Our Honored Guest for the Evening

James C. Eisenach, MD is F.M. James, III Professor of Anesthesiology, Section of Obstetric Anesthesia; Professor of Physiology & Pharmacology, at Wake Forest University, Winston-Salem, NC; and Editor-in-Chief, *Anesthesiology*. Dr. Eisenach first pursued graduate training obtaining his MS degree from the California Institute of Technology, followed by his MD degree at the University of California, San Francisco School of Medicine. He completed residency training in Anesthesiology at the Mayo Clinic, followed by a fellowship in Obstetric Anesthesiology at Wake Forest University. He has remained on the faculty at the Wake Forest Department of Anesthesiology from completion of his fellowship until today.

Dr. Eisenach, the overall director of the Pain Mechanisms Laboratory, has a long history of scientific review and administration and of NIH funded research. He is the recipient of over $20 million in NIH support and $2 million in industry support since coming to Wake Forest School of Medicine in 1985, and is currently PI on two RO1 grants, one of which received MERIT status in 2011. He was the PI for the pivotal multi-center trial leading to FDA approval of epidural clonidine for intractable neuropathic pain associated with cancer. Dr. Eisenach holds 5 INDs from the FDA for novel analgesic drugs and two patents for novel treatment for pain. He is a past member and chairman of the Anesthetic and Life Support Drugs Advisory Committee at the FDA. In addition to serving on several review committees for the NIH, VA, and national anesthesiology societies, he has been the Vice-Chairman for Research in the Department of Anesthesiology since 1998. Dr. Eisenach has trained 52 post-doctoral and undergraduate fellows and students since 1985. He has organized two major pain symposia at IASP international meetings.

We are pleased that Dr. Eisenach is participating in Academic Evening 2015 as Poster Judge, Awards Presenter, and Speaker. The title of Dr. Eisenach’s presentation is “Desperation and Deception in Medical Science and Practice.”
Program

4:15pm - 4:30pm  Poster set-up

4:30pm - 5:15pm  Poster Session

5:15pm – 6:15pm  Moderated Poster Session

6:15pm – 6:30pm  Poster take-down & move to dining room

6:30pm – 7:15pm  Dr. Eisenach Presentation:  
“Desperation and Deception in Medical Science and Practice”

7:15pm – 8:15pm  Dinner Service

8:15pm – 8:30pm  Best of Meeting Awards & Presentations
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Sanjay Bhananker
David Dorsey
Nathalia Jimenez*
Nicholas Kassebaum*
Stephen Kolwicz
G. Burkhard Mackensen*
Phil Morgan
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*Faculty Moderators

STAFF

Candy Acosta
Tim Adams
Julie A. Baker
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Fred Hilerio
Andrea McAuliff
PRESENTING FELLOWS

**ACGME Fellows**
Winston Choi
Paul Jacobs
Tatyana Shkolnikova

**Faculty Fellows**
Carlos Delgado Upegui
Jeffery Fujii
Rachel Kutteruf
Ben Maniwatana
Sarah Robinson
Nadav Sheffy

**Research Fellows**
Naveen Bojjireddy
Jacqueline Ho
Chi Fung Lee
Liladher Paudel
Nathan Roe
Dan Shao
Eric Smith
Pei Wang
Huilang Zhang
JiangJiang (Chris) Zhu

**Children’s Research Fellows**
Leslie Itsara
Melanie Noel
Renjini Ramadasan Nair

PRESENTING RESIDENTS

Christopher Barnes
Charles Carspeckn
Anda Cornea
Rachel Douglas
David Frey
Elizabeth Hansen
Michael Holland
Joshua Kohtz
Li Li
Michelle McGauvran
Chinwe Nwaneshiudu
Stephanie Pan
Michael Patz
Matthew Pennington
Aalap Shah
Anthony Tran
Andrew Walters
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5. Elizabeth Visco; Aalap C Shah; Housestaff Quality and Safety Committee Working Group: Post-Operative Transfer-of-Care at HMC: A Multidisciplinary Quality Improvement Initiative

6. Daniel Oh; Aalap C Shah; Anna Xue; Bala G Nair; John D Lang: A Hand-Off Tool to Facilitate Transfer of Care from Anesthesia to Nursing in Intensive Care Units

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Background: The primary aims of this quality improvement project were to increase reporting of medication errors and to implement targeted countermeasures to reduce the incidence of medication errors by anesthesiologists. The Institute of Medicine has called for development of strategies to prevent medication errors, which are a major cause of preventable harm. (1) Although the field of anesthesiology is considered a leader in patient safety, existing data on medication errors are limited and few reliable error prevention strategies for anesthesia providers have been developed. (2, 3)

Methods: Using Toyota Production System quality improvement methodology, a multidisciplinary team observed 120 hours of medication practice in the operating room and conducted a Failure Mode and Effects Analysis (FMEA) to systematically deconstruct and evaluate each medication handling process step and score possible failure modes to quantify areas of risk. Five targeted countermeasures were identified and implemented over 12 months including: (1) medication tray reorganization, (2) standardization of medication cart layout with human factors and design expert consultation, (3) standard two-provider check for infusions, (4) anesthesia cart and syringe label redesign, and (5) development of a medication practice guideline with regular education. An anonymous error reporting system was developed, and biweekly reminders were sent to all anesthesia providers to collect data. Preexisting data from hospital and departmental QI reporting mechanisms were included.

Results: Prior to the initial FMEA event and the initiation of this project, an average of 0.124 medication errors were reported per 1000 anesthetics. After the initial FMEA event and initiation of anonymous error reporting mechanisms, self-reported medication errors increased to 1.451 errors per 1000 anesthetics. Similarly, 1.3 near miss events per month were reported, where none had previously been captured. The countermeasures were subsequently implemented, and after this a consistent decrease in medication errors was observed. Six months after countermeasure implementation, the medication error rate decreased to 1.1 per 1000 anesthetics. A run chart describing medication error rates per 1000 anesthetics over time demonstrates a significant decrease in error rates with seven consecutive points below the mean since countermeasure implementation.

Discussion: Medication error reporting improved with the initiation of this quality improvement project, which may be attributed to increased medication safety awareness by providers and improved reporting mechanisms. After implementation of countermeasures a trend of decreasing medication error rates was observed, suggesting that targeted medication practice changes may improve medication safety in the operating room.
Background: Anesthesia drug errors occur at a rate of approximately 0.5-1% of anesthetic procedures, based on studies of self reported errors (1). We reported data in 2003 based on completion of a paper form following each anesthetic (2). We reported data in 2014 based on self reporting in Docusys AIMS for each anesthetic; these data showed very little change compared to 2003. Recently we implemented a bar code-based Anesthesia Drug Safety System (3) in which all syringes are labeled with bar codes, and these bar codes are scanned using Smart Anesthesia Manager prior to administration. In this study we have compared the drug errors reported before and after introduction of the Anesthesia Drug Safety System.

Methods: Using Smart Anesthesia Manager, quality assurance “hardstops” including drug error data were recorded from every anesthetic from April 1, 2014 to March 9, 2015, a total of 18,477 cases. Errors were classified using the same taxonomy as in previous studies from New Zealand and the University of Washington. Of these cases, 11,629 were performed before and 6,848 were performed following the implementation of the Anesthesia Drug Safety System.

Results: The rate of error prior to introduction of the drug safety system was 0.43% (50 errors/11,629 cases). Following introduction of the Anesthesia Drug Safety System the rate of error was 0.22% (15 errors/6848 cases) (p=0.0099).

Discussion: The rate of self reported drug error in New Zealand was reported in 2001 as 0.75% of anesthetics (1). In 2003 we found a very similar rate of 0.68% at the University of Washing Medical Center (2). In early 2014 we found the error rate reported in our Smart Anesthesia Manager CQI hardstop data to be 0.44% (17 errors/3891 cases). Later in 2014 and early 2015, prior to the introduction of the anesthesia drug safety system we found the rate to be 0.43% (50 errors/11,629 cases). After introduction of the Anesthesia Drug Safety System, the rate declined to 0.22% (15 errors/6848 cases). The Anesthesia Drug Safety System is designed primarily to prevent vial swap errors (by reading the bar code on the vial prior to making a label) and syringe swap errors (by reading the bar code on the syringe prior to drug administration). These types of errors should be recorded as “substitutions”, however the reduction in errors was not accounted for by a decline in “substitutions”. This suggests that the reduction was due to something else or to underreporting. There is the possibility that the anesthesia drug safety system has
had a non-specific effect to increase vigilance and prevent drug errors in general, not only those related to vial or syringe swaps. There is also the possibility that improved syringe labeling has reduced errors by mechanisms other than preventing substitution (syringe swaps). Further surveillance and investigation is required.  
Keywords: drug error, bar code, syringe label, anesthesia information system  

3: Performance of Anesthesia Pre-Induction Safety Steps  
Cynthia Wu, BA; Srdjan Jelacic, MD; Bala Nair, PhD; Kei Togashi, MD; Andrew Bowdle, MD, PhD  
Anesthesiology & Pain Medicine, University of Washington  

Background: Anesthesiologists perform a series of steps in preparation for each case, some of which may be missed inadvertently or even intentionally for the purpose of greater efficiency. We conducted a study to determine the incidence of missed critical steps during preparation for the induction of anesthesia.  

Methods: We performed a prospective observational study on selected general and gynecological surgery cases at the University of Washington Medical Center between October 2014 and February 2015. Trained observers started data collection prior to patients entering the operating room (OR) and continued observations until 15 minutes after induction. Observers checked for the availability of back-up airway devices (laryngeal mask airway, bag valve mask, Eschmann catheter, and 2nd laryngoscope) and resuscitation drugs (atropine, epinephrine, phenylephrine, ephedrine, and succinylcholine), whether daily anesthesia machine leak test was performed, and if suction was functional prior to the patient entering the OR. They also recorded the presence of electrocardiogram, pulse oximetry, blood pressure, and end-tidal carbon dioxide (EtCO2) signals on the monitor and if the intravenous (IV) line was functional prior to induction. Finally, the observers recorded whether allergies and airway concerns were addressed during the Surgical Care and Outcomes Assessment Program (SCOAP) checklist.  

Results: Total of 48 missed steps were recorded in 420 cases observed. In 32 (7.6%) cases, one of the back-up airway devices was missing, which included 1 case without laryngeal mask airway, 10 cases without bag valve mask, 4 cases without Eschmann catheter, and 17 cases without a 2nd laryngoscope available. Phenylephrine syringe was not available in 3 (0.7%) cases while ephedrine syringe was not available in 1 (0.2%) case. Suction was not functional in 7 (1.7%) cases while the IV was not functional in 3 (0.7%) cases. In 2 (0.5%) cases, the EtCO2 monitor was off prior to induction. Allergies were not addressed in 67 (16.0%) cases while airway concerns were not addressed in 83 (19.8%) cases during SCOAP checklist.  

Discussion: Anesthesia providers miss critical steps during their preparation for the induction of anesthesia. Implementation of pre-induction checklists may ensure that these steps are never missed in order to prevent compromises in patient safety.  

Acknowledgments: E. Patchen Dellinger, MD; Daniel J. Boorman; Laura Sissons-Ross
4: Evaluation of Compliance with the Surgical Care and Outcomes Assessment Program 1 Safety Checklist

Srdjan Jelacic, MD; Bala Nair, PhD; Kei Togashi, MD, MPH; E. Patchen Dellinger, MD; T. Andrew Bowdle, MD, PhD; et al.

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Background: Many hospitals are mandating the use of perioperative checklists based on the World Health Organization (WHO) Surgical Safety Checklist, which was associated with improved patient outcomes. However, only few studies have explored the compliance of using the surgical checklist. The compliance consists of frequency of checklist use (at least 1 checklist item addressed) and completeness (every checklist item addressed). Although the frequency of checklist use is thought to be high in our hospital, there has not been a systematic study of completeness of checklist use. We hypothesized that compliance with the completeness of checklist use is low.

Methods: We performed a prospective observational study on selected general and gynecological surgery cases at the University of Washington Medical Center between November 2014 and February 2015. Trained observers evaluated the frequency and completeness of the Surgical Care and Outcomes Assessment Program (SCOPA) (1) surgical safety checklist use prior to the induction of anesthesia. Trained observers also recorded the level of team participation on a scale consisting of excellent, good or poor. The distraction level was recorded on a scale consisting of none, minimal, moderate or severe. The observers also recorded whether the checklist was read from a poster, a large flat panel screen, a desktop monitor or recalled from memory.

Results: The SCOAP (1) surgical safety checklist was used in all of the 305 cases observed. The average number of checklist items performed in the observed cases was 9 (65%) out of 14. The most commonly performed checklist items were the confirmation of patient (99%) and procedure (99%). The rest of the checklist items were performed in less than 90% of cases. The surgical safety checklist was read completely without interruptions in only 12 (4%) out of 305 cases. Posters were used for following the checklist in 79% of the cases while the surgical checklist was recalled from memory in 12% of the cases. In 7% of the cases the posters were used for part of the checklist performance while the remaining checklist items were recalled from the memory. The team participation level was poor in 58 (19%) cases. The distraction level was at least moderate in 110 (36%) cases.

Conclusion: Although the frequency of surgical checklist use is 100%, compliance with performing each checklist item is low. The intended use of surgical safety checklists involves adherence to every checklist item, which remains to be achieved at our institution. There is growing evidence that compliance with the use of the surgical checklist is related to successful implementation and dissemination strategy. (1) Computerized aviation-style surgical checklists may also have a role in improving the completeness of checklist use by improving checklist visualization and
navigation. (2, 3) Additionally, it allows for easy modifications while facilitating hospital-wide checklist compliance reporting.

**Acknowledgments:** Daniel J Boorman; Laura Sissons-Ross; Cynthia Wu

**References:**

**Keywords:** surgical checklist, safety, compliance

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### 5: Post-Operative Transfer-of-Care at HMC: A Multidisciplinary Quality Improvement Initiative

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**Background:** Transfers of information represent high-risk error-prone patient care episodes and typically occur while there are many competing demands on attention. Standardization and electronic documentation of the post-operative transfer-of-care (TOC) has been recommended to improve handover quality. Through a collaborative, multidisciplinary quality improvement initiative, we introduce and implement a team-based checklist approach to the post-operative TOC at our medical center. Furthermore, we will conduct a pre/post implementation analysis of checklist utilization and evaluate its impact on information reporting, validated handover quality assessment measures and nursing task burden.

**Methods:** Intervention: As a quality improvement initiative undertaken at Harborview Medical Center, Seattle, WA, this project was considered exempt from review. A structured checklist containing the smallest number of items thought essential during intraoperative handoff was developed by the authors, with the intent of physical implementation in each patient bay in the recovery room. An electronic form (PACU RN PowerNote), was also drafted in parallel with the checklist and approved through our compliance committee for inclusion in the electronic health record. Chief resident surveys and an educational video were created as educational supplements to increase familiarity and utilization of the checklist.

**Data Collection:** From January 2015 to September 2015, we are employing two trained individuals to observe post-operative TOC of elective inpatient procedures at a single recovery room at Harborview Medical Center. PACU nursing staff were given a validated handoff domain assessment tool (Handoff CEX; Horwitz 2011) and chief residents were queried with confidential surveys to elicit recommendations for checklist content. Data collection was divided into three phases: pre-implementation
(January 27-March 27), implementation (March 27-May 31), and post-implementation (June 1–September 30) phases.

**Results:** Pre-implementation audits (n=78) confirmed surgeon presence for 57.7% of handovers. Average TOC time was 3.81 +/- 2.05 minutes, and average length of stay (LOS) (i.e. PACU time until discharge ready) was 90.17 +/- 43.39 minutes. Information items with frequent omissions included PONV prophylaxis (41.0%) and last antibiotic dose (29.1%). Frequently omitted items related to anticipatory guidance included a pain-management plan (54.4%) and call triggers (2.5%). Handoff CEX scores (n=204) included scores of 5 or greater in all domains; overall satisfaction score was 7.70 +/- 2.13 (median 9). PACU RNs sent an average of 1.03 +/- 1.46 pages to the surgeon or anesthesia provider for order clarifications.

Implementation data collection is underway, and post-implementation analyses will commence in June 2015.

**Discussion:** Although survey tools used to evaluate TOC demonstrate excellent scores in all domains, pre-implementation data review suggests that certain items are frequently missed from the verbal handover report given by the surgeon and/or anesthesia representative. The results also highlight the deficiency of anticipatory guidance measures communicated to the recovery room nurse. Baseline data quality measures, such as LOS and handover time, will be useful when evaluating the effect of this process improvement on post-implementation efficiency.

**Keywords:** Handovers, Post-Operative Transfer of Care, Patient Safety

### 6: A Hand-Off Tool to Facilitate Transfer of Care from Anesthesia to Nursing in Intensive Care Units

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**Background:** Patient care handoff from one team of providers to another is a critical moment highly prone to medical errors. Transfer of care at the end of surgery between anesthesia and nursing team in a typical example when inaccurate and incomplete transfer of relevant clinical information could increase the risk of inadvertent medical mistakes. We describe the development of a tool that provides a customized report of intraoperative data to both anesthesia and recovery teams to facilitate safe hand-off. Additionally, the tool also notifies the recovery team of patient transport, providing the recovery team with the handoff summary prior to the patient actually arriving at the recovery bed. Advance notice and upfront availability of handoff information could better prepare the recovery team for a smoother transfer of care.

**Methods:** We developed an AIMS-based handoff tool that can be evoked at the touch of a key on the AIMS computer. The tool presents the most current AIMS data summarized into main categories – medications, anesthetic techniques and lines, fluid input/output and labs. At the end of surgery, prior to leaving the operating room, the anesthesia provider selects the disposition location and initiates a “print summary and page recovery” action. This automatically prints the transfer summary report in designated recovery room printers and sends a text page to recovery staff notifying that a patient is leaving the operating room. The recovery room staff collects and
reviews the summary report in preparation for handoff. During handoff, the anesthesia and recovery room team uses the transfer summary report as a reference document.

We piloted the handoff tool to facilitate transfer of care in the intensive care units (ICU). The Cardiac, Surgical and Medical ICU staff was presented with the transfer tool and the associated workflow of receiving the patient transport notification page, collecting the handoff summary report and utilizing it to facilitate transfer of care. Similarly, the anesthesia team was also trained in the use of the handoff tool. Volunteer medical students were recruited and trained to observe the transfer of care and collect data pertinent to the handoff process.

**Results:** Handoff process was observed in 7 instances when the transfer summary sheet was not used (controls) and in 14 instances when the transfer summary sheet was used (intervention). In general, omission of critical data elements was slightly less when using the transfer summary - Urine output: 3/14 (intervention) vs. 3/7 (controls), Blood loss: 5/14 (intervention) vs. 4/7 (controls) and Fluids & infusions: 2/14 (intervention) vs. 3/7 (control). Duration of the handoff process was similar for control and intervention cases though the intervention group had primarily cardiac ICU cases while the control group comprised of mainly surgical ICU cases. The use of the handoff tool to facilitate patient transfer could be easily integrated into the clinical workflow without disruptions.

**Discussion:** Pilot observation and data indicate that an AIMS-based handoff tool could be easily integrated into the clinical workflow and could potentially facilitate safer patient transfer. Further studies are required to prove the effectiveness of such a tool.

**Keywords:** Handovers, Post-Operative Transfer of Care, Patient Safety

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### 7: Process Improvements To Improve Initiation Of Epidural Infusions And Decrease Pacu Pain Scores
Michelle M. McGauvran, MD; Aalap Shah, MD; Bala Nair, PhD; Laurent Bollag, MD
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**Introduction:** To improve patient outcome in selected surgeries, preoperative epidural catheters placed for post-operative pain management should ideally be started at the beginning of surgery. An audit of epidural start times in our institution revealed that infusions were started pre or intra-operatively only 57% of the time. We evaluated effects of process enhancements including pharmacy and system-based interventions as well as electronic alerts on compliance in starting epidural infusions prior to the completion of surgery.

**Methods:** Areas of improvement included the pharmacy (improved order processing time and infusion delivery to the theaters) and equipment supplies (daily restocking) in addition to an Anesthesia Information Management System (AIMS) based decision support system using repeated pop-up reminders.

**Results:** A survey sheet with patient-reported pain scores were filled out by the PACU nursing staff. Epidurals that were noted to be started intraoperatively constituted “timely “ initiation. Survey data was collected during the pre-intervention and post-intervention phase.
97 survey sheets were collected during the entire data collection period; one survey sheet was excluded as it was returned blank. There were no differences regarding LOR or level of epidural between the pre- and post-intervention group. Pre-intervention, 67.5% epidural infusions were started pre or intra-operatively. Post-intervention, 85.7% of epidural infusions were started pre or intra-operatively (p=0.045). The maximum PACU pain scores differences proved significant with p=0.027. Averaged observed pain scores at the time of recovery room departure also proved significant with p=0.023. Maximum score – Recovery room departure score between the two groups was p =0.532. Anesthesia providers and PACU nurses taking care of patients whose epidural infusions were not started intraoperatively were invited to provide an explanation on the data collection form.

**Discussion:** Epidural workflow improvements including pharmacy and equipment changes combined with electronic alerts resulted in an 18% increase in timely initiation of epidurals. Maximum pain scores in PACU between timely initiation and non-timely initiation proved clinically significant as well with a p=0.027 as well as averaged observed pain scores with a p value of 0.023. There were no differences in the median pain score change (Maximum score – Recovery Room departure score) between the two groups (p=.532) as well as no differences regarding pain scores when comparing patients before and after the workflow package was implemented.

**Conclusion:** Given the dearth of data, baseline studies of the effects of timely (i.e. intraoperative) epidural infusions as well as a study investigating the impact of a timely epidural infusion start of neuraxial analgesia on post-operative narcotic and adjunctive pain medication requirements, and antiemetic usage are needed.


**Keywords:** epidural infusion, regional, quality improvement

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### 8: Early Epidural Use and Post-Operative Analgesia in Patients Undergoing Elective Surgery

Aalap C Shah, MD; Michelle McGauvran, MD; Bala G. Nair, Ph.D; Laurent Bollag, MD

**Introduction:** To improve patient outcome in selected surgeries, preoperative epidural catheters placed for post-operative pain management should ideally be started at the beginning of surgery.1 Delayed epidural initiation may increase postsurgical pain, incidence of opioid related side effects in the PACU and recovery room (PACU) time. A Quality Improvement audit of epidural start times in our institution revealed that infusions were started pre or intra-operatively only 57% of the time. Prior to planned process improvements (i.e. intra-operative electronic epidural infusion reminder, pharmacy workflow changes), we evaluate the effect of early (i.e. intraoperative) initiation of epidural infusions on post-operative pain control and opioid medication usage.

**Methods:** After obtaining IRB approval, we conducted a retrospective review of all procedures associated with the placement of a pre-operative neuraxial (epidural)
catheter for elective surgery between October 2012 and October 2014. Emergent
and obstetric cases were excluded from this study. We collected data regarding
demographics, inpatient procedure (type, length), and epidural placement and
infusion characteristics (concentration, rate, time of initiation). In addition, data
regarding intra- and post-operative opioid and anti-emetic medication use,
hypotension, post-operative nausea/vomiting, and PACU length of stay was
collected. Patients were primarily grouped whether or not their epidural infusion was
started intraoperatively. Primary outcome measures included patient-reported scores
obtained upon admission to the PACU (FIRST), prior to transfer out of the PACU
(LAST), and the maximum reported pain score during their PACU stay. (MAX).
Secondary outcome measures include post-operative opioid consumption and the
incidence of nausea and vomiting in the PACU. All statistical analysis was performed
using SPSS v.18.0 (IBM Corp., Armonk, NY, USA).

Results: We collected data on 2199 procedures from October 2012 to October
2014. The median procedure length was 218 minutes. Of these 2199 procedures,
1238 epidurals (Group 1) were started while the patient was undergoing their
procedure (56.3%), while 961 procedures were started after surgery was over (or not
started at all.) There was a statistically insignificant trend towards lower FIRST pain
scores (4.78 +/- 3.68 vs. 5.08 +/- 3.68; p=.058) and MAX pain scores (5.97 +/- 3.26
vs. 6.26 +/- 3.16; p=.083). Longer procedures (with accompanying increasing
duration of epidural infusions) were associated with a lower FIRST median pain
score (4 vs 6; p=.016), with the largest difference noted between procedures >= 6
hours versus those less than 4 hours. However, LAST pain scores (prior to leaving
the PACU) were unchanged (3.41 vs. 3.39; p=.843), regardless of procedure length.

Conclusion: Preliminary results suggest only mild improvements in patient-reported
pain scores upon emergence from anesthesia, with essentially no difference in
scores when the patient is ready to transfer to the floor. While further analysis is
needed to elucidate the impact of pre-existing chronic pain, as well as the opioid and
side-effect-sparing properties of epidural infusions, the anesthesia provider is
recommended to evaluate the potential for intraoperative epidural infusion initiation
on a case-by-case basis.

