6 mice. Two days later, sepsis was induced by cecal ligation and puncture. One group was treated with androstenediol (1mg/kg) (n=10), a second group received the vehicle intralipid (n=10), once daily. Untreated animals receiving vehicle or either androstenediol served as controls (n=10). Four hours after sepsis induction, mice were exsanguinized and MCP-1, MCP-3 and MIP-1 β levels were determined in blood plasma. Neutrophil infiltration in lung tissue was determined by Ly6G staining. Results: Chemokine levels significantly rise in both treatment groups compared to the corresponding control group. In the androstenediol treated shock/sepsis group, levels of all three chemokines are significantly reduced compared to the vehicle treated shock/sepsis group. Likewise, neutrophil influx is slightly diminished after androstenediol treatment. Conclusions: Androstenediol attenuates chemokine levels and therefore immune cell attraction in this shock and sepsis model. Thus, androstenediol seems to be a potential therapeutic agent as these modulations might contribute to protection in traumatic and septic settings.

P198

ESTROGEN REDUCES GUT DYSFUNCTION AND MORTALITY FOLLOWING HEMORRHAGE AND ABDOMINAL AORTIC CROSS-CLAMPING. ZF Ba*, KI Bland*, IH Chaudry, Center for Surgical Research & Dept. of Surgery, Univ. of Alabama at Birmingham, AL 35294.

Major abdominal vessel injury and rupture of abdominal aortic aneurysm remain a major cause of mortality. Moreover, aortic cross-clamping (ACC) that may be required to repair the aorta shuts down blood flow to organs below the ACC site and increases additional morbidity. To reduce the post-ACC complications, a model of trauma-hemorrhagic shock (T-H) plus abdominal ACC (T-H-ACC) was developed. Male rats underwent midline laparotomy and were bled to and maintained at a BP of 40 mmHg until 40% of shed blood volume was returned in the form of Ringer's lactate. Rats were then resuscitated with 4x the shed blood volume with Ringer's lactate. A bolus of 17 β -estradiol (E2; 1 mg/kg) or vehicle was administered intravenously at the onset of resuscitation. The abdominal aorta was clamped above the celiac artery for 30 min at 15 min after the onset of resuscitation. The results indicate that T-H-ACC caused lower CO, higher plasma lactic acid and higher mortality compared to shams or T-H alone. Gut permeability increased markedly in this model of T-H-ACC and was accompanied with mucosal damage and lower intestinal blood flow. However, E2 administration prior to ACC clapping markedly improved CO, decreased lactic acid, attenuated gut permeability, improved mucosa and decreased the mortality rates compared to T-H-ACC receiving vehicle. These results indicate that E2 administration following T-H and prior to ACC prevents increased gut dysfunction and mortality under those conditions. Thus, E2 is a useful adjunct even in the model which requires ACC after T-H (NIH RO1 GM39519).

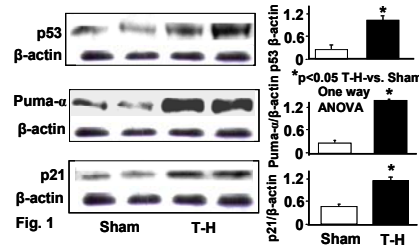
Groups	Mortality (%)	CO (mg/ml)	Lactic Acid (mg/ml)
Sham	0	27.5 \pm 2.6	8.5 \pm 1.0
T-H	32.4	15.4 \pm 1.7*	7.0 \pm 0.7
T-H-ACC	87.5	10.4 \pm 2.7*	67.0 \pm 4.2*
T-H-E2-ACC	25.0	15.5 \pm 0.6*#	22.6 \pm 0.7*#

P199

P53 MEDIATES TISSUE DAMAGE AFTER TRAUMA-HEMORRHAGE VIA MITOCHONDRIAL-DEPENDENT APOPTOTIC SIGNALING. F Moeinpour*, M Athar*, L Figueiredo, G Liu*, E Abraham*, KI Bland*, IH Chaudry. Departments of Surgery, Dermatology and Medicine, University of Alabama at Birmingham, AL 35294.