Keywords: Post-operative Analgesia, Regional Anesthesia, Opioid-Sparing

9: Choice of local anesthetic and peripheral nerve block characteristic
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Background: Surgical procedures on the upper extremity are often performed under
brachial plexus blocks. Established literature suggests that such blocks can typically
provide 10-20 hours of postoperative analgesia. The existing practice at Harborview
Medical Center varies by practitioner, who may employ different local anesthetic (LA)
solutions, sometimes with adjuvant medications added (e.g., dexamethasone) in an
attempt to prolong the duration of the block. What effects these variations actually
have on block duration, postoperative opioid requirement, and other block
characteristics, is unclear. This quality improvement project seeks to describe the
block characteristics with the various commonly used LA solutions.
Methods: Patients who underwent outpatient finger, hand, wrist, or arm surgery from January 26, 2015 through April 9, 2015 at Harborview Medical Center and preoperatively had received a single-shot brachial plexus nerve block were identified from the anesthesia record. At 4 to 10 days postoperatively, a follow-up telephone call was made to each patient. Questions were asked to determine the (1) length of time until complete resolution of the block, (2) length of time before the first oral analgesic, (3) total amount of oral analgesic (in oxycodone equivalents) taken during the first 24 and 48 hours, (4) presence of any residual symptoms that might suggest nerve injury, and (5) overall patient satisfaction with the block.

Results: Successful follow-up data was obtained for 69 patients. Mean block duration for all patients (n=69) was 11.34 ± 1.05 hours. Patients who received 0.5% ropivacaine (n=44) reported a block that lasted on average 3.95 hours longer than those who had received a mixture of 0.25% ropivacaine and 1% mepivacaine (n=24). Patients who also received perineural (n=6) or intravenous dexamethasone (n=3) as an adjunctive medication tended to report a longer block. Oral analgesic requirement was generally inversely correlated with block duration. Seven patients reported dissatisfaction with the block, largely because the duration was shorter than was expected. There were no reports of residual symptoms in any of the patients.

Discussion: These findings, based on existing practice at Harborview, support the notions, previously established in literature, that (1) adding a shorter-acting local anesthetic, i.e., mepivacaine, to a longer-acting local anesthetic, i.e., ropivacaine, shortens overall block duration; and (2) adding dexamethasone as an adjunctive medication can prolong block duration. Our study was not adequately powered to evaluate the incidence of nerve injury following block, which in the literature is thought to be extremely rare. Patient satisfaction with blocks may be improved by carefully setting realistic expectations with the patient. The shortage of preservative-free dexamethasone has limited its use in our practice.

Keywords: peripheral nerve blocks, local anesthetics, dexamethasone, brachial plexus
10: Association of Intraoperative and Postoperative Blood Glucose Levels in Non-cardiac Surgery Patients
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2Biostatistics, The Mountain-Whisper-Light Statistics LLC
3Anesthesiology & Pain Medicine, Virginia Commonwealth University

Background: Postoperative hyperglycemia has been associated with poor surgical outcome. The effect of intraoperative glucose management on postoperative glucose levels and the optimal glycemic threshold for initiating insulin are currently unknown.

Methods: A retrospective cohort study of non-cardiac surgery patients that required intraoperative glucose management was undertaken with data extracted from electronic medical records. In patients that required glucose management, intraoperative glucose levels and insulin therapy were compared against postoperative glucose levels during 3 periods—first postoperative level within 1 hour, within the first 12 hours, and 24 hours of the postoperative period. Logistic regression models that adjusted for patients and surgical factors were used to determine the association between intraoperative glucose management and postoperative glucose levels.

Results: In 2440 patients that required intraoperative glucose management, an increase in mean intraoperative glucose level by 10 mg/dL was associated with an increase in postoperative glucose levels by 4.6 mg/dL (CI [4.1, 5.0], p<0.001) for the first postoperative glucose measurement, 2.4 mg/dL (CI [2.0, 2.8], p<0.001) for the mean first 12-hour postoperative glucose and, 2.2 mg/dL (CI [1.8, 2.5], p<0.001) for the mean first 24-hour postoperative glucose levels. Patients with diabetes (regression coefficient = 12.2, p<0.001) and intraoperative steroid use (regression coefficient = 10.1, p<0.001) had positive effect on elevated postoperative glucose levels. Intraoperative hyperglycemia (> 180 mg/dL) was associated with postoperative hyperglycemia during the first 12 hours (OR = 2.14, p< 0.001) and the first 24 hours (OR = 2.22, p< 0.001). When compared with starting insulin for an intraoperative glucose threshold of 140 mg/dL avoiding hyperglycemia, initiation of insulin for a hyperglycemia threshold of 180 mg/dL was associated with an increase in postoperative glucose levels (7 mg/dL, p< 0.001) and postoperative hyperglycemia incidences (OR = 1.53, p=0.01). Both intraoperative (0.4% Vs 1.2%, p=0.4) and postoperative (1.8% Vs 2.2%, p=0.8) hypoglycemia (< 60 mg/dL) incidences were fewer when insulin was initiated at a glucose threshold of 140 mg/dL versus 180 mg/dL, though the results did not reach statistical significance.

Discussions: Higher intraoperative glucose level is associated with higher postoperative glucose level. Intraoperative hyperglycemia increases the odds for postoperative hyperglycemia. Adequate intraoperative glucose management by initiating insulin infusion when glucose level exceeds 140 mg/dL to prevent hyperglycemia is associated with lower postoperative glucose levels and lower incidences of postoperative hyperglycemia. Incidences of hypoglycemia did not increase when initiating insulin at a threshold of 140 mg/dL.

Acknowledgments: This research was partly supported by a Patient Safety Innovation Program Grant provided by the University of Washington.
11: Intraoperative Glucose Levels and Postoperative Complications in Non-cardiac Surgery Patients
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Background: Studies associating hyperglycemia with poor surgical outcome have thus far focused on cardiac surgery patients and postoperative glucose levels. The association between intraoperative glucose levels and surgical outcome is poorly understood in non-cardiac surgery patients.

Methods: We undertook a retrospective cohort study of adult patients (N = 995, 455 diabetic – both type 1 & 2 and 540 non-diabetic) that required intraoperative glucose management in non-cardiac surgery (≥ 3 hours duration). Intraoperative glucose levels and glycemic variability (measured in standard deviation - SD) were compared against ICU length of stay, incidences of surgical infections, and pulmonary & cardiovascular complications. Logistic regression models that adjusted for patients and surgical factors were used to determine the association between intraoperative glucose levels and glycemic variability and postoperative outcomes.

Results: Compared against a reference set of cases with well controlled mean glycemic levels < 140 mg/dL, cases with high mean glucose levels > 180 mg/dL was associated with increased ICU length of stay (β = 2.17, p =0.01) and higher odds of surgical infections (OR = 3.87, p=0.004) and pulmonary complications (OR = 5.20, p=0.02) in patients without diabetes. However, in diabetic patients the same associations were not observed. Compared with a reference set of cases with low glycemic variability (SD < 15), cases with high glycemic variability (SD > 35) was associated with increased ICU length of stay (β = 1.82, p =0.03) and higher odds of pulmonary complications (OR = 8.70, p=0.04) in patients with diabetes. However, a similar trend observed in non-diabetic patients did not reach statistical significance.

Discussions: These results indicate that in diabetic patients, high intraoperative glycemic variability, and in non-diabetic patients, high intraoperative glycemic levels are associated with poor surgical outcome.

Acknowledgments: This research was partly supported by a Patient Safety Innovation Program Grant provided by the University of Washington.

Keywords: Blood glucose, Intraoperative, Hyperglycemia, Postsurgical complications
Background: Percutaneous edge-to-edge mitral valve repair (PMVR) with the MitraClip procedure has been shown effective for the reduction of mitral regurgitation (MR) severity in patients with degenerative MR (DMR). In recent years, PMVR has been more frequently applied in patients with functional MR (FMR). Only limited data are available on acute dynamic changes of the mitral valve (MV) annular geometry after PMVR. This study assessed if tracking of dynamic geometric changes using three-dimensional transesophageal echocardiography (3DTEE) imaging permits assessment of immediate morphologic and functional characteristics of the MV following PMVR.

Methods: With Institutional Review Board approval, we retrospectively analyzed 27 patients who underwent the MitraClip procedure, with 18 in the DMR group and 9 in the FMR group. All patients underwent a comprehensive intraoperative TEE exam (iE33 system; Phillips Medical Systems) with both pre and post-procedural 3DTEE images of the MV. Using available quantification software (4D MV assessment™ 2.0, Tomtec), offline analysis of the MV was performed in triplets and then averaged. 3D annulus area was measured at end systole. Annular displacement distance, annular displacement velocity, and the sphericity index (ratio of anterior-posterior to Anterolateral-posteromedial diameter) were calculated throughout systole. Results were analyzed using two-sample Wilcoxon rank-sum (Mann-Whitney) test.

Results: The procedure was completed successfully in all patients and quantitative 3D assessment using the 3D tracking software was feasible in all patients before and after MitraClip insertion. 3D MV annulus area, annular displacement distance and the sphericity index remained unchanged in both DMR and FMR when compared to baseline. Annular displacement velocity was decreased in both groups post MitraClip, with larger changes observed in the DMR group.

Conclusion: To our knowledge, this preliminary study is the first to describe acute changes in MV annular velocity in patients with DMR and FMR undergoing PMVR. 3DTEE permits quantitative tracking of dynamic signatures of the MV before and after the MitraClip procedures. This technology could possibly be used to improve pre-procedure dynamic analysis of MV anatomy and geometry to enable better patient selection and procedural guidance. Future studies should aim at comparing dynamic 3DTEE quantification with other imaging modalities and assess its potential impact on interventional technique and patient outcomes.


Keywords: MitraClip, mitral valve repair, Tomtec, mitral regurgitation
**13: Transcatheter Melody Valve-in-valve Implantation for Bioprosthetic Mitral Valve Dysfunction**

Winston Choi, MD; Thomas K. Jones, MD; Srdjan Jelacic, MD; Donald Oxorn, MD; G. Burkhard Mackensen, MD, PhD; et al.

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<table>
<thead>
<tr>
<th>Background:</th>
<th>The prevalence of structural valve deterioration (SVD) is increasing. This is due to an increase in the aging population, and the increasing use of bioprosthetic valves in younger patients. As the number of failing bioprostheses rises, a commensurate need for less invasive valve re-replacement will occur. Transcatheter valve-in-valve (TVIV) implantation is an attractive alternative to open surgery for high-risk patients with severe bioprosthetic mitral valve (BMV) dysfunction. This case series describes our institution’s experience with TVIV implantation using the Melody® valve for treatment of severe BMV SVD in patients with prohibitive surgical risk.</th>
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<tr>
<td>Methods:</td>
<td>With IRB approval, a retrospective chart and echocardiographic review was performed for four consecutive patients who underwent TVIV implantation with the Melody® valve for severe, symptomatic BMV dysfunction. The Melody® valve is a bovine jugular valve supported by a balloon-expandable stent. All patients underwent arterio-venous rail guided transcatheter delivery of a 22 mm Melody valve with transapical exteriorization in an antegrade, trans-septal fashion. Image guidance was accomplished with fluoroscopy and transesophageal echocardiography. All patients were considered to have excessive surgical risk by the multidisciplinary heart team.</td>
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<td>Results:</td>
<td>Patients ranged in age from 61 to 80 years. Time from last BMV implantation averaged 10 yrs. The average STS risk score was 17.2 ± 7.4%. Successful deployment of a Melody® valve via Ensemble delivery system occurred in all patients with no device malfunctions in the perioperative period. Diastolic trans-mitral gradients decreased from an average of 14.3 ± 4 mmHg to 3.8 ± 1.3 mmHg, and mitral valve area increased from an average of 1.5 ± 0.5 cm² to 2.8 ± 0.6 cm². There was no mortality at 30-day follow up. All patients had mild or less mitral regurgitation, with no perivalvular regurgitation. There were no vascular or pulmonary complications. All but one patient experienced significant symptomatic relief. This patient developed a delayed hemolytic transfusion reaction and demonstrated mild dynamic LVOT obstruction, in the setting of a previously placed mechanical aortic valve.</td>
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<tr>
<td>Discussion:</td>
<td>In this preliminary case series, the transcatheter Melody® valve-in-valve implantation provided safe, short-term relief of severe BMV dysfunction in these selected high-risk patients. Safe delivery and proper alignment of the device was reliably achieved with the arterio-venous rail technique and transapical exteriorization of the guide wire. Long-term valve durability, morbidity and mortality are under investigation. Careful orientation of the prosthesis is important in order to minimize the risk of LVOT obstruction.</td>
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<td>Keywords:</td>
<td>Melody bioprosthetic valve deterioration mitral stenosis</td>
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Introduction: Hypovolemia in the setting of moderate or severe cerebral vasospasm in patients with aneurysmal subarachnoid hemorrhage (SAH) increases the risk of delayed cerebral infarction (DCI). Thus, consensus recommendations are to maintain euvolemia. Our primary aim was to examine the correlation between 1) the change in calculated total body solute (an estimate of the extracellular fluid volume) over time and 2) the shock index (SI) at a single point in time with the change in directly measured blood volume (BV) over time and BV status at a single point in time.

Methods: This retrospective study was approved by the University of Washington IRB. All adult patients in whom a BV was measured while in the neuroscience ICU of a large academic medical center were included. BV was measured as part of routine care using iodinated I131 albumin injection and the BVA-100 (Daxor Corp, New York, NY). Total body solute (TBS) was estimated at the time of BV measurement by multiplying the calculated total body water (TBW) by the sum of the serum Na+ and K+ plasma concentrations. TBW on admit was estimated with Watson’s formula (http://www.medcalc.com/tbw.html) accounting for the patient’s sex, height and weight. Daily water balance was estimated using nurse entered daily fluid balances. SI was calculated as heart rate/systolic blood pressure in mmHg. Absolute volumes and numbers representing total solute quantities were normalized as appropriate by dividing by patient mass. Data were analyzed with Excel for Mac 2011 (Microsoft, Redmond, WA). Linear regression was performed relating 1) repeated calculations of total body solute over time to repeated blood volume measurements in the 12 patients who had more than one BV measurement, and 2) shock indices and total blood volume for each patient who had at least 1 BV measurement and is reported as a coefficient of variation (r2).

Results: Overall, 56 patients were included. BV ranged from 75-150% of ideal. Shock indices ranged from 0.3 to 1.1 mmHg-1min-1. SI and BV were not correlated (r2=0.054, p=.09). Despite this, it appears that at values of shock index exceeding 0.85 mmHg-1min-1, the probability of hypervolemia becomes very small. Linear regression of change in total Na++K+ between measurements was very poorly correlated with change in blood volume between measurements (r2=.03, p=0.6).

Conclusion: When used to assess the state of the ECFV, change in calculated TBS and SI did not correlate with total circulating BV. Knowledge of this uncertainty should inform our care plans in the neurosurgical ICU, where relying on these values to direct care could reasonably be expected to lead to unpredictable results.

Keywords: blood volume, volume status, hypovolemia, shock index, electrolyte balance
Background: Patients with pulmonary hypertension (PHTN) presenting for elective surgery are at significantly higher risk for adverse perioperative outcomes, including increased hospital length of stay, right ventricular failure, cardiac arrhythmia, persistent postoperative hypoxemia, coronary ischemia and death. The diagnosis of PHTN is based on costly echocardiographic examination and right heart catheterization and should be reserved for high risk patients. No studies have assessed the role of self-reported functional classification on PHTN severity stratification, and few studies have achieved a sufficiently large patient sample size. We evaluate the predictive value of self-reported exercise tolerance on echocardiogram findings, outcomes, and length of stay (LOS) after non-cardiac, non-obstetric surgery.

Methods: We queried the University of Washington AMALGA database for all PHTN seen in pre-operative anesthesia clinic for non-cardiac, non-obstetric procedures from April 2007 through September 2013. Inclusion criteria mandated an echocardiogram <1 year prior to the procedure and available patient-reported functional status (< or >= 4 METs). Univariate analyses were used to compare functional status with echocardiographic findings, complication rates, and length of stay (LOS). To date, we have collected information on 539 procedures in 315 PHTN patients (51.1% female, average age 61.8 +/- 14.4 years) with pre-operative evaluations and functional status (FS) classification, out of an estimated total of ~1500 patient charts.

Results: Poor self-reported exercise tolerance (FS < 4 METs; 151 patients) was associated with ASA class >2 (p=0.015) and increased BMI (34.6 +/- 16.1 vs 29.4 +/- 10.3; p=.001) as well as comorbidities including a history of ventricular dysrhythmias (p<0.001), DM2 (p=0.004), OSA (p=0.003), angina (p=0.008), lower baseline oxygen saturation on pulse oximetry (96.4% vs. 97.5%, p=0.010) and higher resting heart rates (79.4 vs. 72.7 bpm, p<0.001) Decreased left ventricular function (p=.055), severe pulmonary hypertension (p=.039) and increased right atrial pressures (p<.005) were seen in a greater proportion of poor functional status patients. There was a statistically insignificant trend towards higher complication rates > 30 days post-hospital discharge in the FS < 4 METs subgroup (14.6% vs. 7.0%; p=0.041). Patients with FS < 4 METs demonstrated a greater LOS (7.21 +/- 13.20 vs. 4.75 +/- 9.91 days, p=0.047). In the entire cohort, six patients (2.0%) died within 30 days of their procedure. Logistic regression yielded poor functional status and an open surgical approach as independent predictors of LOS > 7 days.

Discussion: Patient-reported functional status demonstrates associations with cardiopulmonary comorbidities and decreased left ventricular function in PHTN patients. Further data collection and multivariate analyses can help elucidate the role of reported exercise tolerance as an independent cost-effective predictor of cardiac
function and post-operative complications. Development of a risk stratification approach can guide decision-making regarding escalated diagnostic workup and management prior to surgery.

Keywords: Pulmonary Hypertension, Functional Status, Post-Operative Outcomes

16: Estimated Blood Loss During Dilation and Extraction by Anesthetic Type
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Introduction: Dilation and evacuation (D&E) procedures are commonly performed in the first and second trimesters with GA neuraxial, or monitored anesthetic care (MAC). The most common complication is bleeding especially in second trimester resulting in greater risk of transfusion and mortality. This retrospective study investigates differences in estimated blood loss (EBL) between D&Es performed under GA vs MAC.

Materials and Methods: Anesthetic and operative reports (n = 235) for D&Es performed at UWMC in OR over four years were reviewed. Cases were analyzed by anesthesia type, EBL as reported by anesthesia, vs OB provider. 22% of cases did not have a numerical EBL, and only 4% were neuraxial or TIVA, all these were discarded from statistical analysis.

Results: D&Es performed under general anesthetic, the mean EBL was 287mL ±82mL (95% CI). Of the 108 procedures performed under MAC mean EBL was significantly less at 105 mL ± 27mL (95% CI) as shown in the chart below. A two-sided two sample t-test demonstrated a statistical difference in EBL (p = 6.53X10-5). None received transfusion and EBL did not vary significantly between providers.

Discussion: D&E cases under MAC had less blood loss compared with those under GA which could be attributed to many factors. Volatile anesthetics are known to have effects on uterine tone, GA is more likely to be performed if the case is complicated (abnormal placentation, molar pregnancy, increased gestational age). Further research is needed to elucidate the effects of anesthesia on blood loss adjusted for complication and other cofounders.

References:

Keywords: Dilation, extraction, MAC
17: Programmed Intermittent Epidural Bolus Versus Continuous Epidural Infusion For Labor Analgesia
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**Background:** Evidence that an epidural bolus provides a better spread of the injectate in the epidural space than a continuous infusion has emerged (1). Programmed intermittent epidural bolus (PIEB) results in lower local analgesia dosing, reduced motor block, instrumentation rates and physician-administered top-ups for breakthrough pain (2). In July 2014, each L&D room was equipped with a CADD®-Solis PIB Ambulatory Infusion System. We compared our continuous epidural infusion (CEI) protocol (10ml/h bupivacaine 0.0625%-fentanyl 2mcg/ml, 5ml PCEA bolus, 10min lock-out) with a PIEB setting using the exact same hourly & PCEA dose and lock-out time. The 1st PIEB was set to start 45min after initiation of analgesia with spinal dose (CSE), followed by 10ml PIEB q60min, 5ml PCEA bolus, 10min lock-out and a reset of the PIEB. The bolus rate was 250 ml/h (max speed with standard tubing). We hypothesized that PIEB would result in less physician-administered top-ups compared with CEI & PCEA.

**Methods:** Data was collected from April to December 2014 allowing a ‘before & after’ comparison. Demographics, anesthetic interventions (time to 1st physician-administered top-up, number of top-ups) and obstetric data (duration of 2nd stage, time to delivery, delivery mode) were recorded.

**Results:** Data from 240 cases were analyzed (120 PIEB vs 120 CEI). There was no difference in demographics, time from spinal analgesia to delivery, duration of 2nd stage or mode of delivery between groups (24% cesareans with PIEB vs 27% with CEI; p>0.05). There was no difference in the number of women requesting a top-up (50 with PIEB vs 45 with CEI group; p>0.05), median time until top-up or hourly top-up rate (Figure).

**Conclusions:** Contrary to our expectations, there was no difference in number or timing of top-up request between groups. This may be explained by the rather long interval between programmed boluses (60min), the 45min interval between spinal dose and 1st PIEB dose, and the low volume of PCEA bolus (5ml); this setting was chosen to keep the exact same hourly dose and PCEA settings. This pilot emphasizes the many variations in programming that need to be further tested, such as evaluating the analgesic effects of a shorter interval (45min) and larger PIEB & PCEA bolus (8ml). It also remains to be defined whether longer intervals offer other advantages besides improved analgesia such better voiding and maternal temperature profiles.


**Keywords:** PIEB, epidural, infusion, bolus, labor, analgesia
Introduction: Thrombocytopenia occurs more frequently in pregnancy with the majority caused by preeclampsia, gestational thrombocytopenia, and idiopathic thrombocytopenic purpura (ITP). Gestational thrombocytopenia and ITP are characterized by stable, but low, levels of fully-functional platelets. Preeclampsia-associated thrombocytopenia, on the other hand, is characterized by falling levels of abnormally-functioning platelets. Uncertainty exists as to the absolute platelet count required to safely perform neuraxial anesthesia and analgesia in the two different situations.

Methods: A retrospective chart review was performed at the University of Washington Medical Center from October 2012 through February 2015. All patients with platelet counts less than 100 x 10^9.L^-1 at the time of initiation of labor analgesia or surgical anesthesia, or the start of active labor if no labor analgesia was used, were identified. For each patient, the following data was recorded: cause of thrombocytopenia, platelet count, anesthetic technique, mode of delivery, and any neurologic deficits identified during hospitalization.

Results: 64 patients were identified having 67 deliveries. 17 had gestational thrombocytopenia, 10 had no diagnosis but likely had gestational thrombocytopenia, 9 had ITP, 6 had other diagnoses, and 22 had preeclampsia alone or the syndrome of hemolysis, elevated liver enzymes, and low platelets. Neuraxial analgesia or anesthesia was administered to 83% of the non-preeclamptic patients with platelet counts 50-99 x 10^9.L^-1. Neuraxial anesthesia was administered to 53% of the preeclamptic patients with platelet counts 50-99 x 10^9.L^-1, and to 100% of patients who desired it with platelet counts 75-99 x 10^9.L^-1. No neuraxial hematomas were identified.

Discussion: Our series adds to the body of literature supporting the safety of neuraxial anesthesia and analgesia for patients with platelet counts 50-99 x 10^9.L^-1 from causes other than preeclampsia. For pre-eclampsia-associated thrombocytopenia, there is no consensus on a safe cut-off given the dynamic nature of the disease. These findings support the suggestion that counts above 75 x 10^9.L^-1 immediately prior to needle placement are sufficient for preeclamptic women. Given the impossibility of doing randomized controlled trials to answer this question, retrospective reviews are our next best method to identify safety parameters for neuraxial analgesia and anesthesia in this potentially high-risk population.

Keywords: Regional Anesthesia, Obstetrics, Thrombocytopenia, Preeclampsia
19: Pregnancy outcomes following injury related to hospital trauma designation in Washington State

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Background: Approximately 8% of all pregnant women experience a traumatic injury during pregnancy, but there has been no prior evaluation of a state trauma system’s effect on maternal and neonatal outcomes. This study examined the association of treatment in a designated trauma hospital versus a non-trauma hospital on outcomes of injured pregnant patients.

Methods: This population-based retrospective cohort study linked the Washington State Birth Events Records Database and Comprehensive Hospital Abstract Recording System (from 1995 to 2012) to ascertain all pregnant patients who were hospitalized with an injury during pregnancy. The cohort was dichotomized by exposure to trauma versus non-trauma hospitals for injury care. We analyzed the association between trauma hospital designation and risk of adverse maternal and neonatal outcomes using logistic regression to estimate odds ratios (OR) and 95% confidence intervals (CI), adjusting for injury severity score.

Results: We ascertained 3,429 injured pregnant women for analysis. Women treated in trauma hospitals had an adjusted odds ratio (aOR) of 0.68 (95% CI: 0.47 – 0.99) for placental abruption, 0.62 (95% CI: 0.51 – 0.74) for preterm labor, and 0.82 (95% CI: 0.69 – 0.98) for cesarean delivery. Neonates of women treated in trauma hospitals had an aOR of 0.55 (95% CI: 0.40 – 0.75) for meconium at delivery and 0.66 (95% CI: 0.48 – 0.90) for fetal distress.

Discussion: Injured pregnant women treated at designated trauma hospitals had several improved maternal and neonatal outcomes. Trauma hospital treatment, with a greater focus on maternal resuscitation and monitoring, may explain these findings. This information may help guide future triage decision rules for injured pregnant patients.

Acknowledgements: The authors wish to thank the WA State Department of Health for data access, Mr. Bill O’Brien for programming and data management, and Dr. Jin Wang at the Harborview Injury Prevention and Research Center for providing the ICD-MAP-90 software used to calculate ISS scores. Dr. Krishnamoorthy is supported by an institutional training grant (National Research Service Award T32 GM086270).

Keywords: Pregnancy, Trauma, Outcomes
Background: Traumatic brain injury (TBI) is a major public health problem and leading cause of death and disability worldwide. As falls have now surpassed motor vehicle collisions as the leading cause of TBI in the United States, the incidence of isolated severe TBI is rising. No literature exists on risk factors for mortality in isolated severe TBI, a disease that has a distinct pathophysiology from severe TBI in the setting of multisystem trauma. We determined demographic, clinical, and facility-level risk factors for in-hospital mortality in this patient population.

Methods: We examined data from the National Trauma Databank (NTDB), excluding children, patients with non-isolated TBI, patients hospitalized for less than 48 hours, and patients who were transferred to other facilities. We described the mortality experience of our patient cohort, stratified by clinical, demographic, and facility-level characteristics. We used multivariable Poisson regression with clustered robust standard errors to analyze the association between demographic, clinical, and facility-level characteristics and in-hospital mortality. We performed multiple sensitivity analyses to test the robustness of our model assumptions.

Results: 40,590 patients were included in our analysis. The cumulative incidence of in-hospital mortality was 10.2%. In multivariable analysis, older age (RR 3.92, 95% CI 3.54 – 4.34), male gender (RR 1.17, 95% CI 1.09 – 1.25), admission hypotension (RR 1.83, 95% CI 1.61 – 2.09), need for mechanical ventilation (RR 4.18, 95% CI 3.64 – 4.80), high injury severity (RR 1.86, 95% CI 1.41 – 2.45), and poor initial neurologic grade (RR 3.06, 95% CI 2.74 – 3.43) were associated with a higher risk for mortality.

Discussion: There is a high burden on in-hospital mortality after isolated TBI, even in patients that survive the first 48 hours of hospitalization. Admission hypotension and the need for mechanical ventilation during hospitalization represent potentially modifiable risk factors for mortality after isolated severe TBI, above and beyond other demographic, clinical, and facility-level characteristics. Future research should aim to examine the impact of therapeutic and preventative measures for cardiopulmonary dysfunction after isolated severe TBI.

Keywords: TBI, outcomes, facility, risk factors
Immediate Post-operative Complications in Geriatric Trauma

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Background: It is expected that by 2050, there will be over 90 million adults over 65 years in the United States and trauma is the fifth most common cause of death in this group. Approximately 1/3rd of the elderly with Injury severity score (ISS) >15 die in hospital, and some of these deaths may be preventable. While intra-operative and peri-operative late complications of trauma in the elderly have been studied, there is a paucity of data about immediate post-operative complications in geriatric trauma patients. We examined the prevalence of common early post-operative complications among elderly injured patients who survived trauma surgery.

Methods: After IRB approval, we conducted a retrospective study at the University of Washington’s Harborview Medical Center, the only level one trauma center serving four US states (Washington, Alaska, Montana and Idaho). All charts of non-critical trauma patients requiring surgical intervention for trauma over a period of 19 months between May 2012 – November 2013 were examined. Intubated patients or those needing direct critical care admission from the operating theatre were excluded. Patients 65 years and over were compared with younger non pediatric patients with regards to immediate post-operative complications and resource utilization in the Post Anesthesia Care Unit (PACU). Multivariable Generalized Linear Models (GLM) were used to model the association between patient age and three outcomes: PACU time, PACU hypotension and need for intensive care unit.

Results: A total of 3,543 patient’s charts were examined. Of those, 3089 (87%) patients were under 65 years. 74.1% of the patients under 65 years were male compared with only 51.7% in the 65 years and over but ISS scores were similar (12.5). Average time to discharge readiness from PACU was also similar between groups (75 minutes for < 65 years and 78 minutes for ≥ 65 years). PACU hypotension (systolic pressure < 90mmHg for < 65 years and < 110mmHg for ≥ 65 years) occurred in 4.7% vs. 36.9%, respectively. Adjusting for cofounders including gender, ISS, operative time, BMI and intraoperative hypotension, PACU time increased by 8.4 minutes for each increasing year of age (p =0.01), and there was a 5% increase in the odds of PACU hypotension for every year of age above 18 years (P < 0.005). PACU hypoxia (O2 saturation < 90%) was less frequent in patients < 65 years (3.6% vs. 6.4%). Need for ICU was 12.8% for patients < 65 years vs. 24.5% in the older group. ICU length of stay was not significantly associated with age in this moderately injured trauma patient population. When stratified by age, there was a 32.4% need for ICU among the over 85 year age group.

Discussion: The rate of immediate post-operative complications such as PACU hypotension and hypoxia is significantly higher in the elderly trauma population, irrespective of gender or injury severity. Especially concerning is the 9X higher rate of PACU hypotension among elderly geriatric trauma patients who are not critically ill on admission. There may be a need for special care pathways geared toward the moderately injured geriatric trauma patient.

Keywords: trauma, geriatric anesthesia. PACU
22: Frequency and Outcomes of Emergency Department ICP Monitoring in Severe Pediatric TBI
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Background: The relationship between intracranial pressure (ICP) monitoring and outcomes in patients with severe traumatic brain injury (TBI) remains unclear. There are no data on the use of Emergency Department (ED) ICP monitoring in children with severe TBI. We aimed to examine the frequency and outcomes of ICP monitoring in the ED in children with severe TBI.

Methods: A retrospective multicenter cohort study encompassing five regional pediatric trauma centers affiliated with academic medical centers examined children under 18 years with severe TBI (admission Glasgow Coma Scale score ≤ 8, ICD-9 diagnosis codes of 800.0-801.9, 803.0-804.9, 850.0-854.1, 959.01, 950.1-950.3, 995.55, maximum head abbreviated Injury Severity Score ≥ 3) who received tracheal intubation for at least 48 hours in the ICU between 2007 and 2011. The main outcomes were in-hospital mortality and Discharge Glasgow Outcome Scale (GOS) score. A dichotomous measure of discharge GOS was used: “poor” (major impairment-vegetative state) versus “favorable” (baseline, minor-moderate impairment).

Results: Among the 224 patients admitted to the ED, 62 (28%) patients had ICP monitoring. Of those who had ICP monitoring in the ED, 50 (81%) had early ICP placement (within 4 hours of admission to ED). There was a trend towards lower in-hospital mortality with any ED ICP monitoring (aRR 0.56; 95% CI: 0.30, 1.05, p = 0.07). There was no significant difference in discharge GOS scores between patients who had any ED ICP monitoring as compared to those who did not (aRR 1.21; 95% CI: 0.85, 1.71).

Discussion: The frequency of ED ICP placement and monitoring is low and yet, ICP monitoring initiated in the ED may be associated with lower in-hospital mortality in children with severe TBI.

Acknowledgements: The other authors include MS Wainwright (Ann & Robert H. Lurie Children's Hospital of Chicago), JI Groner (Ohio State University College of Medicine), MJ Bell (University of Pittsburgh), CC Giza (Mattel Children’s Hospital, UCLA), and from the University of Washington: DF Zatzick, RG Ellenbogen, LN Boyle, PH Mitchell, FP Rivara, and A Rowhani-Rahbar.

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Keywords: brain injury, intracranial pressure, emergency department, pediatrics, trauma, outcomes
23: Timely Hemodynamic Resuscitation and Outcomes in Severe Pediatric Traumatic Brain Injury

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Objectives: Early resuscitation may improve outcomes in pediatric traumatic brain injury (TBI). We examined the association between timely treatment of hypotension and hypoxia during early care and outcomes in children with severe TBI.

Methods: Hypotension was defined as systolic blood pressure less than 70 + 2 (age in years), and hypoxia was defined as PaO₂ < 60 mmHg or oxygen saturation < 90% in accordance with the 2003 Brain Trauma Foundation guidelines. Timely treatment of hypotension and hypoxia during early care was defined as the treatment within 30 minutes of a documented respective episode in the prehospital or emergency department locations. Two hundred and thirty-six medical records of children under 18 years with severe TBI from five regional pediatric trauma centers were examined. Main outcomes were discharge mortality and Glasgow Outcome Scale (GOS) score.

Results: Hypotension occurred in 26% (60/234) during early care and was associated with in-hospital mortality (23.3% vs 8.6%; p = 0.01). Timely treatment of hypotension during early care occurred in 92% (55/60) by use of intravenous fluids, blood products or vasopressors and was associated with reduced discharge mortality (aRR 0.46; 95% CI 0.24, 0.90) and less likelihood of poor discharge GOS (aRR 0.54; 95% CI 0.39, 0.76) when compared to children with hypotension who were not treated in a timely manner. Early hypoxia occurred in 17% (41/236) and all patients received timely oxygen treatment.

Conclusions: Timely resuscitation during early care was common and associated with lower discharge mortality and favorable GOS in severe pediatric TBI.

Acknowledgements: Monica S. Vavilala, MD had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Keywords: Traumatic brain injury, Pediatric, Outcomes, resuscitation, hemodynamic, timely
Introduction: It is common practice to discontinue anti-platelet medications prior to elective neurosurgery. This study was performed to evaluate whether aspirin taken without cessation prior to emergency neurosurgery has a negative impact on patient outcome.

Methods: This is a retrospective chart review on emergency neurosurgical procedures (craniotomies and Burr holes) performed for traumatic subdural, extradural and intraparenchymal hemorrhage over a 5 year period (2008-2012) in a level 1 trauma center. Demographic data, past medical history including chronic antiplatelet and anticoagulant medications, ASA classification and Glasgow Coma Scale (GCS) score on admission, and surgical and anesthesia intraoperative data were gathered. Patients 50 years and older were included in the study. Exclusion criteria were: 1) chronic preoperative treatment with anticoagulants or antiplatelet agents other than aspirin, 2) concomitant traumatic injuries requiring surgery, and 3) repeat neurosurgery in the same admission. In-hospital mortality was considered as a primary outcome measure. Secondary outcome measures were: 1) perioperative volume of blood products transfused in a period of 48 hours before to 48 hours after surgery; 2) duration of ICU stay; 3) duration of mechanical ventilation, and 4) duration of hospital stay. Patients who received chronic aspirin therapy preoperatively (Aspirin Group) were compared to patients who did not receive aspirin (Non-Aspirin Group) using a logistic regression model to control for patient age, ASA class and admit GCS score, with the dependent variable for each outcome dichotomized to either greater than/equal to or less than a cutoff value chosen to demarcate an adverse vs. a routine outcome. Odds ratios for each category were compared between the aspirin and control group, with a 95% confidence interval not crossing 1.0 considered as significant.

Results: There were 310 patients identified in the cohort (68.2 ± 12 years, 63% male); patients in the Aspirin Group (n=120, 61% male) were older than patients in the Non-Aspirin Group (n=190, 64% male), (72.6 ± 12 vs. 65.5 ± 12 years, p<0.001), and had a higher GCS score (13 ± 4 vs. 11 ± 4, p<0.001). In-hospital mortality was 13% in Aspirin and 24% in Non-aspirin group. The unadjusted OR (0.45, 95% CI 0.24-0.84) shows significantly lower mortality in the Aspirin Group, and this OR is attenuated and becomes non-significant when adjusted for age, ASA class and admit GCS score (OR 0.68, 95% CI 0.34-1.39). Aspirin was a predictor for receiving perioperative platelet transfusion after adjusting for confounders (OR 4.136, 95% CI 2.305-7.421). None of the other secondary outcome variables show significant association with preoperative aspirin.

Conclusions: Chronic aspirin treatment without cessation in patients over age 50 undergoing emergency neurosurgery is not associated with increased mortality, and is associated with increased perioperative platelet transfusion.

Keywords: aspirin emergency neurosurgery craniotomy outcomes
25: Effects of Dexmedetomidine on Hemodynamics during Propofol-Remifentanil Anesthesia for Craniotomy
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Background: Dexmedetomidine is often used as an adjuvant to Total Intravenous Anesthesia (TIVA) with Propofol and Remifentanil for craniotomy. The potential advantages of addition of Dexmedetomidine, a selective α-2 agonist, include hemodynamic stability, reduced administration of vasoactive medications, and a quick and smooth emergence from anesthesia, although there are no data examining these effects. This study aims to compare the emergence characteristics and intraoperative hemodynamics between patients receiving Propofol-Remifentanil (PR) and Propofol-Remifentanil-Dexmedetomidine (PRD) for elective craniotomy for cerebral aneurysm clipping.

Methods: Following IRB approval, we retrospectively analyzed the electronic medical and anesthesia records of adult patients (≥ 18 years) who underwent elective craniotomy for aneurysm clipping at Harborview Medical Center between October 2012 and September 2014. Patients who received either (1) Propofol plus Remifentanil or (2) Propofol plus Remifentanil plus Dexmedetomidine were compared. The primary outcomes were systolic blood pressure and heart rate, intraoperatively, during emergence, and in PACU. Additional endpoints were average infusion dose of vasopressors, average TIVA infusion doses, emergence time from anesthesia, number of treatments for hypertension, incidence of PONV, and initial pain score in PACU.

Results: In preliminary analyses, intraoperative hemodynamic variables including mean systolic blood pressures, mean arterial pressures, and heart rates were found to be similar for each of the two groups. Additionally, phenylephrine infusion rates were similar for the PRD and PR groups (0.33 mcg/kg/min vs 0.36 mcg/kg/min, p=0.255). The PRD group had lower Propofol infusion rates compared to PR group (97.0 mcg/kg/min vs 111.8 mcg/kg/min, p=0.029) and higher Remifentanil infusion rates compared to the PR group (0.17 mcg/kg/min vs 0.15 mcg/kg/min, p=0.039). Surgery and anesthetic duration were longer in the PRD group compared to the PR group (338.6 and 486.0 minutes vs 282.8 and 416.1 minutes, p=0.004 and p=0.002 respectively), but emergence time was similar between groups (29.5 min vs 28.8 min, p=0.857).

Discussion: No differences in intraoperative systolic blood pressure, mean arterial pressure, or heart rate were found between the PRD group and the PR group, and phenylephrine infusion rates were also found to be similar between groups. Despite significantly longer duration of surgery and anesthesia, the emergence time in patients in the PRD group was comparable to the patients in PR group. This could be attributed to lower Propofol doses in the PRD group. It is also possible that this result was affected by a variable not yet examined in this study, such as incidence of prior SAH or type and location of aneurysm. Limitations of the study include its retrospective nature and no control for anesthetic depth. Inaccuracies in data recording with electronic medical records, including missing data, data artifacts, input
errors, and OR timing records, are also limitations of the study. Prospective comparison using a standardized treatment and blinded practitioners would be useful to validate these results.

Acknowledgements: Shu-Fang Newman for data abstraction from anesthesia records

Keywords: Craniotomy, Dexmedetomidine, hemodynamics, emergence, total intravenous anesthesia (TIVA)

26: Injury and Liability Associated with Spine Surgery

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Background: Previous analysis of the Anesthesia Closed Claims Project Database identified spine procedures as the second most common procedure resulting in hemorrhage malpractice claims (1). Recent investigations have identified other devastating complications associated with spine surgery, such as postoperative vision loss (2) and major adverse cardiac events (3). Surgical duration has also been identified as an important risk factor for high severity injuries during spine surgery (4). This study examined malpractice claims for spine procedures and compared them to other surgical procedures as well as factors within spine malpractice claims associated with severe injury.

Methods: After IRB approval, we identified 3097 surgical claims of which 333 were spine procedures that occurred in the year 1995 or later from the Anesthesia Closed Claims Project Database of 10,367 claims. Fisher’s exact test, T-test for equality of means, and Independent Samples Mann-Whitney U Test were used to analyze differences in spine claims with P<0.05 for statistical significance. Payments were CPI-adjusted to 2014 dollars.

Results: Spinal cord injury (45% versus 28%, p=0.002) and eye injury (18% versus 4%, p<0.001) occurred in greater proportion in spine surgeries than other surgeries, while death occurred less often in spine claims (23% versus 33%, p=0.001). A higher proportion of severe permanent injuries were associated with surgical duration greater than or equal to 4 hours compared to less than 4 hours (46% versus 27%, p = 0.003), and included eye injuries (25% versus 9%, p=0.001), positioning injuries (25% versus 11%, p=0.007) and massive hemorrhage (15% versus 5%, p=0.012). Almost all of the eye injuries (n=48, 96%) occurred in cases lasting greater than or equal to 4 hours, including 28 injuries to the optic nerve. Respiratory events, e.g. difficult intubation, were less often associated with longer spine surgeries (6% versus 19%, p=0.002). Anesthesia care was assessed as appropriate for spine procedures in greater proportion than other surgeries (65% versus 58%, p=0.013), but there was no difference in whether a payment was made (56% for both groups) or in the median payment made between spine surgeries and other surgical procedures ($407,625 and $281,325).

Discussion: Spine procedure claims represented greater than 10% of all surgical malpractice claims during the study period. While death occurred in lesser proportion
after spine procedures, other serious injuries, such as permanent severe nerve injury and eye injury, occurred more often in spine surgeries than in other surgical procedures. Longer duration of surgery was associated with a greater proportion of severe nerve injuries in anesthesia malpractice claims for spine surgery. This information can be used to guide surgeons, patients and anesthesiologists in shared decision making and development of strategies to optimize patient care from both a surgical and anesthetic perspective.

References:

Keywords: Closed Claims, malpractice, spine surgery complications

27: Liability Outside the Operating Room: Comparison of NACOR database with Closed Malpractice Claims

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Introduction: With precipitous growth in the complexity and volume of surgical procedures performed outside the operating room (OOR) there is an increasing need to understand anesthesia-related morbidity and legal liability in the OOR cases. Our previous closed claims (CC) review of cases performed OOR from 1990-2006 showed a 2-fold higher rate of deaths arising from OOR anesthetics compared to those performed in the (GOR) setting (1). In contrast, recent data from the National Anesthesia Clinical Outcomes Registry (NACOR) database did not reveal a greater incidence of anesthetic complications during OOR procedures (2). In this study we used the same two national anesthesia databases to compare the extent of malpractice claims (MC) associated with OOR practice to claims encountered in GOR settings in a more contemporary sample.

Methods: We examined the NACOR database of 12,252,846 cases from 2010-2013 [2]. Anesthesia MC for events occurring in 2000-2012 were derived from the CC Project database of 10,357 claims. Inclusion criteria were cases or claims for surgical anesthesia care. Obstetric procedures were not included. OOR locations were limited to gastroenterology (GE), cardiology, or radiology. OOR locations were identified by CPT codes in NACOR cases [2] and by locations as recorded in files for claims [1]. Characteristics of OOR MC's were compared to NACOR OOR cases. OOR MC were compared to GOR MC by Fisher’s exact test for proportions and Mann Whitney U test for inflation-adjusted payment amount.

Results: GE, cardiology and radiology cases together comprised 19% of NACOR anesthesia cases but only 4% of anesthesia MC in the CC registry. GE was the most common OOR location in both MC’s (51%) and NACOR cases (81%). Patients in OOR claims were younger (41% < 50 yr) compared to NACOR cases (30% <50 yr), less healthy (34% ASA 1-2 vs. 60%) and more likely to have sedation vs. general
anesthesia (69% sedation in claims vs. 38% sedation in NACOR cases). Mortality was 61% in the CC cases. Overall mortality in the NACOR sample of OOR cases was 0.02%.

When comparing OOR MC to MC arising from the GOR, respiratory events were more common in OOR locations (53% vs. 23%, p<0.001); inadequate ventilation or oxygenation occurred in one third (31%) of OOR claims. In 35% of OOR claims the injury was possibly, probably or definitely preventable by better monitoring compared to only 17% of GOR claims (p=0.001). Anesthesia care was more commonly assessed as substandard in OOR claims (66%) compared to claims from the GOR (44%, p=0.001). Payment was more common in OOR claims (72% vs 57%, p=0.014). When a payment was made, OOR claim payments were larger (median $554K vs. $285K, p=0.003).

Conclusions: OOR MC represented a smaller proportion of total claims than the proportion of OOR cases in NACOR. This may suggest lower anesthesia liability associated with locations OOR or alternatively result from the rapid recent increase in the number of these cases, not yet reflected in CC registry. In MC from OOR, mortality was higher, payments were more likely, and larger than in GOR claims. Most OOR anesthesia MC involved respiratory events during sedation and were assessed as possibly preventable by better monitoring.

References:

Keywords: Anesthesia outside of the operating room; outcomes; liability

28: The Price of Pain: National Health Expenditures associated with Pediatric Pain-related Conditions
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Background: Pediatric pain is increasingly being recognized as a major cause of morbidity in children; however associated national healthcare expenditures are unknown. The primary objective of this study was to assess the impact of pediatric pain on national healthcare expenditures in comparison to healthcare expenditures for other common childhood conditions including asthma, attention deficit hyperactivity disorder (ADHD), and obesity.

Methods: We analyzed data from a nationally representative sample of 6-17 year old children (n=1,544) in the USA captured in the 2007 National Health Interview Survey and 2008 Medical Expenditure Panel Survey. Healthcare expenditures of children with pain were compared to children without pain and also to children with asthma, ADHD, and obesity.

Results: Across the sample 18% of parents reported that their child had a pain-related condition (migraine, other chronic headache, neck, back, abdominal, and other chronic pain) over the previous 12 months. Pediatric pain was associated with incremental healthcare expenditures of $1,339 (95% CI $248-$2,447) per capita relative to children without pain. Nationally, pediatric pain was associated with $11,8
billion (95% CI $2,18 - $21.5 billion) in total incremental healthcare expenditures. The incremental total healthcare expenditures associated with pain were similar to those of ADHD ($9.23 billion; 95% CI $1,89-$18.1 billion), but more than those associated with asthma ($5.35 billion; 95% CI $0-$12.3 billion) and obesity ($0.73 billion; 95% CI $-6.28- $8.81 billion) in childhood. These findings suggest that healthcare expenditures for pediatric pain-related conditions exert a considerable economic burden on society. Efforts to prevent and treat pediatric pain are urgently needed.

Acknowledgements: This project was partially supported by a faculty fellowship award from the Department of Anesthesiology & Pain Medicine at Seattle Children’s hospital and the first author (CG) was supported by National Institutes of Health Ruth L. Kirschstein National Research Service Award Institutional Research Training Grant T32GM086270 (TP).

Keywords: health care expenditures, pediatric pain

29: The Role of Pain Catastrophizing in Children’s and Parents’ Pain Memories After Surgery
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Objectives: Children’s memories for pain influence subsequent pain experiences. Parents’ memories may also impact children’s pain experiences, by influencing parent-child interactions about pain and children’s cognitions and behaviors. Pain catastrophizing (i.e., tendency to magnify/perseverate on the threat of, and feel helpless in response to pain) in either children or parents has been identified as a factor underlying memory biases over time; however, this has not been empirically examined. This is the first longitudinal study to examine the role of child and parent pain catastrophizing in the development of pain memories following a painful event, in this case major surgery.

Methods: Participants were 49 children (32 girls, Mage=14.70 years, SD=1.98) undergoing major surgery and their parents (89.4% mothers). One week before surgery, children and parents completed measures of pain catastrophizing. Two weeks following surgery (the acute recovery phase), children and parents completed measures of child pain intensity and pain affect. Following surgery (at 2-4 months), children and parents were interviewed via telephone and their recall of child pain intensity and affect were elicited.

Results: Hierarchical linear regression models revealed that over and above covariates (e.g., age, sex, initial pain ratings), parent catastrophizing about child pain accounted for significant variance (22%,37%) in children’s affective and parents’ sensory pain memories. Whereas parent catastrophizing had a direct effect on pain memories, mediation analyses revealed that child catastrophizing indirectly influenced the development of children’s and parents’ pain memories through the child’s pain in the acute recovery phase following surgery (Bindirect=.12,.06).
Conclusions: Children and parents who engage in more catastrophic thinking about child pain prior to surgery develop more distressing pain memories several months later. However, parent catastrophizing demonstrated the greatest influence on children’s and parents’ evolving cognitions about child pain.

Relevance to topics of interest: Findings extend lab-based research on healthy children’s anxiety and pain memory development to clinical samples of youth and their parents.

Learning Objective: Based on the content of this session, attendees will be able to explain the process by which parents’ and children’s catastrophic thinking about child pain before major pediatric surgery influences the development of their memories of pain over time.