P53 plays a key role in eliciting cellular response to a variety of stress signals. Since its role following trauma-hemorrhage (T-H) remains poorly defined, we examined if p53 expression and its transcriptional activity target genes (p21 and puma-21) are altered after T-H. Male C57/BL6 mice underwent T-H (midline laparotomy, 60% blood loss blood pressure at 35 mmHg for 90 min), followed by fluid resuscitation with sacrifice 2 hr thereafter. T-H induced expression of p53, p21 and puma- α (n=4-6 mice/group) (Fig. 1). This was followed by apoptosis induction in tissues. T-H also induced p53 migration to the mitochondria altering its membrane potential that led to cytochrome c release in the cytoplasm and enhancement of apaf-1, caspase-3 and DNA fragmentation. Moreover,

anti-apoptotic Bcl-xL expression was reduced while pro-apoptotic Bad increased. Similar studies in mice treated with pifithrin- α (which inactivates the transcriptional activity of



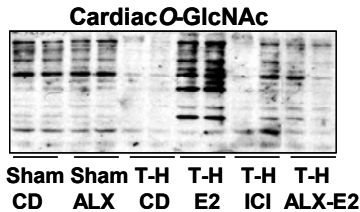
p53) showed that pifithrin- α protected against T-H-induced apoptosis/associated molecular events. In additional studies, p53^{-/-} null mice were used. Interestingly, these mice manifested resistance to T-H-induced tissue damage. We also examined if downstream events to p53 are important in affording protection against T-H-mediated molecular alterations underlying tissue damage. siRNA-mediated silencing of Bcl-xL manifested an increase; Bad silencing resulted in a decrease in caspase-3 and DNA fragmentation. Thus, p53 is a novel therapeutic target for diminishing T-H-induced early tissue injury. (NIH R01 GM37127)

P200

MECHANISM OF CARDIOPROTECTION BY ESTROGEN (E2) FOLLOWING TRAUMA-HEMORRHAGE (T-H): UPREGULATION OF CARDIAC O-GLYCOSALATION. S Yang^{1*}, S Hu^{1*}, L Zou^{2*}, R Raju^{1*}, JC Chatham², KI Bland^{1*}, RB Marchase^{2*}, and IH Chaudry¹.

¹Center for Surgical Research, Department of Surgery, ²Department of Cell Biology, University of Alabama at Birmingham, AL

Although E2 or glucosamine administration following T-H prevents T-H-induced cardiac dysfunction and cardioprotection by glucosamine increased cardiac N-acetylglucosamine (O-GlcNAc), it is unclear whether the E2-mediated cardioprotection is also due to the increase in cardiac O-GlcNAc. T-H was induced in male adult rats (mean BP 40 mmHg for 90 min) and the rats then received E2 (1 mg/kg, iv) or vehicle (cyclodextrin, CD, 20.7 mg/kg, iv) during resuscitation. In additional E2-treated T-H groups, the estrogen receptor (ER) antagonist ICI 182,780 (ICI, 3 mg/kg, ip) or O-GlcNAc transferase inhibitor alloxan (ALX, 25 mg/kg, iv) was administered 30 min before E2. Two hr after resuscitation, left ventricular (LV) performance was determined; heart tissue and plasma harvested, cardiomyocytes isolated, and cardiac nuclei extracted followed by cardiac mitochondrial isolation. Following T-H, LV performance decreased, cardiac O-GlcNAc and nuclear factor E2-related factor 2 (Nrf2) expression, mitochondrial ATP and cytochrome-c oxidase activity decreased. In contrast, cardiac ICAM-1, p-I κ B- α , NF- κ B expression, and myeloperoxidase (MPO) activity increased significantly. E2 restored LV performance, cardiac O-GlcNAc and Nrf2 expression, mitochondrial ATP level and cytochrome-c oxidase activity, and it prevented the increase in cardiac ICAM-1, p-I κ B- α , NF- κ B and MPO activity. T-H-induced decrease in cardiac ER expression was normalized by E2. Both ICI and ALX abrogated the salutary effects of E2 on the above parameters. Thus, the E2-mediated cardioprotection following T-H is due to upregulation of cardiac O-GlcNAc, which is ER-dependent. (supported by NIH R01 GM 39519 [IHC], R01 HL076165 [RBM])



P201

HEART RATE COMPLEXITY IS DECREASED DURING APNEA AND RESPIRATORY ACIDOSIS

Andriy Batchinsky, Ian Black, Corina Necsoiu, John Jones, Leopoldo Cancio. U.S. Army Institute of Surgical Research, 3400 Rawley E. Chambers, Fort Sam Houston, Tx, 78234.

Background: We previously showed that heart-rate complexity (HRC) is decreased following trauma/hemorrhage. We sought to determine the effects of prolonged apnea and respiratory acidosis on HRC. **Methods:** Swine (n=6) were anesthetized (propofol), intubated, paralyzed (pancuronium), and mechanically ventilated in volume-control mode at baseline. Then, tracheal gas insufflation with intermittent pressure release at 25 cm H₂O twice a minute was performed (apnea phase), followed by a return to baseline ventilation. Electrocardiogram (EKG) was recorded to a computer at 500 Hz at 4 timepoints: baseline (PRE), after 30 min of apnea (AP1), end of apnea at 120 min when pH=7.1 (AP2), and recovery (POST). HRC methods measuring irregularity and fractal organization of the signal, and heart-rate variability methods reflecting regular oscillations in the EKG, were used. Statistics by one way ANOVA with repeated measures. **Results:** See table.* p<0.05.

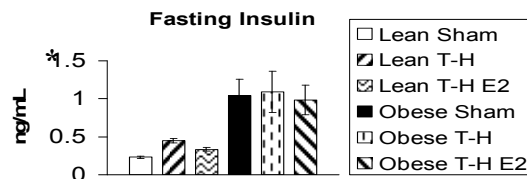
Measures/Timepoint	PRE	AP1	AP2	POST
Mean RRI, ms	523	505	438*	489
Sample Entropy, unitless	0.87	0.61	0.55*	1.08
Fractal Dimension by curve lengths	1.87	1.36*	1.43*	1.81
Bit-per-word Entropy	4.17	3.14*	3.13*	4.06
Signal Distribution Entropy	0.69	0.52*	0.52*	0.68
HFP, ms ²	27	0.14*	1.7*	3
CDM HFA, ms	5.1	0.6*	1.29*	2

Conclusions: Apnea and respiratory acidosis led to reversible decreases in multiple measures of HRC. Also, the power (HFP) and amplitude (HFA) of vagal input to the heart were decreased. Thus, decreased HRC is not specific for trauma/hemorrhage, but may also be useful for monitoring of patients at risk of respiratory arrest.

P202

TRAUMA-HEMORRHAGE (T-H) INDUCES ELEVATED INSULIN CONCENTRATIONS AND INSULIN RESISTANCE IN OBESE RATS. TC Hyatt*, S Yang*, KI Bland*, IH Chaudry. Center for Surgical Research and Department of Surgery, University of Alabama at Birmingham, AL 35294.