Keywords: Surgery, pain, memory, children, parents, catastrophizing

30: Predictors of Pain Trajectories and the Impact on Health Outcomes in Children after Major Surgery

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Background: 1.5 million children undergo inpatient surgery in the U.S. annually, and many experience significant pain and distress. Emerging research suggests that pain may persist in the long-term for some children after surgery, but there are limited data on the course of pain following major surgery and predictors of poorer outcomes. Child and parent psychological factors (anxiety, worries about pain) have been reported in prior studies to relate to postsurgical outcomes but their relationship to pain trajectories is not clear. The aims of this study were to address these gaps by 1) characterizing pain trajectories using repeated assessments in the 12 months following surgery, 2) identifying presurgical child and parent psychological risk factors of persistent pain, and 3) examining relationships between pain trajectories and long-term health and functional outcomes in children after major surgery.

Methods: Sixty children age 10-18 years (M=14.7), 67% female, 83% white, undergoing major spine or pectus surgery without major comorbidity, and their parent/guardian participated in the study. Data were collected from baseline (pre-surgery) to 12 month follow up at 4 time points. Participants completed validated questionnaires assessing psychosocial factors (Pain Catastrophizing Scale- child and parent versions) during the week prior to surgery. Children completed a 7-day electronic pain diary assessing pain intensity (numeric rating scale, 0-10) at baseline, 2-weeks, 4-months, and 1 year after surgery. In-hospital pain scores were collected from the electronic medical record. Mean pain intensity was calculated for each time-point. At 12 months, children repeated assessments of health-related quality of life (Pediatric Quality of Life Inventory), and activity limitations (Child Activity Limitations Interview), and parents reported on children’s health-care utilization (Client Service Receipt Inventory) over the 12 months since surgery.

Results: Group-based longitudinal mixture modeling identified two distinct trajectory groups of postsurgical pain: early recovery (78%), and late recovery (22%). The
early recovery group had progressively decreasing pain after surgery up to 12 months. The late recovery group had increasing pain initially, followed by a gradual decrease up to 12-months. In a logistic regression model adjusting for age and gender, greater parental pain catastrophizing before surgery significantly predicted membership in the late recovery group (OR=1.11, p=.03). Child psychological factors and baseline pain did not predict pain trajectories (p’s>.05). In a multivariate linear regression analysis adjusting for age and gender, membership in the late recovery group was significantly associated with poorer health-related quality of life (β=-10.7, p=.02), and greater activity limitations (β=3.6, p=.04) at 1 year after surgery. **Discussion:** Longitudinal analyses identified two distinct trajectories of postsurgical pain characterized by early versus later recovery of pain. A sizeable subgroup of children (22%) had prolonged recovery of postsurgical pain, which significantly impacted their health outcomes at 12-months. Pain trajectories were predicted by baseline parent psychological factors, suggesting that preoperative interventions modifying parent behaviors and cognitions might be useful in this population. Supported by K23HD078239. **Keywords:** Postsurgical pain, chronic pain, health outcomes, health-related quality of life, pediatric surgery

31: Intervention to Reduce Distress for Parents of Youth Receiving Intensive Pain Rehabilitation
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**Background:** Chronic pain is stressful for children and families. Intensive pain rehabilitation programs require spending several weeks at the hospital, which can add to family burden. These families may benefit from treatment to decrease parental stress. Problem-solving therapy has previously proven effective in reducing parental distress in other pediatric chronic conditions. This study aimed to adapt and evaluate the feasibility of problem-solving therapy (PSST) delivered during an intensive pain rehabilitation program to reduce distress in parents of youth with chronic pain.

**Methods:** In the first phase, an existing PSST manual for parents of youth in outpatient pain treatment was adapted, including modifications to session format and treatment material to suit parents of youth receiving intensive pain rehabilitation. In the second phase, the intervention was tested in an uncontrolled trial to evaluate feasibility determined by response to treatment content, ratings of acceptability, and ability to deliver treatment visits. In exploratory analyses, we examined change in parent distress, parent problem solving skills, and child physical function from pre- to post-treatment and three-month follow-up.

**Results:** The intervention was piloted with 19 parents (15 mothers and 4 fathers, M age = 45.12 years) of youth with chronic pain (M age = 14.4 years. 59% female). Parents completed 1-6 sessions (M = 4.14); 79% completed treatment. Parents had
few no-shows (range 0-1) and rescheduled sessions (range 0-3). Sessions were completed equally in person (52%) and by phone (48%). Therapists rated parents as motivated to participate (M = 8.02/10), receptive to learning (M = 7.97/10), demonstrating good understanding of the PSST process (M = 8.31/10), completing homework (M = 7.63/10), and having good rapport (M = 8.10/10). Parents were moderately satisfied with treatment (M = 32.56/45) and generally found it to be an acceptable treatment (M = 4/5). Parents demonstrated positive change in parent distress from pre-treatment (M = 56.1) to post-treatment (M = 51.7) that was maintained at three-month follow-up (M = 50.0) (p < 0.05). Parents also demonstrated increased problem solving abilities from pre-treatment (M = 100.8) to post-treatment (M = 101.9) that was maintained at three-month follow-up (M = 105.9) (p < 0.05). Children demonstrated positive change in physical function from pre-treatment (M = 16.4) to post-treatment (M = 9.3) that was maintained at three-month follow-up (M = 9.5) (p < 0.05).

Discussion: Results support the feasibility of delivering PSST to caregivers of youth receiving intensive pain rehabilitation. PSST may be a promising treatment for decreasing distress among parents of youth receiving intensive pain rehabilitation. Our future work will use a semi-structured interview to examine parent perceptions of PSST and inform further modifications to the intervention that may enhance treatment satisfaction.

Acknowledgments: This research was supported by the Seattle Children’s Research Institute Center for Child Health, Behavior and Development Small Grant and Stimulus Funds (PI: Law).

Keywords: Chronic pain, pediatric, parent, problem solving

32: Impact of Pain on the Prevalence and Incidence of Activity-Limiting Fatigue among Older Adults
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Background: Fatigue is a common complaint among older adults; however, the dynamics of activity-limiting fatigue have not been well characterized among older adults with and without pain. Accordingly, we sought to determine the prevalence, incidence, and recovery rates of activity-limiting fatigue according to pain characteristics in a national, prospective cohort of older adults.

Methods: Data from the 2011 and 2012 National Health and Aging Trends Study were analyzed. In-person interviews were conducted in 7,592 community-dwelling Medicare beneficiaries ages >=65 years (response rate=71.0%). Participants were asked whether they had been bothered by pain and the location of pain as well as whether “low energy or exhaustion” limited their activities. Poisson and multinomial logistic regression models were used to assess associations of pain with the prevalence, incidence, and recovery rates of activity-limiting fatigue.

Results: Pain was reported by 52.9% of the population at baseline. Comparing participants with and without pain revealed that the prevalence of activity-limiting fatigue was 44.2% and 13.6%, respectively [age- and sex-adjusted prevalence ratio (PR)=3.19; 95% confidence interval (CI):2.87-3.55]. Among participants with 0, 1, 2,
3, and ≥4 sites of pain, prevalence of activity-limiting fatigue was 13.6%, 24.7%, 33.4%, 44.2%, and 65.3%, respectively (P<0.001 for trend). In participants without fatigue at baseline, the 1-year incidence of activity-limiting fatigue was 20.3% and 11.7% among those with and without pain, respectively [age- and sex-adjusted risk ratio (RR)=1.70; 95% CI:1.42-2.04]. Importantly, in those reporting activity-limiting fatigue at baseline, the 1-year recovery rates were 34.5% and 47.5% in older adults with and without pain, respectively [age- and sex-adjusted RR=0.73; 95% CI:0.62-0.84]. Associations were robust to adjustment for several potential confounders, including comorbidity, physical capacity, and symptoms of anxiety, depression, and poor sleep.

**Discussion:** In summary, older adults with pain have a higher burden of activity-limiting fatigue than those without pain, and were more likely to develop (and less likely to recover from) activity-limiting fatigue over a 1-year follow-up period. Considering that fatigue is a powerful predictor of disability in older adults and a key component of frailty, a major geriatric syndrome, multifactorial interventions designed to improve the functional capacity of older adults with pain will need to address fatigue as a comorbid, disabling symptom. Accordingly, further investigation of the biopsychosocial mechanisms underlying the associations observed in the current study is needed.

**Acknowledgements:** The National Health and Aging Trends Study is sponsored by the National Institute on Aging (grant number NIA U01AG032947) through a cooperative agreement with the Johns Hopkins Bloomberg School of Public Health. The National Institute on Aging had no role in the design and conduct of the current study; management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript.

**Keywords:** Pain; Aging; Fatigue; Physical Capacity; Epidemiology; Geriatrics

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**33: Injury and Liability Associated with Implantable Devices for Chronic Pain**

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**Background:** Implantable drug delivery systems (IDDS) and other implantable devices for the management of chronic pain have been used since the early 1990s. Morbidity and mortality from IDDS and spinal cord stimulators typically result during surgical phases (implantation or removal of devices) or maintenance(1,2) with higher mortalities particularly associated with intrathecal drug (opioid) delivery(3). We investigated liability associated with devices used to manage chronic pain.

**Methods:** After IRB approval, we identified 941 chronic pain claims of which 142 were related to devices and care that occurred in the year 1990 or later from the Anesthesia Closed Claims Project Database of 10,367 claims. Fisher’s exact test, chi-square analysis, and Independent Samples Mann-Whitney U Test were used to analyze differences in device claims with P<0.05 for statistical significance.

**Results:** The most common devices were IDDS (n=90, 63%) of which 38 claims were for maintenance of IDDS. Although 64% of all patients with device-related claims experienced temporary or minor injuries, 57% of IDDS maintenance claims (p<0.001) experienced either death (18%) or severe permanent injuries (39%) with
13% of claims resulting in severe permanent brain damage. Death and brain damage in maintenance claims resulted from medication administration errors, e.g., pocket and side port-fills (n=7), programming errors (N=6), and wrong drugs (N=3), while spinal cord injury was the result of delayed recognition of granuloma formation (n=9).

104 claims were for surgical events: 41 (29%) were for spinal cord stimulators (39 for implantation and 2 for removal), 52 (50%) for IDDS (44 for implantation and 8 for removal), and 11% for other devices (tunneled catheters = 8 and peripheral stimulators = 3). Permanent severe injury occurred in 24% of claims related to nerve stimulators. Claims for implantation or replacement of IDDS resulted in death (12%) and permanent severe injury (24%). The most common damaging events for implantation and replacement of all devices were infections (n=24) and needle trauma to cord or cauda equina (n=10) and for removal of devices was retained catheter fragments (n=6).

Care was assessed as less than appropriate in 76% of IDDS maintenance claims, compared to 43% of all other device claims (p=0.001). Payment was made in 62% of IDDS maintenance claims compared to 34% of all other device claims (p=0.003). The median payment was highest for claims for IDDS maintenance ($334,526) and lowest for the removal of IDDS ($67,304).

**Conclusions:** Maintenance of IDDS was associated with death, permanent brain damage, or permanent neurological injury from granuloma and was largely associated with substandard care resulting in payment. The majority of the substandard care associated with maintenance of these pumps involved medication administration errors and failure to recognize progressive neurological deterioration. Surgical phases of device implantation were associated with infections or permanent neurological injury from poor surgical technique involving needle trauma to cord or cauda equina. These findings demonstrate the need for providers to exercise caution in these areas in order to avoid severe complications.

**References:**
1. Deer TR Neuromodulation 2012 15(5)467-82
3. Coffey RJ Anesthesiology 2009 111(4)881-891

**Keywords:** chronic pain, liability, implantable devices

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**34: Predictors of Control Preferences in the Pre-Anesthesia Clinic**

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**Background:** Shared decision making is a model of practice to increase patient engagement in preference-sensitive decisions that improves outcomes and patient satisfaction. There is also a growing interest for greater patient engagement underlined by a provision of the Affordable Care Act. However, patient factors related to engagement styles are not well understood and prior studies of control preference predictors have yielded inconsistent results in various healthcare settings [Florin et al, JCN 2008 & Rodriguez et al, JHC 2013]. In this study, we hypothesized that
female gender, lower education level, and older age will be associated with passive control preferences in which the patient prefers the physician to make medical decisions.

**Methods:** Patients eligible for regional anesthesia for their procedure or postoperative pain management were surveyed prior to their pre-anesthesia clinic (PAC) visit. The anonymous survey included demographic questions and Degner’s control preferences scale [CJNR 1997]. Subjects with active, collaborative, and passive control preferences were compared by t-test and chi-square test with p<0.05 for statistical significance.

**Results:** Lower education level was associated with passive control preferences (p=0.027). Subjects with passive control preferences tended to be older than those with either collaborative (p=0.007) or active (p=0.001) control preferences. However, active versus collaborative control preferences were not affected by age (p=0.320). There was no significant association between gender and control preferences (p=0.731).

**Discussion:** The PAC protocol schedules visits for patients >50 years of age or with comorbidities. Despite the older subject population, our study still showed a tendency for comparatively older subjects to be more passive in their control preferences compared to relatively younger subjects. Our study also found that less educated patients had more passive control preferences. Decision-making is a multifaceted process and patient preferences could change with health progress. Patients’ preferences for participation in medical decision making should be assessed and reviewed frequently so that healthcare providers may adjust and continue to aim to match patients’ control preferences.

**Acknowledgments:** I would like to thank Dr. Karen Posner, Dr. Karen Domino, and Shawn Mincer for their support throughout this project. I would also like to thank the University of Washington School of Medicine for their contributions to this project.

**Keywords:** Shared decision making, control preferences, education level, pre-anesthesia clinic

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**35: Regional Anesthesia Decision Aids Improve Patient Engagement and Understanding**

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**Introduction:** One component of the perioperative surgical home is to provide patients the ability to participate in decisions regarding their care, including shared decision-making.[1] In preparation for implementing shared decision-making in our pre-anesthesia service, our needs assessment found that patients had poor understanding of regional anesthesia and desired information about their anesthesia and postoperative pain management choices. [2] The current study evaluated whether introduction of regional anesthesia (RA) decision aids during the pre-anesthesia clinic visit improved patient engagement and knowledge surrounding their anesthesia options.

**Methods:** After IRB approval, consenting adult English-speaking patients who were likely to be offered regional anesthesia for their procedure or postoperative pain
management had their clinical encounter observed and completed a post-encounter survey. Observations recorded whether RA was mentioned, what was mentioned (nature, risks, benefits, alternatives, understanding, preferences), and if the patient asked questions about RA. The survey included questions about patient desire for information, demographic questions, and the Degner control preferences scale [3] to evaluate whether subjects were active, collaborative, or passive in medical decision-making. A RA knowledge test was administered at the end of the clinic visit, and patients completed anxiety state [4] and decisional conflict [5] assessments. Subjects who received a RA decision aid prior to their clinical encounter (POST) were compared to patients who did not receive a decision aid (PRE) by Fisher’s exact test and t-test with p<0.05 considered statistically significant.

**Results:** The 126 patients were similar PRE and POST in age (mean 58 ± 12), gender (54% male), education level (42% college graduates), and control preferences (72% active or collaborative). In observations of clinical encounters POST introduction of RA decision aids, mention of RA was more common (64% vs. 49%, p=0.015) and patients were more likely to ask questions about RA (70% vs 32%, p=0.001, Figure). In 85 observed visits with mention of RA, POST visits included more of the 6 items tracked (p=0.007). Specifically, alternatives were more likely to be mentioned in POST than PRE visits (78% vs. 52%, p=0.016). POST patients more commonly expressed a desire for written information about their anesthesia choices (71% vs. 40%, p=0.004). POST patients had greater understanding of RA, with knowledge test scores of 53% compared to 30% for PRE patients (p=0.001). There were no differences in anxiety or decisional conflict.

**Conclusions:** When there is >1 clinically appropriate option for anesthesia and postoperative pain management, patients seem primed to engage in shared decision-making if given information about their anesthesia choices. Greater patient engagement and understanding of anesthesia options is consistent with a perioperative surgical home and is easily accomplished through introduction of decision aids during the pre-anesthesia evaluation visit.

**References:**
1. ASA Committee on Future Models of Anesthesia Practice, 7/15/13.
5. Légaré F. Can Fam Physician 2010, e308

Keywords: regional anesthesia, decision aids, shared decision-making, perioperative surgical home, patient engagement

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**36: The Value of Preoperative Laboratory Testing in Orthopedic Surgeries**

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**Background:** Several studies have reported that routine preoperative testing does not change perioperative management nor does it affect perioperative adverse events in patients undergoing elective surgeries.
The aim of this study was 1) to determine whether indicated preoperative laboratory testing affects length of stay (LOS) and mortality rates in patients undergoing orthopedic surgery and 2) to compare the costs between routine testing and indicated testing.

**Methods:** A novel clinical protocol of clinically indicated preoperative testing for patients undergoing elective orthopedic surgery was introduced in March 2012 at the Pre-Anesthesia Clinic at a 500–bed tertiary Veterans Affairs Puget Sound Health Care System–Seattle Division. The protocol included indicated testing guided by clinical history, physical examination, and invasiveness of planned surgery. After Institutional Review Board approval, all the patients who received their preoperative evaluation from July 2010 to November 2013 were included into the cohort. The gathered data included a complete blood count, chemistry 7, liver function tests, anticoagulation tests, albumin, hemoglobin A1C, electrocardiogram, and chest radiography. A retrospective analysis was conducted to compare LOS, in-patient and postoperative mortality rates, and costs associated with testing between the "indicated testing" group of patients who underwent surgery between March 2012 and November 2013 and the historic control of “routine testing” group between July 2010 and March 2012 using VA Informatics and Computing Infrastructure database. Chi-square and ANOVA analyses were used to compare the number of preoperative tests, mortality rates, and LOS between the routine and indicate testing.

**Results:** A total of 2722 orthopedic surgeries in 2473 patients were identified: 1385 (50.9%) surgeries in 1257 patients in routine testing group and 1337 (49.1%) surgeries in 1216 patients in indicated testing group. Patient age (58.34 years SD ± 13.2), gender (91% male, 8.9% female), ASA class (approximately 50% class III), and clinical risk factors were similar between the two groups. There was a significant reduction in the number of tests ordered in the indicated testing period (p=0.000) but no significant differences in LOS (p=0.399), nor in-patient (p=0.399), 30-day (0.363) and one-year (p=0.185) mortality rates between the two groups. Indicated testing resulted in a total cost saving of $50,141.17.

**Discussion:** Routine laboratory testing may be eliminated as it does not have an impact on perioperative outcomes while causing unnecessary spending. Clinically indicated preoperative approach to the ordering of preoperative testing in patients with comorbidities seems to be safe and cost effective.

**Keywords:** preoperative indicative tests cost-effectiveness

**37: Consultation Network Topography in Patients Undergoing Total Knee Arthroplasty.**

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**Introduction:** Consulting subspecialties/services is central to health care delivery. We evaluated a network of consultations done on patients with a history of Total Knee Arthroplasty (TKA) within one year in a single VA hospital. We hypothesized that network topography of subspecialty/services consultations is a function of a population morbidity along with local practice. The goal of the study was to elucidate the structure of a consulting network. In addition we sought to observe the structure of prescription medication network for these patient.
Method: Following IRB approval, a patient cohort comprising of all patients in the VA who had TKA between the years 2011-2012 in the Seattle VA Medical Center was obtained. A directed consultation network was constructed where the 'From Service' and 'To Service' served as nodes and a directed edge between them. A bi-partite network was constructed from the drugs prescribed for each patient and projected onto the drug space. Network coefficients were computed: node degree, betweenness centrality, and eigenvector centrality. Membership in largest connected component was assigned for each node in the network. A community detection algorithm was applied, to detect discrete networks of consultation within the network. The distribution of the node degree was parametrized. Network were plotted showing the most consulted services, and the communities of consulting services which frequently work together.

Results: Total of 171 patients were included where 343 consultation were made. By node degree the most consulted service was non VA care (i.e. seeking care outside the VA) followed by physical therapy prosthetic requests social worker, podiatry. The network demonstrates a scale free network topography (1) with power law degree distribution (alpha to be 2.4). Largest connected component was that of social workers followed by mental health. Plotting betweenness against network degree showing orthopedic surgery to have high betweenness and low degree, podiatry to have high degree but low betweenness and non VA care to have both high degree and betweenness centrality. The projection onto the medication prescription medication subspace shows a scale free distribution where the leading drug classes are opioids, followed by anti-depressants and anxiolytics.

Discussion: Analysis suggests most consultation work is in a few areas namely social workers, mental health, urology and gastroenterology. The most commonly consulted services were the non VA care, physical therapy, musculoskeletal outpatient clinics and social workers. The care in patients undergoing TKA in the Seattle VA medical center can be characterized by many requests for non VA care, while the main bulk of consultation within the VA utilizes physical therapy, musculoskeletal clinics and prosthetics services. Sub-networks are those of social workers and mental health. This is mirrored by a patient population with many musculoskeletal disorders which consumes opioids, NSAIDs and anti psychotic medications. Some services serve as 'gatekeepers' to other services(orthopedics), while others are 'end-users' (podiatry). We suggest applying network analysis for insights across hospitals and patients for comparison.

References:

Keywords: scale free networks, social network analysis, consultation, patient phenotyping
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### 38: Vasopressor Use During Microvascular Free Flap Surgery- A literature review.

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**Background:** Some current otolaryngology textbooks still warn against vasopressor use in microvascular free tissue transfer. The concern is the denervated flap is at risk for failure due to arterial or venous thrombus, vasospasm, poor perfusion, and infection. Provider differences are found regarding the use of vasopressors during free flap surgery. Of 39 otolaryngology surgeons surveyed 23% felt vasopressors contributed to flap failure.

**Methods:** A literature review was done in order to determine if there is any benefit or harm for flap survival and complications with vasopressor use during free flap surgery. PubMed and Google Scholar was searched using relevant key words. 10 human studies 6 animal studies and 4 recent literature reviews were found in English.

**Results:** 7 retrospective reviews in free flap surgery were found. 2 in head and neck surgery (47 and 485 patients) 1 review of breast flaps (n=169), 1 in jejunal flaps (n=110). 3 reviews included all free flap surgeries (n=169, n= 169, n=400). In all articles vasopressor use did not affect flap outcome, increase complications or
predict failure. Two studies measuring blood flow in 25 human flaps found norepinephrine and dobutamine increased free flap blood while epinephrine and dopexamine decreased flow. In 20 patients dobutamine increased flap blood flow better than dopamine. One study showed milrinone had no effect on flap outcome and increased vasopressor use (n=88).

Two swine studies have conflicting results of vasopressor augmenting flap blood flow, with phenylephrine increasing total flow in one study while decreasing in another at higher doses. One rat study showed phenylephrine increased MAP but decreased cutaneous flow but another found it increased flap flow. Dobutamine was beneficial while epinephrine was not in another rat study on flap flow. There are two conflicting rat studies on whether denervation supersensitivity to alpha agonist occurs.

There is limited evidence to guide pressor choice in rotational flap surgery. Epinephrine has been shown to be beneficial in one porcine model while phenylephrine was not.

Review articles using above studies and additional animal studies: Patanni 2009 “No available clinical evidence to support prohibition on pressor use”. Brickman 2012 “No clinical evidence against the selective use of vasoactive agents during free flap surgery. There may be a beneficial effect of the use of dobutamine on free flap perfusion”. Ahmed 2013 “No reliable prospective clinical evidence that supports the absolute contraindication of pressor agents during free flap surgery”. Motakef 2014 “Vasopressors when indicated for hypotension did not increase flap failure or complication rates in breast reconstruction, head and neck surgery, in upper or lower extremity reconstruction. Cumulative dosage and timing of vasopressor administration are not correlated with adverse outcomes (level of evidence 2b) Consider norepinephrine and dobutamine for hypotension in free flap surgery (1b). Dobutamine improved anastomotic blood flow in head and neck free flap surgery (1b)”

Discussion: While animal studies are conflicting. Human retrospective reviews suggest that it is safe to administer vasopressors intraoperatively for free flap surgery. Norepinephrine and dobutamine are recommended first choice, while phenylephrine and ephedrine and other vassopressors have been safely used in usual clinical doses.

Keywords: vassopressors, free flap, outcomes, surgery

39: Use Of Mechanical Support For Failing Single Ventricle Physiology
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Introduction: Medical and surgical advancements allow congenital single ventricle patients to survive into adulthood. As this population ages, more patients suffer from acute or chronic decline in cardiac function. In patients unresponsive to medical therapy there are few treatment options due to the scarcity of transplantable organs and limited experience in the use of implantable assist devices. We present two cases of Impella® (ABIOMED, Inc. Danvers, MA) placement in patients with a history of Fontan palliation.