Both hemorrhagic shock and obesity lead to marked insulin resistance (IR). To determine if trauma/hemorrhagic shock has the same impact on insulin sensitivity in lean and obese animals, male rats were put on a very high fat diet (HFD; 60% kcal from fat) or a control diet (6% kcal from fat) for 6 weeks. Animals were subjected to laparotomy and bled to a mean blood pressure of 40 mmHg. They were then given cyclodextran (vehicle) or 17 β -estradiol (E2) (1 mg/kg), then resuscitated with 4x the shed blood volume with lactated Ringer's. Animals were sacrificed 2 h post-resuscitation. Plasma glucose and insulin were determined and the homeostasis model assessment for insulin resistance (HOMA-IR) used to determine whole-body IR. Preliminary results indicate that plasma glucose was not significantly different between groups ($p=0.053$) but fasting insulin was markedly different (see Figure; $p=0.006$). HOMA-IR calculations showed a significant difference between means ($p=0.006$)--obese animals (9.6 ± 2.7) were more insulin resistant than their lean counterparts (2.1 ± 0.2 ; $p=0.023$). These preliminary findings suggest that after only 6 weeks of HFD, obesity has a significant impact on IR, independent of T-H, and that T-H yields a similar trend among lean vs. obese. E2 treatment in both groups attenuated the increase in IR, although a higher dose may be necessary to achieve significant decreases in IR following T-H among obese. (NIH T32 GM063490)



* $p < 0.05$ vs lean

P203

SHOULD AGE BE A FACTOR FOR LEVEL I TRAUMA ACTIVATION.

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Background: Elderly trauma patients have a higher incidence of medical co-morbidities when compared to their younger cohorts. Currently, the minimally accepted criteria established by the Committee on Trauma (COT) for the highest level of trauma activation (level I) does not include age as a factor. Should patients older than the age of 60 with polytrauma and/or a significant mechanism of injury be considered as part of the criteria for level I activation? Would these patients benefit from a higher level of activation? Methods: A retrospective review of all level II trauma patients admitted to a Level II trauma center between July 1, 2006 and June, 30, 2008 was performed. Factors taken into consideration include: associated co-morbidities, mortality rate, transfusion requirements and injury severity score (ISS). Results: During this 2-year period, 1,028 patients who presented as a level 2 trauma activation were admitted to our institution. Eighty-eight percent were younger than 60 years old, and 12% were older than 60. We found a 2-fold increase in the requirement for blood transfusions in the older (age > 60) population. Additionally, the patients older than 60 demonstrated a 4-fold increase in morbidity and an 18-fold increase in mortality, when compared to the patients under the age of 60. This occurred despite no significant difference in mean ISS.

Age	Mean ISS	% Morbidity	% Mortality	% Transfusion
< 60	9.4	4.2	0.3	9.8
> 60	11.9	17.5	5.6	21.4

Conclusion: Our analysis shows that the patients older than the age of 60 have an increased risk for morbidity and mortality. Age > 60 should be a criterion for the highest level of trauma activation for patients with polytrauma and/or a significant mechanism of injury.

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INDUCTION OF ANTIMICROBIAL PEPTIDES IN BLOOD OF POLYTRAUMA PATIENTS

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Objective: Antimicrobial peptides (AMPs) hold a broad-spectrum activity against bacteria, fungi and viruses. Trauma is still one of the main reasons for death among the population worldwide. Should the seriously injured patient survive over the first days after trauma, there is a high risk of developing bacterial infection and sepsis. In this study we determined the expression and production of human cationic antimicrobial protein (LL-37) and human beta-defensin-3 (HBD-3) in serum samples from patients suffering from polytrauma.

Methods: The blood samples of polytrauma patients and a healthy control group was analysed Elisa. Statistical differences between the groups were evaluated using the t-test. The antibacterial activity was examined by agar diffusion testing.

Results: Compared to the healthy control group, serum of polytrauma patients showed significantly higher levels of AMP expression. The serum of polytrauma patients showed the highest antimicrobial activity of all tested samples.

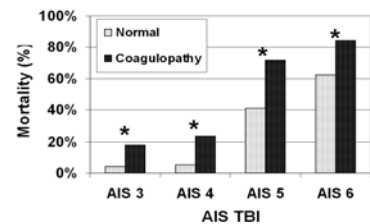
Discussion: Serum samples of polytrauma patients showed high expression of both tested AMPs, which may protect the severely injured patient from overwhelming bacterial infection. An evaluation of AMP production in serum samples states that cause a predisposition to infection of trauma patients will provide the means to initiate a new approach in management of the conditions. There is still a lack of experience in the clinical use of AMPs, and this important aspect should be addressed in future investigations.

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COAGULOPATHY IN ISOLATED TRAUMATIC BRAIN INJURY IS ASSOCIATED WITH IMPAIRED OUTCOME. A. Wafaisade*, R. Lefering*, E. Neugebauer, B. Bouillon*, M. Maegele*. Department of Traumatology, University of Witten-Herdecke, D-51109 Cologne, Germany.

Objective: To assess the frequency of acute post-traumatic coagulopathy in blunt traumatic brain injury (TBI) and whether its occurrence was associated with negative out-come. Methods: Retrospective analysis of DGU Trauma Registry datasets from 3114 patients with isolated blunt TBI using univariate (table) and multivariate analysis. Results: Incidence of coagulopathy in TBI upon emergency room (ER) admission was 22.7%. Its occurrence correlated significantly with injury severity (AIS TBI), GCS \leq 9, hypo-tension at site of accident and ER arrival, amount of i.v. fluids administered during the pre-hospital phase and age. The presence of coagulopathy was associated with higher frequencies of single (61 vs. 39%) and multiple organ failure (36 vs. 19%; both p<0.001). The mortality in patients with coagulopathy was 50.4 vs. 17.3% in non-coagulopathy patients (p<0.001) with an adjusted odds ratio of 2.97 (95% CI: 2.3 - 3.85; p<0.001) in multivariate analysis. Conclusion: Coagulopathy upon ER admission in patients with isolated blunt TBI is associated with increased morbidity and mortality.

	Coagulopathy n=706(22.7%)	No coagulopathy N=2408 (77.3%)	p-value
Mean age (years)	53 \pm 22	49 \pm 21	< 0.001
AIS TBI	4.5 \pm 0.7	4.2 \pm 0.7	< 0.001
AIS body	1.0 \pm 0.9	1.0 \pm 0.9	NS
GCS at scene	6.8 \pm 4.4	9.2 \pm 4.4	< 0.001
BP systol. (mmHg)	125 \pm 45	134 \pm 34	< 0.001
i.v. fluids pre-hospital (ml)	1197 \pm 1006	893 \pm 704	< 0.001



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REGULATION OF AROMATASE EXPRESSION IN ASTROCYTES FOLLOWING INJURY.

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Introduction: Traumatic brain injury (TBI) is a complicated, multi-phased disease process. Following injury, astrocytes are activated to protect various neuronal populations. As estrogen is a potent neuro-protectant, we propose that upregulation of aromatase expression within astrocytes should convert testosterone to beneficial estrogen, and this enzyme may be regulated differently by various insults (oxidative stress, glucose deprivation). **Objective:** To elucidate aromatase expression in response to different stressors in astrocytes, and its potential role in testosterone conversion to local estrogen production following brain injury. **Methods:** Rat glioma (C6) cells were incubated with 400 μM of H_2O_2 for up to 24 hours. C6 cells were then serum deprived for up to 48 hours. Changes in aromatase levels were detected using RT-PCR and Western analysis methods. Media testosterone levels were measured to determine if increased aromatase expression correlated with a decrease in testosterone levels. **Results:** Treatment of the C6 cells with H_2O_2 , resulted in a 40% decrease in aromatase RNA levels compared to the control group. In serum deprived C6 cells, there was a significant increase (~90%) in aromatase levels after 48 hours, corresponding to a 43% decrease in the levels of testosterone at 48 hours. **Conclusion:** Following injury of astrocytes with H_2O_2 , there is a decrease in aromatase expression and likely local estrogen production, which might render the brain more vulnerable to toxic insults. In contrast, serum/glucose deprivation led to a significant increase in aromatase production. These results will further our knowledge of aromatase and its role in local estrogen production. The vastly different responses based on injury types, as well as the genetic makeup of an individual patient, may help explain significant differences between the outcomes of clinically similar brain injuries.

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PREDICTORS OF MORTALITY FOR PENETRATING INJURIES EXCLUDING ISOLATED HEAD INJURY.