Case Presentation: Patient 1 is a 19-year-old male with a history of hypoplastic left heart syndrome treated with staged Norwood, Glenn, and lateral tunnel Fontan palliations. He presented in acute decompensated systolic heart failure with progressive multi-organ dysfunction despite medical treatment. An Impella CP® device was placed on hospital day 2. The patient’s inotropic support was weaned and he was subsequently diagnosed with hemophagocytic lymphohistiocytosis and Epstein Barr viremia and began appropriate treatment. On hospital day 13 the Impella CP® was removed. He was discharged home on hospital day 27. Patient 2 is
a 31-year-old male with a history of double inlet left ventricle and l-transposition of the great vessels treated with a lateral tunnel Fontan and a Damus-Kaye-Stansel modification. He was transferred to our institution after a witnessed cardiac arrest and remained unstable with end organ dysfunction despite medical treatment. An Impella 5.0® was placed on the day of hospital transfer. The patient remained dependent on the Impella® device and underwent HeartWare® (HeartWare®, Framingham, MA) implantation and Impella® removal on hospital day 11. At the time of this submission, Patient 2 remains hospitalized as he continues to recover from his multi-organ dysfunction.

**Discussion:** The Impella CP® and Impella 5.0® are percutaneously delivered catheter-based left ventricular assist devices (LVADs) that provide temporary circulatory support. They function through an axial flow pump that offloads the left ventricle and improves coronary perfusion through a reduction in myocardial workload and increased cardiac output. Device functionality is dependent on a competent aortic valve. The use of an Impella® as a support strategy for failing atrio-pulmonary Fontan circulation has been described in pigs.(1) In other experimental models Impella® devices were singularly inadequate for cavopulmonary support due to severe flow recirculation.(2) The use of the Impella® in these experimental situations (1,2) is very different than using the Impella® for decompensated systolic heart failure. For direct cavopulmonary support Impella® usefulness appears uncertain. LVAD implantation in heart failure patients with Fontan palliations has been reported in certain children and adults.(3) To our knowledge, however, our patients represent the first examples of Impella® placement for refractory cardiogenic shock in patients with prior Fontan palliations. Patient 1 was successfully weaned from the majority of his vasoactive support and then safely decannulated. Patient 2 utilized the Impella® device as a bridge to implantable LVAD. As the congenitally palliated heart population continues to age, these types of support strategies will likely become more commonplace.

**Keywords:** Impella, Fontan, LVAD, single ventricle physiology

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**40: Diffuse Intramyocardial Air after David-V Aortic Root Reconstruction**

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**Introduction:** Coronary air embolism (CAE) is a common intraoperative complication of cardiac surgery. The right coronary artery distribution is most commonly affected because the right coronary ostium is superior when the patient is supine. CAE may produce coronary ischemia that may be detected by seeing myocardial dysfunction in the affected coronary distribution with intraoperative TEE. However, it is extremely uncommon to actually visualize air within the myocardium on TEE. In this case, an apparently large right CAE resulted in bright echos, presumed to be air emboli within the inferior left ventricular myocardium.

**Case Description:** A robust, otherwise healthy 57 year old male with dilation of his proximal ascending aorta and severe aortic insufficiency underwent elective aortic root graft reconstruction, aortic valve reimplantation and coronary artery transposition (David V procedure). Post bypass he suffered persistent ventricular fibrillation despite repeated defibrillation and antiarrhythmic drug therapy. Immediate cardiac massage was administered and cardiopulmonary bypass was reinstituted. TEE demonstrated right ventricular and inferior left ventricular hypokinesis, consistent with right coronary ischemia. There were prominent bright echos in the inferior wall of the left ventricle strongly suggesting diffuse intramyocardial air in the right coronary distribution. Following a period of resuscitation and successful defibrillation the patient was weaned from cardiopulmonary bypass with inotropic support and inhaled nitric oxide. At that time the bright echos suggesting
intramyocardial air were gone. Emergent cardiac catheterization demonstrated an essentially normal coronary angiogram.

The postoperative course was complicated by an acute myocardial infarction on postoperative day one. Cardiac catheterization demonstrated severe vasospasm in the PDA, RCA and OM1. An intra-aortic balloon pump and inotropes were required. The LVEF, which was normal preoperatively, declined to 27% and later improved to 42%. RV systolic function was severely reduced, consistent with RV infarction, and failed to improve during the postoperative course. The patient survived but suffered substantial myocardial damage.

**Discussion:** Coronary air embolism is common during cardiac surgery. Despite the usual efforts to deair the heart prior to separation from bypass, some air may remain in the left ventricle. TEE is often used to guide deairing and was used for that purpose in this case. Embolization of the coronaries, especially the right coronary from residual air in the left ventricle may result in coronary ischemia, but this is usually transient. Massive CAE, resulting in air visualized on TEE as bright echos within the myocardium, is extremely rare.

Although returning to cardiopulmonary bypass successfully treated this patient’s air embolism and coronary ischemia, with resolution of the bright appearance of the inferior wall myocardium, the patient went on to suffer an early postoperative myocardial infarction from coronary spasm in a similar distribution to the original air embolism. We speculate that the coronary spasm was caused by coronary endothelial damage or instability produced by the original air embolism.

**Keywords:** coronary air embolism, endothelial damage, transesophageal echocardiography

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41: Intra-aortic Balloon Pump for Acute Flail Mitral Regurgitation
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**Introduction:** 25 year old female with history of hepatitis C, deep vein thrombosis, and polysubstance abuse who presented with left sided weakness, confusion, and headache in the setting of possible overdose.

**Case Description:** Her initial CT scan showed right malignant MCA infarct, and she was emergently taken for decompressive craniotomy. A large mitral valve vegetation was noted on ECHO, and blood cultures were positive for Enterococcus faecalis and Candida albicans.

Her subsequent course was complicated by recurrent embolic stroke and moderate ARDS, and on day 7 of hospitalization, she acutely developed tachycardia, hypotension, and hypoxia. Repeat ECHO showed flail anterior leaflet from likely chordae rupture. Nitroprusside therapy was initiated for afterload reduction, with marked reduction in oxygenation, likely from impaired hypoxic pulmonary vasoconstriction. An intra-aortic balloon pump was inserted for afterload reduction and to bridge to valve replacement surgery, with improvement in her oxygenation. Unfortunately, arterial thrombi developed leading to ischemic lower limbs. At this point, her family elected to transition to comfort care.

**Discussion:** There are several causes of acute mitral regurgitation, including infective endocarditis. Many risk factors exist for the development of endocarditis and its associated complications. The complication of acute mitral regurgitation is definitively treated with surgical repair (valve repair or replacement), however that is not always a safe option, as in the case of our patient. Strategies for afterload reduction include medical management and invasive procedures such as intra-aortic balloon pump.
42: Bilateral Paravertebral Catheter Placement For Mediastinal Chest Tube Pain
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Introduction: There is substantial evidence for the effectiveness and safety of paravertebral blocks for post-thoracotomy pain management. However, little data exists with respect to paravertebral blocks for cardiac surgery patients and none for mediastinal chest tube related pain.

Case Description: Recently a patient presented to the acute pain service post-operative day 1 status post coronary artery bypass grafting (CABG) with severe mediastinal chest tube insertion site pain. Two mediastinal chest tubes were present on each side of midline in the vicinity of the xyphoid process. Bilateral paravertebral catheters were placed at T7 under ultrasound guidance. Within five minutes of placing the blocks and bolusing each catheter with 15cc of .5% ropivacaine the patient’s pain diminished from 10/10 at rest at the site of the mediastinal drains to between 0 and 3 at rest and with deep breathing, respectively. Incentive spirometry improved immediately from 250cc to 750cc after block placement. On 2 subsequent mornings the patient’s pain scores remained between 0 and 4 with deep inspiration. The patient’s PCA usage remained minimal and no opioid related side effects were noted. Incentive spirometry recorded values remained at or above 1000cc. The paravertebral blocks were removed after his mediastinal chest tubes were discontinued. He remained hemodynamically stable for the duration of the paravertebral block infusions.

Discussion: Paravertebrals were placed preferentially because the future of this patient’s hemodynamic and anticoagulation status were unclear. At the thoracic level paravertebral blocks offer these two distinct advantages over epidurals. In addition, a very select target dermatomal distribution was causing significant distress for the patient. While paravertebrals tend to spread to a smaller number of dermatomes than epidurals, they can have a profound analgesic effect if placed correctly and appropriately. This has been demonstrated in a multitude of surgeries: most prominently thoracic and breast surgery. The potential uses for this block have yet to be fully explored. This case exhibits one important example.

Keywords: paravertebral pain mediastinal cardiac chest tube

43: Difficult Positive Pressure Ventilation via Tracheostomy in an Adult with Airway Malacia
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Introduction: Tracheomalacia and tracheobronchomalacia (TBM) are rare disorders of weakened airway prone to excessive compression typically during expiration. Although continuous positive airway pressure (CPAP) or positive end-expiratory pressure (PEEP) is generally recommended to sustain airway caliber [1], we present a case in which positive pressure ventilation was insufficient and possibly even counterproductive.

Case Description: A 57-year-old woman with Weill-Marchesani syndrome, TBM, and a permanent tracheostomy presented for a suspension microlaryngoscopy,
tissue excision, and tracheostomy replacement. She was induced with alfentail and sevofluorane and was ventilated via an endotracheal tube through her tracheostomy. She was given intermittent positive pressure ventilation (IPPV), but had difficulty maintaining adequate ventilation, oxygenation, and tidal volumes even with PEEP addition. These respiratory metrics, however, markedly improved with spontaneous respiration, but worsened again when IPPV was re-trialed. The endotracheal tube was checked to ensure it was neither kinked nor obstructed. Thus, the patient was allowed to spontaneously breathe for the remainder of the case.

Discussion: It is unusual to experience difficulty ventilating through a tracheostomy given the direct airway access. In this case, the insufficient ventilation and tidal volumes likely resulted from expiratory airway collapse from general anesthesia and IPPV in the setting of severe TBM. The airway collapse likely effected air trapping and auto-PEEP, which generated high peak inspiratory pressure, which in turn, impaired oxygenation. Both ventilation and oxygenation, however, improved as sedation was weaned and spontaneous breathing restored, likely due to the resolution of auto-PEEP. We liken the airway malacia physiology to that of an anterior mediastinal mass [2] in terms of the increased risk to airway collapse, and recommend a similar approach in its anesthetic management.

References

Keywords: tracheobronchomalacia, tracheostomy, auto-PEEP, high inspiratory peak pressure

44: Severe COPD, COPD Exacerbation, and a Supraglottic Mass: How to Optimize Airway Management?

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Case Description: The patient is a 72yo gentleman with a c/o dyspnea transferred from an OSH where he was treated with antibiotics and duonebs for concern of COPD exacerbation vs. aspiration PNA and wanted Seattle VAMC to further evaluate him for a known supraglottic mass. Just days earlier the OTO had evaluated him and on laryngoscopy found a mass that involves the entire epiglottis, false vocal cords, and arytenoids. Upon arrival he was brought to the pre-operative clinic for a scheduled appointment. The OTO service were planning to do a microdirect laryngoscopy with bx of bilateral larynx, rigid and tracheostomy. In clinic, the patient’s vitals were stable with an O2 saturation of 100%. He denied increased sputum production or fever and admitted to increasing hoarseness, fatigue, and SOB. The patient was in a wheelchair, cachectic, had extreme kyphosis, was somnolent, and his stridorous breathing could be easily appreciated from across the room. Upon auscultation there was no wheezing but there was decreased breath sounds throughout the lungs.

Discussion: His decreased breath sounds and acute decompensation were suggestive of COPD exacerbation for which he needed to be medically optimized. However, his somnolence was suggestive of high CO2 levels secondary to gas trapping from the supraglottic mass. We needed to medically optimize this patient so that he could tolerate an awake fiberoptic intubation in the AM. He was placed on continuous O2 pulse oximetry, Q4H albuterol/ipratropium duonebs with racemic epinephrine was ordered, and Heliox was placed at the bedside. The next morning he was successfully intubated and a tracheostomy was placed.
45: The Adventure of the Missing Glottis: Teaching Direct Laryngoscopy
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Introduction: We report the case of a 56 year-old female patient who presented to our institution for planned elective T10-ilium fusion in prone position. The patient had a 30-pack year history of tobacco use as well as a diagnosis of asthma made at age 50. During preoperative interview the patient was incidentally noted to have mild hoarseness of voice.

Case Description: Induction of anesthesia was carried out with propofol, fentanyl, and rocuronium; the patient was easily bag-mask ventilated. Direct laryngoscopy (DL) with a Macintosh 3 blade was performed by the anesthesia intern; he described his Cormack-Lehane view as grade 1 but stated he was unable to pass the 7.5 oral ETT through the cords. Intubation was taken over by the senior anesthesia resident, who visualized bilateral arytenoids and a right-sided mass obstructing the view of the right vocal fold. A Glidescope was then used to visualize the glottis and to demonstrate the finding to the surgical team (Figure 1). The patient was then intubated with a Glidescope and an Eschmann using a 6.0 oral ETT, to facilitate an in-OR ENT consultation. The surgery was cancelled. In the PACU, the patient continued to have hoarseness but no stridor; she has follow-up scheduled with our ENT colleagues at the time of this abstract.

Discussion: This case demonstrates limitations of teaching direct laryngoscopy when the instructor is unable to simultaneously view the airway. The ideal tool would allow the trainee to visualize the airway with an instrument that mimics direct laryngoscopy while simultaneously displaying video laryngoscopy to the teacher. Two devices, the GlideScope Direct and C-MAC provide the learner with a DL view in the manner of Macintosh Blades and have cameras which allow the teacher to visualize the learner’s view, and two studies investigate their use in teaching modalities.

Keywords: trainee, indirect laryngoscopy, vocal cord lesion

46: The Use of IABP in Cerebral Vasospasm and Stress Cardiomyopathy
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Introduction: We report the case of a 55 year-old female with severe aneurysmal subarachnoid hemorrhage (aSAH) who developed cerebral vasospasm. Vasospasm of bilateral MCAs and ACAs was diagnosed by transcranial Doppler (TCD) & CTA on post-bleed day 8 (PBD), and required astronomical doses of vasopressors to maintain adequate cerebral perfusion pressure (CPP). Angiography showed severe narrowing of the right M1, left M1 and bilateral A1 segments. Vasospasm persisted despite multiple attempts at balloon angioplasty (Figure 1). On exam, the patient was moaning to pain with purposeful movement in her LUE.

Case Description: On PBD 9, a TTE demonstrated severe LV dyskinesis with an EF of 14%, consistent with stress-induced cardiomyopathy. Given poor neurologic exam and presence of heart failure, lithium dilution cardiac output (LiDCO) monitoring was initiated and a continuous central venous oximetry catheter was placed. It was hypothesized that the cardiac index was too low while the systemic vascular resistance was too high. Dopamine and phenylephrine were weaned off. Dobutamine and vasopressin were started in addition to norepinephrine. The
infusions were titrated to the following hemodynamic goals: CI>2.2, SVR<1200dyn x s x m-5, pulse-pressure variation<8%, and ScvO2>65%.

Unfortunately the patient was comatose with extensor posturing. Cardiology was consulted for placement of an intraaortic balloon counterpulsation pump (IABP) in an attempt to improve CPP. Once IABP was placed, NE and dobutamine were weaned to a lower HR for better augmentation. The patient’s neurologic exam improved as her hemodynamics continued to improve. Repeat TTE demonstrated improving LV function, and IABP was eventually weaned off.

**Discussion:** This case highlights two important concepts in the management of cerebral vasospasm with concomitant stress-induced cardiomyopathy: 1) the effective use of LiDCO and continuous ScvO2 to achieve goal-directed optimization of hemodynamics using fluid therapy and vasoactive infusions; and 2) the use of IABP as bridging therapy to augment CPP as the stress-induced cardiomyopathy recovers.

**Keywords:** IABP, balloon pump, vasospasm, stress cardiomyopathy

**47: Combined spinal epidural for cesarean section in a patient with intracranial hemorrhage**

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**Introduction:** Intracranial hemorrhage (ICH) is a rare complication of pregnancy and has a mortality rate of 40%. Anesthetic management must focus on hemodynamic stability, and requires determination of possible cerebral spinal fluid (CSF) flow obstruction or raised intracranial pressures (ICP). In these cases, unexpected dural puncture can have catastrophic consequences. However, with an individualized assessment, multidisciplinary collaboration, and understanding of the underlying lesion, neuraxial anesthesia can provide a more favorable risk/benefit profile than a general anesthetic.

**Case Description:** A previously healthy 24-year-old, G2P1, 92kg woman presented at 32 weeks’ gestation with sudden onset of severe headache and nausea refractory to medication. She denied visual changes or neurologic deficits. Her pregnancy was uncomplicated; and vital signs and exam remained normal. CT scan demonstrated intraventricular hemorrhage without evidence for ventriculomegaly or obstructive hydrocephalus. MRI and MR angiogram demonstrated a arteriovenous malformation (AVM) involving the corpus callosum and right frontal lobe measuring 4x3 cm with a large vein draining into the vein of Galen posteriorly making it a Spetzler-Martin grade 3-4 AVM.

The patient was transferred to HMC NICU for neurological monitoring with an obstetric consult and fetal heart tones remained reassuring. A caesarean section was scheduled for 24 hours after her second dose of betamethasone, and neurosurgical management was deferred until after delivery.

Anesthesia was conducted with a successful combined spinal-epidural (CSE) at the L3-4 level using a 17-G Tuohy needle with loss of resistance at 6.5cm. A 25-G Sprotte needle punctured the dura and clear CSF was noted. 12.5mg of hyperbaric bupivacaine, 100mcg of Morphine, and 10mcg of Fentanyl were administered via spinal needle. A catheter was threaded to 11cm at the skin, and a T4 sensory level was obtained. The Cesarean section proceeded uneventfully with Nicardipine to maintain tight BP control. Additionally two fentanyl boluses through the epidural and IV of 50mcg each were required after successful delivery. Her post-op pain was well controlled, and she received her first of three embolizations two days later and was discharged on hospital day 15 without gross neurologic changes.

**Discussion:** In a patient with a ruptured AVM, strict hemodynamic stability is necessary to prevent hypertension and subsequent fatal hemorrhage. Hypotension...
must also be avoided to prevent fetal-placental hypo-perfusion. Neuraxial anesthesia can avoid the risks of rapid hemodynamic changes and aspiration that can be seen during induction and emergence. However, the risk of dural puncture and subsequent CSF leak may result in traction on dura resulting in rebleeding and possible herniation. This risk however is very small if there is no evidence of CSF flow obstruction or increased ICP. A spinal intentionally punctures the dural membrane but leaves a very small puncture site. A careful CSE technique was agreed upon as the anesthetic of choice. Although a combined technique appears to present more complications than either epidural or spinal alone, the technique offers many advantages and may actually decrease risks associated with either technique when used alone.

**Keywords:** Anesthesia; Arteriovenous malformation; Intracranial hemorrhage; Cesarean section; Combined spinal epidural

48: Purple Urine in a Critically Ill Burn Patient Requiring Reintubation

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**Case Description:** A 61-year-old man is found in a house fire with smoke inhalational injury as well as 30% partial thickness burns to his buttock and lower extremities. The patient is intubated on the scene and transferred to a Burn Intensive Care Unit (ICU) for management. Upon arrival, a foley catheter placement produces 300mL of “deep purple-colored urine." The anesthesia team is called for reintubation because of an endotracheal tube cuff tear. Over the next three days, the change in urine color is reassuring and confirms the cause of this patient’s acute chromaturia.

**Background:** Acute chromaturia can be associated with numerous conditions that can affect the anesthetic management: rhabdomyolysis, acute kidney injury, hemolytic anemia, propofol infusion syndrome, and others. Hydroxocobalamin, a relatively recent FDA-approved treatment for cyanide toxicity, is another cause of acute chromaturia. It is important that the anesthetic provider be able to recognize the distinctive urine color change associated with hydroxocobalamin treatment to aid in medical decision-making regarding management.

**Discussion:** Cyanide toxicity is among the most rapidly lethal poisons and can cause death within minutes to hours of exposure. The most common cause of cyanide poisoning in industrialized countries is domestic fires. It occurs as a product of combustion from materials containing nitrogen and carbon, such as foam rubber, wool, plastics, and other common household synthetic materials. It is reported that significant levels of cyanide are present in up to 35% of all fire victims. Prior to 2006, the only approved antidote in the United States was the multicomponent cyanide antidote package - consisting of amyl nitrite, sodium nitrite, and sodium thiosulfate. Unfortunately, nitrites can cause hypotension and excessive methemoglobinemia, precluding their use for smoke inhalation and sodium thiosulfate may act too slowly to be of benefit. In 2006, the US Food and Drug Administration (FDA) approved a hydroxocobalamin formulation as a new anecdote for known or suspected cyanide poisoning called Cyanokit. This new treatment has the benefit of not producing methemoglobinemia or hypotension and has been demonstrated to be safe for administration to smoke inhalation victims.

Acute chromaturia can be associated with numerous conditions that can affect the anesthetic management of a burn victim. The anesthesia provider should recognize the distinctive color of urine after treatment with hydroxocobalamin as it represents a transient and benign side effect of the treatment.

**Keywords:** chromaturia; hydroxocobalamin; Cyanide toxicity; Cyanokit
49: A case of a cyanotic appearing female after laparoscopic hysterectomy: was it the methylene blue?
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Introduction/Case description: A 42-year-old woman presented at our institution to undergo laparoscopic hysterectomy for abnormal uterine bleeding. The patient’s medical history is remarkable for dyslipidemia, diabetes mellitus, gastroesophageal reflux disease, migraines, and post-traumatic stress disorder. Anesthetic management consisted of induction with lidocaine, fentanyl, etomidate, and sevoflurane maintenance. Forty-five minutes before the conclusion of the operation, she received 50 mg of methylene blue for ureteral localization. At the end of the case, the patient’s face and body appeared blue in color despite normal vital signs, including oxygen saturation above 95%. She was extubated uneventfully, but this blue became more pronounced upon arrival to the PACU. Although the patient was without complaints, with oxygen saturation above 90%, her appearance was concerning for cyanosis and a cooximetry ABG was sent, which showed a PaO2 of 103 and 2.2% methemoglobin (reference range 0-1.5%). The resolution of the discoloration occurred approximately one hour after arrival to the PACU. Vital signs remained normal, and she was discharged from the hospital in good condition the following day.

Discussion: This case illustrates how methemoglobinemia caused by methylene blue should be considered in the differential diagnosis of someone who appears cyanotic after administration. Although methylene blue is a treatment for methemoglobinemia, it can also cause this condition in certain contexts such as G6PD deficiency. Methemoglobin levels in this patient were not high enough to cause her discoloration, though there are also rare reports of methylene blue administration leading to a cyanotic appearance without any hypoxia or methemoglobinemia.

Keywords: methemoglobin, G6PD deficiency, cyanosis, methylene blue

50: High Dose, Prolonged Aminocaproic Acid Infusion in the Setting of Massive Post-Operative Retroperitoneal Hemorrhage following Splenectomy
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Introduction: ε-Aminocaproic acid is an anti-fibrinolytic agent that inhibits the action of plasmin, and has an evidence based clinical role in the attenuation of certain types of perioperative hemorrhage. We present a case of massive postoperative hemorrhage after splenectomy, wherein a prolonged high dose infusion of aminocaproic acid helped achieve hemostasis when bleeding was refractory to procedural efforts to control the source and to blood product transfusion.

Case Description: A 55 year old male with past medical history significant for Acute Myelogenous Leukemia (AML) and resultant massive splenomegaly, anemia and thrombocytopenia underwent planned splenectomy for upcoming allogenic bone-marrow transplant. Intra-operative course notable for a three liter blood loss. On POD#1 he acutely became hypotensive with SBPs in the 60-70s with concurrent several hundred ccs/hr of frank blood output to his abdominal drains with resultant Hct decline to <20. Massive transfusion was initiated and the patient was emergently taken to IR for splenic artery embolization. Despite this, the patient continued to have continuous high volume bloody output from his surgical drains and massive transfusion needs, and was thus taken back by the general surgery team on POD#2 for exploratory laparotomy, which found diffuse retroperitoneal hemorrhage from the splenic bed. Despite efforts to extensively ablate this, the
patient continued to have uncontrolled post-operative hemorrhage, by now having received 14 units of PRBCs, 9 FFP, 4 platelets and recombinant Factor VII. In consultation with hematology, we initiated a 5g load of E-ACA followed by a 1g/hr infusion continuously. Fibrinogen level then rose from less than 200 to 500 mg/dL and this was correlated with a marked decrease in his surgical drain output. This infusion was kept throughout POD 3, with only 1 additional PRBC and 2 platelets needed. By POD#4 the E-ACA dosing was decreased to 1g/q3hrs as his condition continued to improve drastically and his transfusion needs resolved. On POD#5 E-ACA was discontinued all together and patient was successfully extubated. **Discussion:** Despite best efforts with IR splenic artery embolization, surgical re-exploration of splenic bed and massive blood product transfusion, this post-operative patient continued to have life threatening diffuse retroperitoneal hemorrhage. While atypical, the use of a prolonged, high dose E-ACA infusion for 48 hours was used to successfully manage massive post-operative hemorrhage in a patient with a significantly coagulopathic patient in the setting of limited surgical and procedural options for achieving hemostasis in a patient with a diffuse source of bleeding. **Keywords:** aminocaproic acid high dose prolonged massive hemorrhage
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51: Regulation of Mitochondrial Structure and Function by an HNF4 Nuclear Receptor
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**Background:** Lipid sensing nuclear receptors (NRs), such as the PPARs, LXR, and HNF4α, are ligand regulated transcription factors that directly bind and respond to metabolic signals and hormones. The sensitivity of NRs to small molecule ligands, combined with their widespread involvement in metabolism and metabolism related disease, make NRs attractive targets for pharmacological intervention. The development of potent and specific drugs, however, would benefit from a more
thorough understanding of the complex cellular physiology coordinated by lipid sensing NRs. Despite their popularity, lipid sensing NRs have not been extensively studied in model organisms such as C. elegans, highlighting an opportunity to learn more about NR function using this powerful experimental model.