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Introduction: Many clinical trials have used admission systolic blood pressure (SBP) to determine inclusion criteria to identify shock. Inaccurate estimates of mortality in clinical trials have led to underpowered results. Our hypothesis was that measures of coagulopathy and shock upon admission such as International Normalized Ratio (INR), base deficit (BD), and hemoglobin (Hb) when added to SBP improve prediction of mortality. Methods: Patients with penetrating combat-related injuries who were transfused blood products were included. Patients with isolated severe traumatic brain injury (Head AIS ≥ 3) and those with any of the variables analyzed missing were excluded. Logistic regression with stepwise elimination was used with receiver operator curve (ROC) analysis for admission SBP, Hb, BD, and INR to determine their ability to predict in-hospital mortality. Results: There were 840 patients included with a median (IQR) age of 24 years (21-28), Injury severity score of 19 (11-29), and mortality of 90/840 (10.7%). The AUC for in-hospital mortality increased significantly ($p < 0.05$) as BD, and INR were included in the prediction model compared to models that did include only SBP and Hb (models 3-5 vs. 1-2).

Model	Admission Variables	AUC (95% CI)
1	SBP	0.61 (0.54-0.67)
2	SBP + Hb	0.63 (0.56-0.7)
3	SBP + Hb + BD	0.76 (0.71-0.81)
4	SBP + Hb + INR	0.75 (0.69-0.82)
5	SBP + Hb + BD + INR	0.79 (0.74-0.85)

Conclusion: The addition of admission BD and INR as inclusion criteria may improve the ability to select patients with expected mortality for trials in penetrating traumatic injury which exclude isolated severe head injury. Approaches similar to this are needed to improve study design of large clinical trauma trials.

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IS HYPOTHERMIA IN TRAUMA PROTECTIVE?

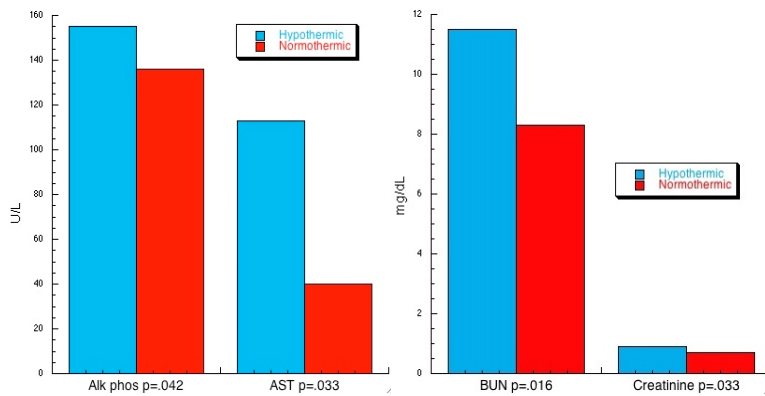
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Objective: We have previously demonstrated survival benefit to induced hypothermia in a model associated with delay to care. In the current study, we wished to evaluate the effects of hypothermia in a porcine model of polytrauma and uncontrolled hemorrhagic shock.

Methods: 16 pigs were instrumented and randomized to normothermic (39° C, n=7), or hypothermic (34° C, n=9) groups. The model included pulmonary contusion (captive bolt gun), hemorrhage to SBP of 50 mm Hg, and then liver injury using a crush clamp.

Animals received limited resuscitation for a 1-hour phase with fluids to SBP of >80mm Hg, and ice packs or warming blankets to achieve goal temperatures, followed by full resuscitation by protocol for 20 hours. Survivors were observed for 24 hrs.

Results: There were no differences in survival between the groups (mortality: hypothermic n=1/9, normothermic n=2/7). Markers of organ injury were elevated in the hypothermic group at 24 hours after injury.



Conclusions: Hypothermia in a model of uncontrolled hemorrhagic shock and short time to definitive care was associated with markers of increased organ injury.