Methods/Results: The C. elegans NHR-49 protein is orthologous to the mammalian HNF4α, a lipid sensing NR that participates in liver and kidney function by regulating fatty acid and sugar metabolism, particularly in response to fasting. Like its mammalian counterpart, NHR-49/HNF4 regulates dependent expression of numerous genes involved in lipid and sugar metabolism.

NHR-49/HNF4 is involved in longevity, fasting response, cold tolerance, hypoxia sensitivity, and mitochondrial morphology. Here we present data demonstrating that NHR-49/HNF4 mitochondrial morphology in the intestine by coordinating the expression of genes involved in ceramide/sphingolipid metabolism with the expression of fatty acid desaturase genes. In regards to the hypoxia phenotype, we show that nhr-49(-/-) mutants are resistant to hypoxia and that this resistance is a result of impaired fatty acid desaturase expression.

Discussion: Because it is clear that the structure and regulatory mechanisms of NHR-49 and the mammalian HNF4 are well conserved, nhr-49 mutants has revealed a novel role for NHR-49/HNF4 receptors in mitochondrial structure and hypoxia sensitivity, thus our C. elegans system has generated novel insight into the physiological function of HNF4 receptors.

Keywords: Mitochondria, Nuclear Receptor, Sphingolipid, Desaturase, Hypoxia

52: A high-throughput screen of protective compounds against non-autonomous hypoxic injuries in C.elegans

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Background: Stroke is one of the leading causes of disability and mortality worldwide. However, an approved neuroprotective drug is still lacking. In stroke, neurons are thought to die immediately from direct hypoxic injury by cell autonomous mechanisms, whereas others may die from factors released from the primarily injured cells. A major limitation in identifying these factors is the inability of current in vivo models to selectively target a set of cells for hypoxic injury so that the primarily injured cells and the innocent bystanders are clearly delineated.

Methods: To develop such a model, we generated transgenic C. elegans strains where a small amount of either neuron or muscle cells were made sensitive to hypoxia.

Results: Hypoxic targeting of these relatively non-essential cells produced widespread innocent bystander cell injury, behavioral dysfunction and eventual organismal death. Furthermore, in a high-throughput screen using this novel model, we have identified several lead compounds that protect against cell non-autonomous hypoxic organismal death.
**Discussion:** We have developed strains and methods that provide the first genetically tractable approach to study hypoxic injury where delayed secondary cell injury can be definitively observed and manipulated. Using these methods, we were surprised to find that widespread necrotic-like cell injury, severe behavioral deficits and organismal death can be produced by targeting less than three percent of somatic cells, none of which are normally required for organismal viability. Our results suggest that a potent and/or amplifying cell non-autonomous mechanism is responsible for the secondary cell injury. Thus, a potential mechanism that a specific signal (we will call this type of signaling molecule a hypoxiakine) from hypoxically injured but not dead cells promotes secondary cell death has been proposed. Moreover, we have identified protective compounds which protect against innocent bystander delayed hypoxic injuries using this novel model. Further characterization mechanisms underlying hypoxic protection of those compounds is under process.

**Acknowledgements:** The work was supported by National Institute of Neurological Disorders and Stroke R01 NS045905, by the International Anesthesia Research Society Frontiers in Anesthesia Research Award, and by the Dr. Seymour and Rose T. Brown Professor endowment fund.

**Keywords:** hypoxia, stroke, neuro-protection, drug screen

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**53: Genetics of Isoflurane-induced Neurotoxicity**

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**Introduction.** Early developmental exposure to volatile anesthetics leads to anesthetic induced neurotoxicity (AIN) in nematodes, rodents, and humans (1). Although neuronal apoptosis, mitochondrial dysfunction and reactive oxygen species (ROS) production have been implicated in AIN, the precise mechanisms by which anesthetics cause neurotoxicity are unknown. We have taken a genetic approach to identify the molecular mechanisms underlying AIN. We have identified interacting pathways that control AIN and which may be useful to alleviate AIN.

**Methods.** Chemotaxis. C. elegans L1 larvae were age synchronized and exposed to isoflurane at their EC95s from hours 4-8 after hatching. Larvae were removed from the anesthetic, and grown to adulthood. Chemotaxis on day one of adulthood was used as a measure of integrated neuronal function (1). Preconditioning (PC). Synchronized L1 larvae were exposed to isoflurane at their EC50s for hours 1-2 after hatching, followed by air for 3 hours and then exposure to isoflurane and chemotaxis studies as described above.

**Results.** Mutations in the insulin-like receptor, DAF-2, induces a stress response and completely attenuates AIN in C. elegans. Studying the downstream targets of DAF-2 identified several genes that affect AIN. These included the kinase GCN-2, the heat shock protein HSP-4 and 3 superoxide dismutases (SOD-1, SOD-2 and SOD-3). Both DAF-2 and HSP-4 were upregulated by exposure to isoflurane. These results suggested that AIN might be alleviated by a preconditioning exposure to isoflurane. Preconditioning completely alleviated AIN in the wildtype N2.

**Discussion.** The sum of our data earmark highly conserved pathways, including mitochondrial dysfunction, ROS generation, and the ER unfolded protein response
(UPR) as key regulators of AIN. A causative role for ROS generation in AIN has been suggested previously. Loss of sod-1 (cytoplasmic), sod-2 or sod-3 (both mitochondrial) alleviated AIN in worms. Loss of any of these superoxide dismutases would not be expected to decrease ROS and reduce ROS damage. Rather, chronic loss of these enzymes may serve to induce a stress response to moderate increases in superoxide. The preconditioning results further indicate that a stress response can be induced by anesthetic pretreatment that protects against AIN. Since isoflurane exposure causes upregulation of hsp-4, we hypothesize that a mild increase in the UPRER leads to protection from AIN. We are testing this model by measuring the PC effect in gcn-2 and hsp-4 mutants.

References.

Keywords: C.elegans, anesthetic neurotoxicity, DAF-2, UPR

54: Variable GST-14 Response to Mitochondrial Redox Stress in C. elegans.
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Background: C. elegans is a powerful genetic model to study mitochondrial disease. Mutant strains defective for the electron transport chain (ETC) and oxidative stress pathways are useful to study the contribution of redox imbalance to lifespan and disease. Previous work from our lab showed that the antioxidant and detoxification pathway enzyme GST-14 plays a role in the reduced lifespan of the complex I mutant gas-1. We analyzed the expression of GST-14 in other short- and long-lived mitochondrial mutants to understand its significance.

Methods: We crossed a transgenic C. elegans strain with gst-14 promoter driven GFP expression (fcIS1; Pgst-14::gfp) to various mitochondrial mutant strains. The homozygous double mutants (fcIS1;gas-1, fcIS1;isp-1, fcIS1;nuo-6, fcIS1;mev-1 and fcIS1;clk-1) were analyzed by microscopy and qPCR to understand the spatial and temporal expression pattern of GST-14.

Results: GST-14 is expressed at low levels in the fcIS1 worms (wild-type, expressing the reporter) and all five double mutants during the larval stages in the body wall muscles. There was an induction of increased pharyngeal expression in fcIS1;gas-1, fcIS1;isp-1 and fcIS1;nuo-6 commencing from day 1 of adulthood. The GFP fluorescence was maximal in fcIS1;gas-1 followed by fcIS1;isp-1 and fcIS1;nuo-6. The increased expression in the pharynx waned off by day 4 of adulthood in fcIS1;nuo-6, decreased significantly by day 10 of adulthood in fcIS1;isp-1, but remained high in fcIS1;gas-1 throughout adulthood. The GFP fluorescence in fcIS1;mev-1 was similar to or lesser than that of fcIS1 for the duration of life. Results with fcIS1;clk-1 are ongoing and will be presented.
Discussion: Increased expression of GST-14 was seen to be maladaptive since knocking down the gene rescued gas-1 lifespan. The lack of induction in the short-lived Complex II mutant mev-1 implicates alternative mechanisms involved in shortening its lifespan. The induction seen in the long-lived mutants isp-1 and nuo-6 during adulthood indicates that GST-14 induction might be one of the robust resistance mechanisms triggered by mitochondrial stress.

Acknowledgement: NW Mitochondrial Research Guild

Keywords: GST-14, mitochondrial stress, redox imbalance, C.elegans

55: Leigh Syndrome And The Regional Transcriptome Of The Ndufs4-KO Mouse Brain

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Background: Knocking out the NDUFS4 subunit of mitochondrial complex I of the respiratory chain, either in all cells of the mouse or just in its central nervous system, causes a progressive neuropathy closely resembling human Leigh Syndrome. Surprisingly most brain cells tolerate the primary mitochondrial defect while only a few distinct brain regions develop inflammation and subsequent neurodegeneration. This region specific susceptibility to degeneration could be caused by region-specific variation in gene expression. We therefore identified genes which are differentially regulated between KO and controls and simultaneously differ in their expression patterns between degeneration resistant and degeneration prone regions. Here we present preliminary findings for olfactory bulb (O), - the most sensitive region -, contrasted against the unaffected region (R) comprised of midbrain and the remaining forebrain.

Methods: Total RNA was isolated from 25 to 30 day old ndufs4 KO and controls (wt or het) and sent to GeneWiz for RNAseq. Genes with significant differential expression between KO and controls within a region were considered for further analysis. For the majority of these the fold change was very small and therefore probably biologically irrelevant. Subsequently we focused on genes with at least 1.75x up (or down) change in the KO in either R or O if the change in the respective other region was either negligible or in the opposite direction.

Results: 53 of ~20,000 genes fulfilled the above criteria. In no case did the change in expression between genotypes exceed 4-fold. Among the candidates, receptors and related proteins stood out. The most promising group is involved in retinoic acid (Crabp1, Rxrg, Rabr) and dopaminergic (Drd2, Drd1) signaling. These genes are down regulated in KO R. Other interesting candidates are the serotonin receptor Htr1d and tachykinin (Tach1), the precursor of neurokinins including substance P.

Discussion: Knocking out a subunit of complex I of the mitochondrial respiratory chain can be expected to affect energy metabolism which indeed had been reported. Furthermore, KO synaptosomes from O, but not from R, have reduced respiratory capacity (own unpublished results). Surprisingly none of the genes identified here for their possible role in neuro¬degeneration encode enzymes of energy pathways. Thus, either neurodegeneration is not caused by lack of metabolic spare capacity, or this capacity is not regulated at the level of transcription.
We observed reduced expression of receptors Rarb, Rxrg, Drp1, Drp2 in the degeneration resistant KO R. Retinoic acid and dopaminergic signaling are reportedly linked (Krze’el et al.). Considering reduced signaling may be neuroprotective we are planning to dampen retinoic acid signaling pharmacologically in the KO using antagonists of Rxr and Rar

Acknowledgements: Valeria Vasta from Sihoun Hahn’s laboratory prepared RNA. Funding was provided by SCRI/CDT.

Keywords: Ndufs4, Leigh, neurodegeneration, transcriptome, retinoic acid, dopamine

56: Specific Brain Regions Control Anesthetic Sensitivity in Ndufs4(KO) Mice
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Background: We have built upon our previous studies using a mouse model of mitochondrial complex I disease, Ndufs4(KO) to understand how volatile anesthetics (VAs) work. Similar to patients with complex I disease, Ndufs4(KO)s are hypersensitive to VAs (1-3). We hypothesized that an energetic defect depresses neuronal activity in CNS regions critical to anesthetic response. We have now characterized the role of specific brain regions on anesthetic behavior.

Methods: All studies were approved by the institutional IACUC committee. To analyze particular brain regions, adeno-associated virus (AAV) expressing Cre-recombinase was injected into mice containing floxed Ndufs4 at ages P34-8. Control mice were injected either with saline or mutant Cre-recombinase, resulting in removal of Ndufs4 only in certain areas of the brain. Three weeks following injection, mice tested for sensitivity to isoflurane and halothane (4).

Results: Mice with injections into the vestibular nucleus (VN) show resistance to isoflurane (n=6; EC50(KO)=1.5; n=6 EC50 (WT)=1.13; p<0.005). Preliminary injections into the central medial thalamus (CMT) show an increase in sensitivity (n=2; EC50(KO)=.98; n=6 EC50 (WT)=1.34; p not yet determined). Finally, we targeted the subpeduncular tegmental nucleus and pontine reticular nucleus (also known as the mesopontine tegmental anesthetic area (MPTA)). Three test mice display normal sensitivity to isoflurane (1.25%) and halothane (1.36%).

Discussion: Loss of complex I function in the VN causes anesthetic resistance, while loss in the CMT may cause increased anesthetic sensitivity. The vestibular nucleus may affect anesthetic response via motor output or by altering the awake state as the parafacial zone within the VN promotes sleep (5). It has been proposed that the CMT promotes a conscious state by mediating cortical synchronization (6). Future plans include dissecting the neural circuitry within the VN and CMT to determine the respective mechanisms of resistance and sensitivity to anesthesia.

Acknowledgements: This funding was supported by an R01 to Margaret Sedensky (R01GM105696).

References:
Introduction: Mitochondrial function has been linked with anesthetic sensitivity in mice, humans, and nematodes (1-3). We reported that, in mice, the loss of the NDUFS4 protein in the mitochondrial respiratory chain (MRC) caused striking sensitivity to two volatile anesthetics and to propofol, but conferred resistance to ketamine (1). The present studies are the first to identify electrophysiologic changes in the CNS of the Ndufs4(KO) mouse.

Methods: All studies were approved by the institutional IACUC committee. We characterized changes in neuronal activity in global KO and WT animals in the hippocampus, and the central medial thalamus (CMT). Using whole-cell patch-clamp recordings we measured baseline activity in the two regions of WT and Ndufs4(KO) mice. We exposed hippocampal slices at 30°C to isoflurane at a concentration that anesthetizes Ndufs4(KO) mice but not controls (0.6% isoflurane). We then measured activity at a concentration which anesthetizes control animals (1.2% isoflurane) but is lethal to Ndufs4(KO) animals.

Results: Intrinsic activity properties did not differ between WT and KO hippocampal CA1 pyramidal cells. In addition, the frequency of spontaneous excitatory postsynaptic currents (sEPSCs) was not significantly different between control and Ndufs4 KO animals. However, when exposed to 0.6% isoflurane the frequency of sEPSCs decreases dramatically in the KO cells but not in control cells. Exposure at 1.2% isoflurane shows that sEPSC frequencies are decreased in both control and KO slices. However, sEPSCs did not recover in KO slices. No differences were noted between control and KO cells for amplitude or decay time. No significant differences between KO and control cells were seen in inhibitory GABAergic postsynaptic currents (sIPSCs). In both cases, isoflurane exposure caused a similar increase in decay times as has been reported by others.

Discussion: A striking difference is seen in sensitivity of sEPSCs to isoflurane in the mutant compared to wildtype; at 0.6% isoflurane sEPSCs are markedly decreased in Ndufs4(KO) but not in WT. At 1.2% isoflurane, a concentration that anesthetizes the WT animal but is lethal to the KO animal, sEPSCs are decreased in hippocampus from both genotypes, but fail to recover in the KO. We have previously noted that isoflurane sensitivity is controlled entirely by loss of Ndufs4 in glutamatergic neurons. We interpret our results to show that excitatory neurotransmission is selectively sensitive to isoflurane in the mutant. Inhibition of the energy requiring glutamatergic

57: Electrophysiologic changes in the Anesthetic Hypersensitive Mouse Mutant, Ndufs4

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Introduction: Mitochondrial function has been linked with anesthetic sensitivity in mice, humans, and nematodes (1-3). We reported that, in mice, the loss of the NDUFS4 protein in the mitochondrial respiratory chain (MRC) caused striking sensitivity to two volatile anesthetics and to propofol, but conferred resistance to ketamine (1). The present studies are the first to identify electrophysiologic changes in the CNS of the Ndufs4(KO) mouse.

Methods: All studies were approved by the institutional IACUC committee. We characterized changes in neuronal activity in global KO and WT animals in the hippocampus, and the central medial thalamus (CMT). Using whole-cell patch-clamp recordings we measured baseline activity in the two regions of WT and Ndufs4(KO) mice. We exposed hippocampal slices at 30°C to isoflurane at a concentration that anesthetizes Ndufs4(KO) mice but not controls (0.6% isoflurane). We then measured activity at a concentration which anesthetizes control animals (1.2% isoflurane) but is lethal to Ndufs4(KO) animals.

Results: Intrinsic activity properties did not differ between WT and KO hippocampal CA1 pyramidal cells. In addition, the frequency of spontaneous excitatory postsynaptic currents (sEPSCs) was not significantly different between control and Ndufs4 KO animals. However, when exposed to 0.6% isoflurane the frequency of sEPSCs decreases dramatically in the KO cells but not in control cells. Exposure at 1.2% isoflurane shows that sEPSC frequencies are decreased in both control and KO slices. However, sEPSCs did not recover in KO slices. No differences were noted between control and KO cells for amplitude or decay time. No significant differences between KO and control cells were seen in inhibitory GABAergic postsynaptic currents (sIPSCs). In both cases, isoflurane exposure caused a similar increase in decay times as has been reported by others.

Discussion: A striking difference is seen in sensitivity of sEPSCs to isoflurane in the mutant compared to wildtype; at 0.6% isoflurane sEPSCs are markedly decreased in Ndufs4(KO) but not in WT. At 1.2% isoflurane, a concentration that anesthetizes the WT animal but is lethal to the KO animal, sEPSCs are decreased in hippocampus from both genotypes, but fail to recover in the KO. We have previously noted that isoflurane sensitivity is controlled entirely by loss of Ndufs4 in glutamatergic neurons. We interpret our results to show that excitatory neurotransmission is selectively sensitive to isoflurane in the mutant. Inhibition of the energy requiring glutamatergic
cycle is the most likely mechanism and is under study. We are also extending our slice studies to the central medial thalamus and vestibular nucleus.

Acknowledgments: We would like to thank our collaborators in the laboratory of Dr. Nino Ramirez at the Center for Integrative Brain Research at Seattle Children’s Research Institute for their insight and expertise in electrophysiology.

References:

Keywords: Isoflurane, Ndufs4, mitochondria, sEPSCs

58: The Role of Hypocretin in Post-Traumatic Brain Injury (TBI) Sleep Disorders

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Background: Disorders of sleep and wakefulness have been reported in up to 73% individuals who have experienced traumatic brain injury (TBI). Specifically, increased sleep need and excessive daytime sleepiness are often reported post-TBI. At present, the etiology of post-TBI sleep disturbances is not well understood, and behavioral and pharmacological therapies have limited efficacy. Here, we investigate the effects of TBI on sleep-wake behavior and on hypocretin and melanin-concentrating hormone (MCH), two neuropeptides important for regulating sleep and wakefulness.

Methods: Adult male C57BL/6 mice (n=6-10/group) were implanted with EEG recording electrodes and baseline recordings were obtained. After baseline recordings, TBI was induced using controlled cortical impact (CCI). EEG recordings were obtained from the same animals at 7 and 15 days post-surgery. A separate set of animals (n=6-8/group) underwent sham or TBI surgery and were sacrificed 7 or 15 days later. Brains from these animals were used for immunohistochemistry to determine the number of hypocretin or MCH-producing neurons in the hypothalamus.

Results: At 15 days post-surgery, wakefulness was decreased and NREM sleep was increased during the dark period in moderately injured animals. There were no differences between groups in REM sleep time, nor were there any differences between groups in sleep behavior during the light period. There was a main effect of injury severity on hypocretin-producing neurons, such that more severe injury resulted in fewer hypocretin-producing neurons. There were no significant differences among groups in MCH-producing neurons. We conclude that moderate TBI reduces wakefulness and increases NREM sleep during the dark period. Moderate TBI also decreases the number of hypocretin-producing neurons, which may be one mechanism underlying observed changes in sleep after TBI.

Acknowledgements: Funding was provided by the Department of Anesthesiology & Pain Medicine, University of Washington, Seattle, WA.

Keywords: hypocretin, MCH, sleep, TBI
Background: Peripheral inflammation following chronic insufficient sleep or overnutrition (i.e., excessive caloric intake) is associated with impaired insulin sensitivity and glycemic control. Overnutrition, such as with a high-fat diet (HFD), also induces insulin resistance in part via hypothalamic inflammation. Whether neuroinflammation elicited by insufficient sleep impairs glucose regulation remains unknown. We tested the hypothesis that neuroinflammation contributes to deficits in glucose regulation following insufficient sleep and overnutrition.

Methods: Adult C57BL/6J mice were housed in novel sleep disruption devices and assigned to one of four groups: undisturbed sleep and chow diet; undisturbed sleep and HFD; 18-h sleep fragmentation (SF) and chow diet; or 18-h SF and HFD. After 3 or 9 days’ exposure to these conditions, blood samples and metabolic tissues were collected and analyzed for selected cytokines using a Luminex bead-based assay (n=7-8/group). Plasma corticosterone concentrations were measured via enzyme immunoassay. Glucose tolerance tests were performed to assess glycemic control in additional mice (post-day 3: n=11-12/group; post-day 9: n=5-6/group).

Results: Three days of SF or HFD significantly impaired glucose tolerance (p < 0.05). After 9 days, effects of SF on glucose tolerance persisted (p = 0.003) whereas effects of HFD did not (p > 0.05). Post-hoc tests revealed that at both time points, only mice exposed to both SF and HFD demonstrated significantly impaired glucose tolerance relative to chow-fed, rested mice (p < 0.05). Three days of SF significantly elevated levels of pro-inflammatory cytokines in the hypothalamus and brainstem, altered cytokine signaling in plasma, and elevated plasma corticosterone levels (p < 0.05). After 9 days, pro-inflammatory responses to SF largely subsided, but elevations in brainstem interleukin-6 and reductions in plasma corticosterone were observed in response to HFD (p < 0.05). At both time points, plasma leptin concentrations increased with HFD and decreased with SF (p < 0.05).

Discussion: SF or HFD impairs glucose tolerance and exposure to both factors results in even greater impairment. SF induces a more rapid pro-inflammatory response than does HFD, however the functional effects of these responses remain unclear as they do not correspond well with patterns of glucose intolerance.

Acknowledgements: This work was supported by the University of Washington Dept. of Anesthesiology and Pain Medicine and by NIH grants 1F32DK103491-01 and 2T32DK007247.

Keywords: insufficient sleep; sleep fragmentation; glycemia; neuroinflammation
60: High-Altitude Pulmonary Edema Is Associated With A Uniform Distribution Of Alveolar Ventilation

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Background: Uneven hypoxic pulmonary vasoconstriction HPV is thought to incite high-altitude pulmonary edema (HAPE) by increasing capillary pressure. We have shown using magnetic resonance imaging (MRI) that those susceptible to HAPE(S) but not HAPE-resistant(R) develop increased spatial heterogeneity of pulmonary perfusion in hypoxia consistent with uneven HPV as a characteristic of HAPE susceptibility. Why HPV is spatially uneven is unknown but may result from heterogeneously distributed alveolar PO2 stemming from heterogeneity in baseline ventilation.

Methods: We tested the hypothesis that ventilation is more heterogeneous in S than R using multi-breath inert gas washout (MBW) in normoxia and hypoxia (FIO2 = 0.125), where indices Scond and Sacin, represent heterogeneity in ventilation from conductive and respiratory airways respectively. Specific ventilation imaging (SVI), a functional MRI technique, was used to measure regional specific ventilation; with the RD of SVI used to quantify heterogeneity. Data were obtained in S (n=6, 1F, 5M), with a history of physician-diagnosed HAPE, and R (n =7, 1F, 6 M), frequent sojourners to > 3,500m without illness.

Results: Contrary to our hypothesis Sacin tended to be more uniform in S than R (S 0.09±0.01, R 0.11±0.03, p=0.08), and Scond and Sacin did not change significantly with hypoxia (p=0.19, 0.72, respectively). S had significantly lower ventilation heterogeneity in normoxia on SVI than R (1.30±0.60 vs2.30±0.87, p=0.04).

Conclusions: Increased ventilation heterogeneity in normoxia is not a feature of HAPE S, and does not increase with hypoxia. This suggests that the basis for uneven HPV in HAPE involves a vascular mechanism. The finding of a more uniform distribution of ventilation in S may suggest a unique prominence of collateral ventilation.

Keywords: High altitude pulmonary edema (HAPE), hypoxic pulmonary vasoconstriction (HPV), lung fMRI, hypoxia

61: Hyperoxia Following Morphine Worsens Hypoventilation in Opioid-Naive and -Tolerant Rats.

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Background: Accidental deaths in outpatients receiving chronic opioid treatment are largely responsible for opioid-related deaths being a leading cause of accidental death. Although long known to involve respiratory arrest, mechanisms that increase risk with increased opioid dose and length of treatment remain unclear. Repeated opioid administration produces tolerance to their analgesic effects, often prompting increased dosing, but opioid depression of ventilation may not gain tolerance as found for analgesia. We have previously shown that rats receiving chronic morphine
show marked tolerance to both morphine sedation and analgesia, but the magnitude of hypoventilation (up to 50%) and the profound loss of hypercapnic sensitivity (up to 80%) following morphine was very similar to the morphine-naïve rats. In both groups, recovery of ventilation following morphine occurred without recovery of the hypercapnic response, suggesting that hypoxic sensitivity was essential for maintenance and recovery of opioid-depressed ventilation. However, in the tolerant rats the magnitude and rate of increased ventilation during recovery was decreased as compared to naive rats. This study addresses the hypothesis that the magnitude of hypoxic drive following opioid administration is dose-dependent, and greater in the opioid-tolerant rats.

**Methods:** Using a procedure that we previously found to produce populations of morphine-naïve or analgesia-tolerant rats, 44 animals were treated twice daily with either saline (n = 30) or morphine (n = 14), respectively, for three days (escalating doses). On the forth day they were tested for minute ventilation and the ventilatory response to normoxic-hypercapnia (ramp increase from 0 to 5% inspired CO2 with 21% O2 over ~ 2 min) or hyperoxic-hypercapnia (same CO2 with 95% O2) during sedation, at 30 min following administration of morphine at 5 (morphine-naïve) or 10 mg/kg (morphine-naïve and -tolerant).

**Results:** For the morphine-naïve rats, as compared to those that received normoxia, those that received hyperoxia showed a significant reduction in the response to hypercapnia following 10 but not 5 mg/kg morphine, and substantially reduced ventilation following both 5 and 10 mg/kg morphine (-21% and -29%, respectively). Morphine-tolerant rats showed a larger average depression of the hypercapnic response with hyperoxia as compared to normoxia, but also larger variability that precluded a statistically significant result, and a much more substantial hypoventilation when exposed to hyperoxia as compared to normoxia following 10 mg/kg morphine (~39%).

**Discussion:** The results clearly show the importance of hypoxic drive in maintaining ventilation following morphine (when hypercapnic drive is essentially eliminated), and suggest that opioid-tolerant rats experience greater hypoxic drive (and possibly hypoxia) following morphine as compared to opioid-naïve.

**Acknowledgement:** This study was funded by the Alcohol and Drug Abuse Institute.

**Keywords:** Opioid, tolerance, respiratory control, hypoxia, hypercapnia
respiratory depression, and if tolerance to respiratory depression is dependent on MOR inactivation through GRK3 or JNK2-mediated signaling.

**Method:** The purpose of this study was first to examine the presence of respiratory depression with fentanyl and morphine using non-invasive pulse oximetry in a mouse model, to determine whether there was acute tolerance to opioid-induced respiratory depression, and observe the effect of genetic knockout of GRK3 or JNK2. Adult male C57BL/6 mice were injected with 2 doses of either fentanyl or morphine 5 hours apart, and oxygenation was monitored by pulse oximetry.

**Results:** Animals injected with a single dose of either fentanyl or morphine demonstrated rapid and profound decreases in oxygenation as measured by pulse oximetry. Additionally, the oxygen desaturation was transiently corrected by administration of 95% oxygen suggesting a mechanism of opioid-induced hypoventilation. Upon injection with a repeat dose of either fentanyl or morphine 5 hours later, oxygen desaturation was also present, albeit an attenuated effect as compared to the first injection (demonstrating acute tolerance). Genetic knockout to GRK3 or JNK2 had no effect on this response. Collectively, these findings demonstrate the presence of acute tolerance to opioid-induced respiratory depression. These findings also suggest that GRK3 and JNK2 mediated signaling, which is known to lead to MOR inactivation by fentanyl and morphine respectively, had no effect on acute tolerance to opioid-induced respiratory depression.

**Acknowledgement:** This work was funded by DA030074

**Keywords:** fentanyl, morphine, respiration depression, tolerance

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**63: Metabolomic Characterization of Kappa Opioid Receptor Antagonism**

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**Introduction:** The kappa opioid receptor (KOR) system has been implicated as the critical stress-response activator for anxiety, depression and drug seeking behavior (1). Specific KOR antagonists such as Norbinaltorphimine (Nor-BNI) may promote stress resilience and thus have therapeutic potential (2); however the signaling pathways that underlie their duration of action are incompletely understood. Our aim was to use targeted metabolomic analysis to characterize the physiological state of neuronal tissue of mice treated with norBNI prior to stress exposure with those treated with vehicle alone.

**Methods:** A group of mice (n=28) were either administered an intraperitoneal dose of normal saline or 10 mg/kg norBNI. Half of each group were then stressed with a validated standard swim test over three days or not stressed. At the end of three days, hippocampal and prefrontal cortex tissue were harvested from each mouse then immediately frozen at -80C to quench the tissue’s metabolism. Tissue samples were methanol extracted and filtered to obtain metabolic and protein fractions. The metabolic extractions were run on an Agilent 1290 Infinity Liquid Chromatography
(LC) system using a HILIC column with a Trip Quad 6410 Mass Spectrometer. The integrated peak intensity for each metabolite was obtained and normalized to the protein concentration. Data analysis was performed using MetaboAnalyst software. 

**Results:** Mass spectrometric analysis detected 42 distinct metabolites (amino acids, TCA cycle, glycolysis, and nucleotide intermediates) in each tissue sample, which were validated using retention times and external standards. In hippocampal tissue, which have ion channels known to be regulated by KOR, glutamine and threonine were increased (FC 2.89 and 2.08 respectively, p<0.05, Wilcoxon Sum Rank test) in stressed mice versus stressed mice pretreated with norBNI. In the prefrontal cortex, lysine was increased (fold change 2.5, p value = 0.01) in stressed mice treated with norBNI versus stress alone. There were no statistically significant changes in tryptophan or glycolysis metabolites though stress induced changes in TCA intermediates across all samples. 

**Discussion:** This study identified specific amino acids in hippocampal and prefrontal cortex tissue that may be important in norBNI’s KOR antagonism. Changes in glutamine, threonine and lysine concentrations may give insight into how KOR pathways alter the physiologic stress response through phosphorylation downstream of the GCPR or help identify upstream protein signaling events. Further work to characterize the correlations between the various metabolites with proteomic data using statistical modeling will help better understand the complex neuropharmacology of kappa opioid signaling. 

**References:**

**Keywords:** kappa opioid signaling, neuropharmacology, metabolomics

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**64: Metabolite Profiling using Smart Isotope Tags and Ratio Analysis: NMR and MS Heterospectroscopy**

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**Background:** Urine is a convenient bio-fluid capable of providing a complete metabolic picture. Global metabolite profiling of human urine is, therefore, promising for the diagnosis of many human diseases. Given the large number of carboxylic acids in urine, it is of interest to develop advanced nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) methods to better analyze the carboxylic acid sub-metabolome. Additionally, the difficulty in comparing and correlating metabolite data between NMR and MS limits efforts to exploit their combined strengths. Towards this goal, we are developing a new method, consisting of smart isotope tags and ratio analysis NMR and MS Heterospectroscopy (RANHSY), which we apply towards an enhancing metabolite profiling in human urine.
**Methods:** Concentrated urine samples from healthy human volunteers were investigated. Metabolites containing carboxylic acid groups were separated from amines using an ethyl acetate extraction protocol (Peng and Li, Analytica Chimica Acta, 2013, 803, 97). The first aliquots of extracted metabolites were tagged with a 15N-cholamine smart isotope tag (Tayyari et al., Anal. Chem., 2013, 85, 8715) and the second aliquots were tagged with 14N-cholamine in the same manner. 15N-tagged metabolites as well as standard compounds were investigated by 1H-15N heteronuclear single quantum correlation (HSQC) NMR spectroscopy at 800 MHz, and by LC-MS, while 14N-tagged metabolites were investigated by LC-MS only.

**Results:** Metabolites extracted into ethyl acetate were tagged successfully with 15N-cholamine. Analysis of their 1H-15N HSQC NMR spectra showed as many as 2000 unique 1H-15N correlations, indicating ~1000 metabolites, assuming roughly two acid groups per metabolite. A majority of metabolites contain at least one carboxylic acid group that reacts with 15N-cholamine to generate 15N-amide bonds with unique 15NH peaks, with chemical shifts that depend on the metabolite structure. Importantly, the detection sensitivity gained herein is enormous as compared to the results obtained after the direct (without extraction) 15N-cholamine tagging. In a demonstration experiment, we applied the RANHSY method to NMR and MS methods, which enabled isolation of MS and NMR peaks from the same compounds. Further analysis of both 15N- and 14N-tagged urine samples by LC-MS and the connection of NMR and MS data using RANHSY will also be reported.

**Discussion:** We have previously shown that the smart tag approach provides an enormous increase in resolution and sensitivity for NMR based metabolomics analysis. However, amines can compete with 15N-/14N-cholamine in their reactions with carboxylic acids during the isotope tagging process, and their presence can create problems in both identification and quantitation of acid metabolites by NMR or MS. Extraction of acid metabolites into ethyl acetate at high salt concentration and low pH ensures the removal of amines. The high ionization efficiency of MS and enhanced sensitivity and resolution of NMR offered by the “smart isotope tag”, 15N-cholamine, facilitates the collection of quantitative data, and connects the metabolite detected by MS to NMR data, and vice versa.

**Acknowledgment:** Authors acknowledge NIH (National Institute of General Medical Sciences 2R01GM085291) for funds to support this research.

**Keywords:** smart isotope tag, RANHSY, metabolites

**65: Globally Optimized Targeted Mass Spectrometry: Reliable Metabolomics Analysis with Broad Coverage**

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**Background:** Mass spectrometry (MS) is an essential tool in metabolomics for investigating alterations in metabolism and metabolic pathways in biological systems. Global profiling and targeted detection are the two most important methods in MS-based metabolomics. However, neither is ideal, and they have somewhat complementary advantages and disadvantages. Global profiling has wide metabolite coverage, but it often suffers from missing values, poor quantitation, and complicated data processing. Targeted MS has the significant advantages of high data quality,
great selectivity, and excellent quantitation; however, a major limitation of targeted MS in metabolomics is its limited metabolite coverage. Therefore, there is a high demand in metabolomics to improve these detection technologies towards better qualitative and quantitative capabilities.

**Methods:** We have developed a new approach, Global Optimized Targeted (GOT)-MS, with the advantages of both targeted detection and global profiling. GOT-MS was developed based on a single liquid chromatography triple quadrupole (LC-QQQ) mass spectrometer in both positive and negative ionization modes. Aqueous metabolites were extracted from a serum sample and separated using hydrophilic interaction liquid chromatography (HILIC). The key step in GOT-MS is a global search of precursor and product ions, which was performed in the mass range of 60-600 Da.

**Results and Discussion:** We first performed selected ion monitoring (SIM) incremental scanning to take advantage of its high sensitivity and good S/N. Given the unit mass resolution of the MS quadrupole, we used an m/z increment of 0.5. We examined each individual SIM, and the m/z values that produced relatively good peak shapes and S/Ns>3 were selected as precursor ions. We then carried out tandem mass spectrometry scanning with incremental collision energy (CE) to profile product ions. Three CE values were selected: 5 V, 20 V, and 35 V. Most metabolites will fragment under these CEs, and MS/MS spectra under CE of 5 V provide more accurate m/z values for the precursor ions. With both precursor and product ions available, multiple reaction monitoring transitions (MRM) scanning was used to optimize the fragmentor voltage, cell accelerator voltage, and CE.

In the analysis of human serum, 595 GOT-MS precursor ions and 1,890 MRMs were determined from the peaks with reasonable peak shapes and S/Ns>3. Median coefficients of variation (CVs) were below 6% for both ionization modes. Scheduled MRM scanning was used to measure >500 MRMs in a single injection. Although definitive identification awaits further confirmation, metabolite candidates for ~50% of the GOT-MS MRMs could be discovered in the Metlin database. GOT-MS was designed to essentially optimize the detection performance of a single LC-QQQ mass spectrometer, and given the capabilities of QQQ-MS, it should be very selective and highly reliable for quantitative analysis. Because precursor and product ions are globally searched, GOT-MS is applicable for a wide range of metabolic studies to detect not only well-known metabolites but also unknowns. LC retention and fragmentation patterns are useful to resolve the metabolites with similar molecular weight. Faster and more sensitive QQQ instruments will detect even more species.

**Acknowledgements:** Support from the NIH (R01GM085291) is gratefully acknowledged.

**Keywords:** Metabolomics, Globally Optimized Targeted Mass Spectrometry
Expanding the Limits of Human Blood Metabolite Quantitation using NMR Spectroscopy

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Background: Metabolite profiling of human serum/plasma is of major interest for the investigations of virtually all human diseases. A current challenge in metabolomics is the reliable identification and quantitation of many metabolites. Limited resolution and sensitivity combined with the challenges associated with unknown metabolite identification have restricted both the number and the quantitative accuracy of blood metabolites. Focused on alleviating this bottleneck in NMR-based metabolomics, we have comprehensively investigated pooled human serum and provided a new method for identification and accurate quantitation of an unprecedented number of human blood metabolites on a routine basis.

Methods: Blood metabolites from human serum were extracted utilizing the optimized protein precipitation method developed in our laboratory. Comprehensive analyses of the complex metabolite mixture of blood were made using an array of 1D and 2D NMR at 700 and 800 MHz, database searches, and spiking with authentic compounds. A large pool of metabolites including numerous unknown metabolites was identified and accurately quantitated. Further, we have comprehensively evaluated the performance of the two most commonly used serum protein removal solvents, methanol and acetonitrile, for optimal recovery of blood metabolites from the complex serum matrix.

Results: Comprehensive NMR investigations of human blood enabled the identification of 67 blood metabolites. Many of these (~1/3rd) are new compared to those reported previously as a part of the Human Serum Metabolome Database. Investigations focused on the evaluation of quantitation using different organic solvents revealed a surprisingly poor performance for protein precipitation using acetonitrile. One third of the detected metabolites were attenuated by 10-67% compared to methanol precipitation at the same solvent to serum ratio of 2:1 (v/v). Nearly 2/3rd of the metabolites were further attenuated by up to 65% upon increasing the acetonitrile to serum ratio to 4:1 (v/v). These results, combined with the newly established identity for many unknown metabolites in the NMR spectrum, offer new avenues for human serum/plasma based metabolomics.

Discussion: Here, we describe the NMR identification of numerous unknown metabolites in blood and provide a simple NMR approach to quantitate the enhanced pool of blood metabolites on a routine basis. An important aspect of this study is that the characteristic peaks for all identified metabolites are marked for easy identification and quantitation of metabolites in the NMR spectrum. This, we believe, is critical for widespread use of enhanced and NMR-based blood metabolite profiling, since the chemical shift databases alone are often insufficient for unambiguous assignment. Quantitative evaluation of protein precipitation reveals that methanol performs most optimally over a wide range of concentration, while acetonitrile shows a surprisingly poor performance. The ability to quantitatively evaluate nearly 70 blood...
metabolites that represent numerous classes including amino acids, organic acids, carbohydrates and heterocyclic compounds using a simple and highly reproducible analytical method such as NMR may potentially guide the evaluation of samples for analysis using mass spectrometry.

**Acknowledgement:** This work was supported by an NIGMS Grant (2R01GM085291)

**Keywords:** NMR, blood, metabolomics, unknown identification, quantitation

**67: Detection of breast cancer recurrence using LC-MS/MS targeted metabolic profiling**

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**Background:** Breast cancer is one of the most prevalent types of cancer among women worldwide, and a major cause of human morbidity and mortality. According to the American Cancer Society, breast cancer is the leading cancer in US females, and the second most common cause of cancer death in women. Research focused on breast cancer recurrence detection using metabolic profiling approaches is relatively scarce; however, some reports have shown that early detection of locally recurrent breast cancers can improve patient survival significantly. Therefore, we propose a targeted LC-MS/MS serum metabolic profiling approach for the detection of breast cancer recurrence, using a panel of significantly altered metabolites as biomarkers.

**Methods:** Serum samples from 140 patients, with either diagnostics of breast cancer recurrence (REC, n=60), or no evidence of disease (NED, n=80), were collected and analyzed. Samples were separated into discovery and validation sets for testing statistical model robustness. Chromatographic separations were performed using an Agilent HPLC system installed with two hydrophilic interaction chromatography (HILIC) columns, and then targeted data acquisition was performed in multiple-reaction-monitoring (MRM) mode using an AB Sciex QTrap 5500 mass spectrometer.

**Result and discussion:** We established an LC-MS/MS targeted metabolic profiling system for breast cancer recurrence detection using serum samples. Applying this metabolic profiling system, we achieved targeted screening of 156 (99 in negative and 57 in positive mode) multiple reaction monitoring (MRM) transitions, for metabolites located in 25 important metabolic pathways (e.g., TCA cycle, amino acid metabolism, and glycolysis). 103 metabolites were eventually detected, with 12 metabolites showing statistical difference (p<0.05) between breast cancer REC and NED groups in the discovery set of samples (33 REC and 41 NED). PLS-DA was also applied to select most important metabolites (based on VIP score) for multivariate statistical model development, and 11 metabolites, namely aspartic acid, urate, tyrosine, histidine, arginine, sorbitol, citraconic acid, hypoxanthine, F16BP, G1P and carnitine, were found have VIP score > 1.5. These metabolites are involved in multiple important human metabolic pathways, such as fructose and mannose metabolism, tricarboxylic acid cycle, glycolysis, and multiple amino acid
metabolisms. Area under the receiver operator characteristic (AUROC) curve, sensitivity, and specificity of the PLS-DA model using these 11 metabolites together with cancer antigen 27.29 (CA27.29, the traditional protein breast cancer biomarker used in therapy monitoring) was then calculated for the discovery set of samples, to evaluate the diagnostic power of these biomarkers. Excellent AUROC (0.90), sensitivity (0.85), and good specificity (0.80) were obtained, which is superior to results using CA27.29 alone (which showed an AUROC of 0.68 for detecting REC over NED) in this study. Furthermore, the validation set of our samples (27 REC and 39 NED) were also tested using the same metabolites model and we have achieved the AUROC of 0.84, sensitivity of 0.56 and specificity of 0.97. The results suggested that we have discovered the potential usefulness of applying a group of important metabolites biomarkers for breast cancer recurrence detection.

**Keywords:** Breast cancer recurrence, metabolite biomarker, targeted metabolic profiling

### 68: Colorectal Cancer Screening and Progression Monitoring Using Targeted LC-MS Based Metabolomics

Danijel Djukovic, PhD; Jiangjiang Zhu, PhD; Lingli Deng, PhD; Haiwei Gu, PhD; Daniel Raftery, PhD, MBA; et al.

**Background:** Colorectal cancer (CRC) is the third most common cancer and the third largest cause of cancer death in the US, but is highly treatable when diagnosed in its early stages (Stage I or II). Thus, early CRC detection is the most effective approach to reduce CRC deaths. In addition to the importance of early detection, close monitoring of disease progression (DP) can be critical for patients’ prognosis management and treatment decisions. In this study we investigated a targeted LC-MS/MS approach for serum metabolic profiling to detect CRC and to monitor and predict patient therapy, using a panel of significantly altered metabolites as potential biomarkers.

**Methods:** A targeted HILIC-LC-MS/MS method was developed to measure 164 metabolites across 25 metabolic pathways in serum samples. This method was applied to analyze 234 serum samples from CRC patients (n=66), polyp patients (n=76) and healthy controls (n=92) as well as 59 serum samples from 21 CRC patients, including 23 samples from DP patients and 36 from other CRC disease status (e.g., stable disease and complete remission). Univariate and multivariate statistical analyses were applied for metabolite biomarker discovery and model development on a selected set of promising biomarker candidates. Monte Carlo cross validation (MCCV) was performed to evaluate model robustness.

**Results/Discussion:** Mann-Whitney U-test analysis of the CRC samples showed that 42, 48 and 8 metabolites were significantly different (p<0.05) in CRC vs. controls, CRC vs. polyps, and polyps vs. controls, respectively. PLS-DA models clearly separated CRC patients from both healthy controls and polyp patients in this study. Receiver operator characteristic (ROC) curves showed high sensitivities (0.96 and 0.89, respectively, for differentiating CRC patients from healthy controls or polyp patients), good specificities (0.80 and 0.88), low false discovery rates (0.22 and 0.14), and excellent AUROC (0.93 and 0.95). Monte Carlo cross validation (MCCV)
was also applied, demonstrating the robust diagnostic power of this metabolic profiling approach.

For therapy monitoring, univariate analysis showed 36 metabolites, including monosaccharides, amino acids, carboxylic acids and nucleosides, showed differences (p<0.05) between CRC DP compared to other disease status (e.g., stable disease and complete remission), and twelve of these were previously reported by other CRC serum metabolites studies. Highly significant changes (defined as p<0.001) were found in the average levels of seven metabolites, namely fructose, aspartic acid, oxalic acid, lactate, pyruvate, oxaloacetate and orotate. A PLS-DA model was built based on the combination of these seven metabolite biomarkers, and excellent performance was obtained (sensitivity 96%; specificity 75%) and area under the ROC of 0.92, which was superior to the traditional carcinoembryonic antigen (CEA) marker currently used for CRC monitoring (AUROC=0.76) for detecting DP over other disease status.

**Acknowledgments:** This work was supported by AMRMC grant W81XWH-10-1-0540.

**Keywords:** metabolic profiling, colorectal cancer, liquid chromatography, mass spectrometry

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**69: Massive Glutamine Cyclization to Pyroglutamic Acid in Human Serum Discovered Using NMR Spectroscopy**

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**Background:** Glutamine is one of the most abundant metabolites in blood and is a precursor as well as end product central to numerous important metabolic pathways. A number of surprising and unexpected roles for glutamine, including cancer cell glutamine addiction discovered recently, stress the importance of accurate analysis of glutamine concentrations for understanding its role in health and numerous diseases. To date, glutamine has been considered as a relatively stable metabolite under the mild conditions widely used in the analysis of human blood serum, for example in NMR analysis. In the present study we question this assumption, focusing on the stability of glutamine and its proclivity to form pyroglutamic acid.

**Methods:** Blood metabolites from pooled human serum were extracted utilizing ultrafiltration and protein precipitation method developed in our laboratory. The 800 MHz NMR analyses of the intact, ultrafiltered and protein precipitated serum samples were made using the recently developed NMR method in our laboratory that enabled identification of an unprecedented number of blood metabolites. Spiking experiments with standard glutamine and pyroglutamic acid were performed for intact serum, ultrafiltered and protein precipitated samples. Metabolites were quantitated and evaluated comprehensively.
Results: Glutamine massively cyclizes to pyroglutamic acid during analysis using protein precipitation and ultrafiltration, while no such cyclization detected in intact serum. Interestingly, while glutamine cyclization occurs in both ultrafiltered and protein precipitated serum, the cyclization was not detected in intact serum. Strikingly, due to cyclization, the apparent serum glutamine level drops by up to 75% and, concomitantly, the pyroglutamic acid level increases proportionately. Further, virtually under identical conditions, the magnitude of cyclization is vastly different for different portions of samples from the same pool of human serum. However, the sum of glutamine and pyroglutamic acid concentrations in each sample remains the same for all portions.

Discussion: We have identified a surprising phenomenon of glutamine cyclization to pyroglutamic acid that occurs during protein removal. Such, cyclization of glutamine in human blood serum under mild conditions typically used in the metabolomics of human serum/plasma is new. The massive in situ cyclization of glutamine within the ESI source identified recently (Anal. Chem. 2014, 86(12), 5633-5637) represents a major challenge for the analysis of blood glutamine using MS. NMR spectroscopy, which does not encounter the phenomenon of in situ cyclization, enables accurate analysis of blood glutamine and hence potentially represents a reliable analysis method for blood glutamine. The unexpected glutamine cyclization to pyroglutamic acid indicate the importance of considering the sum of apparent glutamine and pyroglutamic acid levels, obtained from the contemporary analytical methods, as the actual blood glutamine level for biomarker discovery and biological interpretations. Further, as observed for human serum in this study, glutamine cyclization may also occur during the analysis of other biological specimens as well, and hence caution is needed in the analysis of this biologically important metabolite.

Acknowledgement: This work was supported by an NIGMS Grant (2R01GM085291)

Keywords: NMR, blood, glutamine, pyroglutamic acid, cyclization, quantitation

70: Understanding the physiological role of γ2-AMPK
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Background: AMP activated protein kinase (AMPK) is an energy sensor of the cell. Activation of AMPK during stress conditions modulates cell metabolism, growth and survival. AMPK is a heterotrimeric complex consisting of catalytic α subunit and regulatory β and γ subunits. AMPK α and β subunits have two isoforms (α1, α2 and β1, β2) and γ subunit has three isoforms (γ1, γ2 and γ3). Point mutations in the γ2 subunit have been reported in humans, which cause glycogen storage cardiomyopathy associated with cardiac hypertrophy and arrhythmias. Molecular mechanisms contributing to this phenotype is elusive.

Methods: To understand the physiology role of γ2-AMPK we have adopted two strategies. 1. To identify novel interacting partners we generated HEK293 cell line stably expressing flag-tagged human γ2-subunit and performed mass-spectrometry of proteins pulled down by anti-flag antibody. 2. To understand the subcellular localization of γ subunits we have generated GFP-γ1, GFP-γ2 (wt and R302Q, T400N, N488I and R531G mutants) and GFP-γ3 constructs; expressed them in
COS7 cells and performed confocal microscopy. We also did bioinformatics analysis and identified nuclear localization sequence (NLS) and nuclear export sequence (NES) in γ subunits. We have also validated the NLS and NES by generating GFP-γ2 either lacking the NLS and NES. We have also validated our confocal studies by cell fractionation studies.

**Results and Discussion:** All the GFP-tagged-γ subunits were uniformly distributed between nucleus and cytosol. Truncated version of GFP-γ2 lacking NLS is cytosolic and GFP-γ2 lacking NES is mostly accumulated in the nucleus, suggesting that these signal sequences are required for nuclear-cytoplasmic shuttling of γ2 subunit. Treatment with leptomycin B that inhibits Chromosomal region maintenance 1 (CRM1) led to nuclear accumulation of GFP-γ2-wt, suggesting that the nuclear-cytoplasmic shuttling is CRM1 mediated process. Since AMPK is stress responsive protein kinase we evaluated whether GFP-tagged γ subunits have altered cellular distribution during stress. Either by activation with AMPK activator (A769662) or under metabolic stress condition (2-Deoxy-glucose or glucose deprivation) only GFP-γ2 (both Wt and mutants) accumulated in the nucleus with no effect on cellular distribution of GFP-γ1 of GFP-γ3. To further strengthen this observation we have performed nuclear-cytoplasmic fractionation studies using Flag-γ2 overexpressing stable cell line. We observed that under metabolic stress conditions α2, β1 and γ2 subunits accumulate in the nucleus and we have also demonstrated that the nuclear accumulation of α2β1γ2 complex is AMPK activity dependent. The nuclear translocation of γ2-AMPK complex was associated down-regulation of the rRNA transcription, suggesting that activation of γ2-AMPK affect ribosome biosynthesis and protein translation. We have identified several novel γ2 interacting proteins that are involved in the transcriptional regulation of rRNA from our proteomic studies of flag-tagged γ2-AMPK expressing cells; we are currently validating these candidates to understand the novel role of γ2-AMPK in stress response.

**Acknowledgements:** The study is funded by grants from the NHLBI.

**Keywords:** cardiomyopathy, AMPK, metabolism, nuclear translocation

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**71: Genetic Deletion of γ2-AMPK subunit Aggravates Cardiac Dysfunction During Pathological Hypertrophy**

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**Background:** AMP-activated protein kinase (AMPK) maintains energy homeostasis and plays critical roles in cell metabolism, growth and survival. AMPK is a heterotrimeric complex composed of catalytic α-subunit and regulatory β- and γ-subunits with multiple isoforms for each subunit. The γ2-subunit of AMPK (γc) is a minor isoform in the adult heart but mutations in this isoform causes glycogen storage cardiomyopathy. Furthermore, we have recently demonstrated that γ2-AMPK is upregulated in the failing hearts of mice and human, suggesting an important role of γ2-AMPK in the development of heart disease.

**Methods:** To investigate the biological role of γ2-AMPK in the heart, we generated a cardiac specific knock-out mouse (γ2KO) for γ2-AMPK subunit. γ2KO mice were
made by crossing transgenic mice (Flox) that flank the exon 6 of γ2-AMPK with loxP sites to βMHC-Cre transgenic mice that restrict Cre recombinase expression in the fetal heart. To determine cardiac specific role of γ2-AMPK under stress conditions, we subjected 8-10 weeks old γ2KO and control mice to transverse aortic constriction (TAC).

**Results:** The mRNA and activity of γ2-AMPK in γ2KO hearts were significantly reduced compared to those of their control hearts (P<0.05, n=4). Decreased glycogen content was also observed in the heart. γ2KO mice were born normal and remained indistinguishable from their respective Flox/Flox control mice in terms of heart weight and cardiac function for up to 6 months under baseline condition. After TAC, γ2KO mice showed greater increase in heart weight and more severe cardiac dysfunction compared to control mice (survival rate 50% vs. 62%, HW/BW 7.2 ± 1.08mg/g vs. 5.7 ± 0.36mg/g, and ejection fraction 17 ± 5.8% vs. 29 ± 4.2%, P<0.05, n=6 for control TAC mice). The greater hypertrophic response was further evidenced by increases in BNP mRNA level, myocardial fibrosis and cardiac myocyte size. The γ1- and γ2-AMPK activity significantly increased in control hearts after TAC but the γ2-AMPK activity was depressed and the γ1-AMPK activity compensated for the decrease in γ2KO hearts, suggesting that the deficiency of γ2-AMPK activity cause pathological cardiac hypertrophy under stress. Protein expression level of hexokinase II (HKII) was down-regulated both in the whole lysates and the cytosol of γ2KO hearts, which resulted in decline in the pentose phosphate pathway (PPP) flux and increase in oxidative stress. Knock-down of γ2-AMPK subunit also increased oxidative stress in neonatal rat ventricle cardiomyocytes (NRVCNs). HKII overexpression abolished the phenylephrine-induced hypertrophy and hydrogen peroxide-induced cell death in γ2-AMPK knock-down NRVCNs.

**Discussion:** These data indicate that γ2-AMPK plays a critical role in response to chronic pressure overload in mice. These results suggest that modulation of γ2-AMPK may be a viable therapeutic approach to protect against pathological cardiac hypertrophy and failure.

**Keywords:** AMPK, γ2 subunit, glycogen, cardiac dysfunction, hexokinase II

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72: Restoring NAD Redox Balance Improves Heart Failure via Cytosolic & Mitochondrial Protein Acetylation

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**Background:** In a mouse model of mitochondrial dysfunction due to cardiac-specific deletion of Ndusf4 (cKO) we found that elevated NADH/NAD ratio and hyperacetylation of mitochondrial proteins led to increased mPTP sensitivity and accelerated heart failure induced by chronic pressure overload with transverse aortic constrictions (TAC).

**Methods and Results:** Either restoring NADH/NAD pharmacologically (by supplying NAD precursor NMN) or genetically (by expressing the key enzyme in the NAD salvage pathway, NAMPT) improved HF in cKO. Comparison of protein acetylome in
WT, cKO and cKO+NAMPT hearts identified a number of proteins with "NAD-sensitive" differential acetylation. Increased lysine acetylation (LysAc) of malate aspartate shuttle (MAS) proteins in cKO reduced MAS activity and increased cytosolic NADH/NAD ratio suggesting mitochondrial dysfunction altered cytosolic redox state via MAS flux. Higher LysAc of proteins involved in Ca2+ homeostasis and mPTP regulation in cKO was associated with higher mitochondrial Ca2+ content and mPTP hypersensitivity, and all could be normalized by restoring NADH/NAD. To test whether elevated NADH/NAD ratio also contributes to the pathogenesis of heart failure in animal models without prior mitochondrial defect we treated mice subjected to TAC with NMN for 4 weeks. TAC increased NADH/NAD ratio and LysAc in the heart; mitochondria isolated from these hearts showed higher sensitivity of mPTP opening during Ca2+ stimulation. NMN treatment normalized all the changes and furthermore, reduced pathological hypertrophy and improved contractile function in TAC-NMN compared to TAC-vehicle. Similarly, NAMPT reduced hypertrophy and contractile dysfunction induced by 2-week isoproterenol stimulation.

Discussions: In summary, our findings suggest that the NADH/NAD imbalance caused by impaired mitochondrial respiratory function contributes to the progression of heart failure via hyperacetylation of key regulatory proteins of stress responses.

Acknowledgements: This study is supported by grants from the American Heart Association (postdoctoral fellowship to CFL) and the NIH (HL110349 and HL067970 to RT).

Keywords: Heart failure, protein acetylation, NAD redox balance

73: Deletion of DGAT1 Reduces Triglyceride Incorporation of Palmitate and Alters Cardiac Metabolism
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Background: Triglyceride (TG) metabolism in heart failure has been well established to be impaired in both human and animal models however its impact on severity and progression of heart failure is unknown. Patients with advanced heart failure display decreased myocardial triglyceride levels and decreased diacylglycerol:acyltransferase 1 (DGAT1), the rate limiting step in triglyceride synthesis, however the significance of DGAT1 to heart failure has not been investigated. Therefore we hypothesized that reduced TG synthesis by DGAT1 would cause metabolic remodeling in patients with heart failure.

Methods: Transgenic mice with a tamoxifen-inducible cardiac specific deletion of DGAT1 (iKO) were utilized to evaluate cardiac triglyceride synthesis by 13C NMR spectroscopy in perfused hearts. Gas chromatograph mass spectrometry (GCMS) was used to detect triglyceride fatty acid composition and 13C labeling rates of specific fatty acids.

Results: 13C NMR spectroscopy revealed a 30% reduction in labeled fatty acid (FA) incorporation into TG in iKO (40 minute peak area 7.86±0.26 vs. 5.73±0.50) with no changes in total TG content. In depth investigation of TG pool labeling by GCMS revealed that iKO mice had reduced labeling of 16:0 FA (42.85±2.83 vs. 33.09±4.75 %13C, P=0.014) with no change in 16:1 18:0, 18:1, 18:2 labeling or overall TG FA.
composition were observed (n=4). Reduced 16:0 incorporation reduced overall fatty acid TG incorporation rate into TG (34.18±5.05 vs. 24.20±3.77 nmol/min/g, p=0.16) albeit not significantly. Fats not incorporated into are oxidized directly as iKO hearts showed increased oxidation of exogenous FA (67.0±4.1% vs. 48.5±5.3%) and reduced glucose oxidation (12.9±4.2% vs. 27.4±4.5%, n=5-7). Using linear regression analysis, a strong correlation between the rate of TG turnover and FA oxidation in both groups was observed (R2=0.82, P=0.002, n=8).

**Discussion:** These observations suggest that DGAT1 preferentially incorporates 16:0 FA into TG and that fatty acids not incorporated into the TG pool are diverted towards oxidative metabolism. Therefore reduced DGAT1 expression in failing hearts whose oxidative metabolism is compromised, may promote lipotoxicity by reducing incorporation of toxic lipids into TG.

**Keywords:** Triglyceride, Heart, DGAT1, Metabolism

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**74: Endogenous DRP1 modulates cardiac respiration through mPTP and independent of fission**

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**Background:** The cardiac mitochondria exhibit a stable morphology with a rather low level of dynamic changes. However, fission and fusion proteins, such as dynamin-related protein 1 (DRP1) are abundant in the heart. Whether these proteins bear other functions in the heart than mitochondrial dynamics regulation are largely unknown. We hypothesize that endogenous DRP1 in the heart regulates mitochondrial respiration independent of fission.

**Methods:** Mitochondrial respiration was determined by measuring the OCR with Seahorse assay or Clark type electrode in adult rat cardiomyocytes or mitochondria isolated from adult mouse heart. Confocal imaging was used to quantify mitochondrial morphology in adult cardiomyocytes and H9C2 myoblasts. To evaluate the role of mitochondrial permeability transition pore (mPTP), we monitored superoxide flashes (SOF) and laser-induced mPTP openings, and used cyclophilin D knockout mice (CypD KO). Mitochondrial ROS and Ca2+ were also monitored.

**Results:** Inhibiting the DRP1 GTPase activity by Mdivi-1 or overexpression of the dominant-negative mutant (DRP1-K38A) induced mild mitochondrial morphological changes in adult cardiomyocytes, and inhibited mitochondrial respiration. Modulation of fission/fusion by overexpressing DRP1 or treating cells with S3, a compound facilitates fusion, exhibited significant morphological changes, but failed to influence respiration. Therefore, endogenous DRP1 activity may regulate respiration in the heart and this effect is dissociated with morphological changes. Further, inhibiting DRP1 activity attenuated the frequency of SOF, indicating decreased transient mPTP openings, delayed laser-induced permanent mPTP opening, and increased mitochondrial Ca2+. Inhibiting DRP1 activity decreased mitochondrial ROS levels. The role of DRP1 inhibition on respiration absents in CypD KO myocytes, suggesting the involvement of mPTP in the modulation of respiration by endogenous DRP1.
Conclusion: These results suggest that endogenous DRP1 positively regulates respiration in the heart. This effect is likely independent of its role in mitochondrial fission. DRP1 regulation of respiration may involve transient opening of mPTP and contribute to mitochondrial Ca2+ and ROS signaling.

Acknowledgments: This study is funded by American Heart Association and NIH. We thank Dr. Yisang Yoon (Georgia Regents University) for DRP1-K38A and Dr. Quan Chen (Chinese Academy of Science) for S3.

Keywords: DRP1; Mitochondrial respiration; mPTP; Mitochondrial fission; Cardiomyocyte

75: β1-AR/CaMKII Signaling Causes Cardiac Myocyte Death via Mitochondrial Calcium Overload

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Background: Activation of β1-adrenergic receptors (β1-AR) plays crucial roles in cardiac physiology and pathology. In the short-term, β1-AR activation mediates the fight-or-flight response to boost cardiac performance, whereas chronic β1-AR activation can lead to detrimental outcomes in the heart. It has been proposed that chronic β1-AR stimulation may increase diastolic calcium, which can induce the expression of hypertrophic genes, mitochondrial dysfunction and cell death. However, the specific mechanisms of chronic β1-AR stimulation-induced mitochondrial dysfunction are largely unknown.

Results: In this study, we monitored mitochondrial calcium, membrane potential, reactive oxygen species (ROS) and permeability transition pore (mPTP) openings in cultured adult cardiac myocytes and found that chronic β1-AR stimulation by isoproterenol (ISO, 100 nM for 12-48 hr) induced mitochondrial calcium accumulation before triggering mPTP opening and oxidative stress. Moreover, these effects are mediated by activation of a downstream kinase of β1-AR signaling, the calcium calmodulin kinase II (CaMKII) and depend on mitochondrial calcium uptake via mitochondrial calcium uniporter. Blocking mitochondrial calcium uptake or inhibiting CaMKII activity ameliorated mitochondrial dysfunction as reflected by maintaining of membrane potential and resistant to triggered mPTP opening. These approaches also prevented ISO-induced myocyte death at the later stage (48 hr).

Discussion: Taken together, we provided direct evidence to show the causal role of mitochondrial calcium overload induced by CaMKII during chronic β1-AR in mitochondrial dysfunction and cardiac myocyte death.

Keywords: β1-adrenergic receptors; CaMKII; Mitochondrial Calcium; Cardiac Myocyte; Isoproterenol

76: Hyperactive Mitochondrial Dynamics Mediates Obesity-Induced Heart Dysfunction

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Background: Metabolic syndrome, featured by obesity and diabetes, is an independent risk factor for cardiovascular disease. Hyperlipidemia, an important
etiologic facet of metabolic syndrome, is proposed to induce fatty acid oxidation, oxidative stress, and mitochondrial dysfunction; eventually leading to insulin resistance and myocyte death in the heart. However, how obesity induces cardiac mitochondrial dysfunction and whether mitochondrial dysfunction plays any role in the development of heart failure are questions that remain largely unanswered. **Methods:** Here, we evaluated mitochondrial function in heart tissue isolated from mice fed a high-fat diet (fat calories = 60%) and adult rat cardiac myocytes supplemented with high concentration palmitate (0.3 mM). **Results:** When compared to the littermate controls, mice fed a high-fat diet for 18 weeks had a significant increase in body weight (145%), blood glucose (129%), displayed glucose intolerance, and heart failure. After only 4 weeks of high-fat diet the cardiac NAD+/NADH ratio decreased 61.2%. Hearts from high-fat fed mice had a 211% increase in transient respiration-coupled ROS-production events localized to individual mitochondria (superoxide flash). Despite increased superoxide flash events, oxidative stress was not detected in the heart tissue of mice on high-fat diet. Rat cardiomyocytes treated with palmitate also displayed a decrease in NAD+, the NAD+/NADH ratio, and increased superoxide flash events. Additionally, palmitate treatment increased mPTP opening, cell death, and decreased reactive oxygen species. Inhibition of FA transport to the mitochondria using the carnitine palmitoyltransferase I inhibitor etomoxir (100 µM) attenuated palmitate induced mitochondrial respiration and superoxide flash events. Intraguingly, morphological changes of mitochondria were hyperactive after palmitate treatment, indicating the activation of mitochondrial dynamism. Investigation of proteins that facilitate mitochondrial dynamics revealed that palmitate treatment increased the expression of both DRP1 and OPA1. Finally, inhibiting the activity of a fission protein, DRP1 or increasing NAD+ levels ameliorated palmitate-induced mPTP opening and myocyte death. These results demonstrate that high fatty acid supply decreases NAD+ levels, which may lead to mitochondrial dysfunction and myocyte death through mitochondrial dynamics. **Discussion:** Taken together, we have elucidated a novel mechanism in which a high-fat diet may disturb mitochondrial redox and morphology. Further research is needed to identify the pathophysiological consequence of activating this pathway and functional changes in cardiac myocytes associated with or resulting from hyperlipidemia, obesity and diabetes. Furthermore, future research should focus on the molecular mechanism(s) that activate DRP1 when fatty acids are in excess. **Acknowledgements:** Diabetic Research Center, grant number P30DK017047; National Institutes of Health, National Heart, Lung, and Blood Institute, grant number HL114760. **Keywords:** Mitochondria, diabetes, high-fat diet, cardiomyopathy, lipid toxicity

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**77: Enhanced Fatty Acid Oxidation and the Response to Exercise in Mice**

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**Background:** Obesity is a serious health concern in the United States, with an incidence of approximately one-third of the population. Although behavior
modification, including reduced caloric intake and physical activity, is a recommended strategy for weight loss, pharmacological interventions remain attractive. Enhancing systemic fatty acid oxidation (FAO), via deletion of acetyl CoA carboxylase 2 (ACC2), was originally shown to favorably affect the obesity phenotype in mice. However, recent studies have challenged those findings. Despite the controversy, ACC2 inhibitors are being pursued for the treatment of obesity. It has not been determined whether enhancing systemic FAO impacts the response to exercise. Therefore, the present study seeks to explore the effects of enhanced FAO, accomplished via systemic deletion of ACC2 (ACC2-null), on exercise performance in mice.

**Methods:** Male ACC2-null mice and littermate controls (CON; n=4-5 each group) were randomly assigned to an exercise training (EX) protocol on a motorized treadmill (60 min/day, 5 days/wk, 10 wks). A cohort of non-exercised CON and ACC2-null mice were used as sedentary controls. Mice were subjected to an endurance exercise capacity (EEC) test to exhaustion pre- and post-EX. Metabolic phenotyping in untrained ACC2-null and CON mice was performed by the Nutrition Obesity Research Center (NORC).

**Results:** No differences were noted in body weight in ACC2-null and CON mice up to 6 months of age. Heart, liver, skeletal muscle, and adipose tissue mass were not significantly different in ACC2-null and CON. Food and water intake were also similar. Cage activity was not different during the dark cycle but tended to be less during the light cycle in ACC2-null (P = 0.059). Plasma levels of glucose, fatty acids, and triglycerides were not changed in ACC2-null mice. When subjected to an EEC test, untrained ACC2-null mice demonstrated a ~30% decrease in total exercise time (78.0 ± 2.9 vs 107.8 ± 9.0 min, P < 0.05). Citrate synthase activity in the soleus was reduced by ~20% in ACC2-null, suggesting a potential negative effect of ACC2 deletion on skeletal muscle mitochondria. However, 10wks of EX abolished the impaired exercise capacity in ACC2-null mice as total exercise time was similar to CON (122.2 ± 4.5 vs 125.1 ± 7.4 min). Trained ACC2-null mice had reduced body weight (~7%) and adipose tissue mass (~15%) compared to CON-EX (P < 0.05).

**Discussion:** Although increased FAO, via systemic deletion of ACC2, impairs endurance exercise capacity in untrained mice, chronic exercise training negates the baseline defect. In young, healthy mice of normal body weight, exercise training combined with ACC2 deletion appears to have a positive effect on body weight and adipose mass. The present data suggest that ACC2 inhibitors, in conjunction with chronic exercise training, have therapeutic potential for the treatment of obesity.

**Acknowledgements:** This work is supported by grants from the American College of Sports Medicine and the American Heart Association. UW-NORC is supported by the National Institutes of Health, NIDDK.

**Keywords:** lipid metabolism; acetyl CoA carboxylase 2; exercise training; obesity
Background: Reduced dietary consumption of saturated fats (SFs) is recommended for the prevention of cardiovascular disease. In particular, the SFs, palmitate and stearate, have been linked to apoptosis and lipotoxicity. However, fatty acids (FA) that enter the cell can be oxidized in the mitochondria or redirected to other endogenous lipids compartments such as diacylglycerol (DG) or triacylglycerol (TG). It is not clear whether high consumption of SF alters the composition of these compartments specifically in the heart. Therefore, the present study assessed the composition of myocardial FA, DG and TG in mice fed high SF diets.

Methods: Male FVB mice (10wks old) were fed two different SF diets for 12wks. One cohort was fed a Western diet (WD, n=4) containing 41% kcal/fat (62% SF) and another cohort was fed a Surwit diet (SD, n=4); containing 58% kcal/fat (93% SF). Age matched mice (n=5) receiving a standard chow diet containing 21.6% kcal/fat (32% SF) were used as controls (CON). Body weight and food intake were measured weekly. Composition of FA, DG and TG in cardiac lipid extracts was assessed by gas chromatography-mass spectrometry (GC-MS). Expression of genes related to TG metabolism was analyzed by Real-Time PCR.

Results: High SF feeding significantly increased body weight by ~25% over CON. No difference in body weight was observed between WD and SD mice. Adipose tissue mass was increased ~3-fold after high SF feeding (CON, 0.66 ± 0.42g; WD, 1.87 ± 0.24g; SD, 2.27 ± 0.29g). Fasting blood glucose levels were elevated to a similar degree in both WD and SD mice compared to CON. GC-MS analysis of the endogenous cardiac lipid compartments showed that SFA comprised: 88% of the total cytosolic FA pool, 77% of DG and 50% of TG, which was constant in CON, WD, and SD hearts. However, further analysis revealed that both WD and SD hearts had significantly less linoleate in the FA, DG, and TG pools (P < 0.05 vs. CON). In addition, the relative abundance of palmitoleate and oleate in TG was increased to a similar degree in both WD and SD compared to CON. Gene expression of stearoyl CoA desaturase 1 (SCD1), an enzyme involved in the conversion of palmitate to palmitoleate and stearate to oleate, was increased ~1.5 fold over CON in both WD and SD.

Discussion: Our results show that high fat diets, with varied total fat and SF content, do not appear to induce different obesity phenotypes in mice. In addition, the amount of SF intake does not significantly affect the relative abundance of SF found in the endogenous cardiac lipid compartments. However, our data suggest that the SFs entering TG storage are converted to an unsaturated form, which could be an adaptive mechanism. Furthermore, high SF diets result in a reduction of the unsaturated FA, linoleate, in all lipid compartments, which may be an early indicator of cardiac stress.

Acknowledgements: This work is supported by funding from the Sao Paulo Research Foundation and the American Heart Association.

Keywords: lipid profiling; lipid metabolism; diet-induced obesity; cardiac lipotoxicity
79: Determining the Role of Branched Chain Amino Acid Utilization in Cardiac Substrate Utilization

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**Background:** Emerging evidence suggests that the metabolism of three essential branched-chain amino acids (BCAA) leucine, isoleucine and valine is significantly altered during the development of cardiovascular and metabolic disease. Catabolism of BCAAs is a key step in maintaining BCAA homeostasis in the body, and recent data from the Tian laboratory suggests that defective BCAA catabolism impairs glucose metabolism, which contributes to insulin resistance and exacerbated I/R injury. Impairment of BCAA catabolism due to the deletion of mitochondrial localized protein phosphatase 2C (PP2Cm), a key enzyme in activating BCAA catabolism, increases levels of BCAAs within the body. The PP2Cm-knock out (KO) mouse, which has elevated BCAA levels in the heart, shows a significant decrease in the relative contribution of glucose to oxidative metabolism and an increase in fatty acid oxidation compared to control mice. Although KO mice at two months have normal blood glucose and insulin levels, at six months these mice develop hyperglycemia and hyperinsulinemia. Additionally, KO hearts have a higher susceptibility to stress-induced heart failure and demonstrate decreased ability to recover after ischemia reperfusion (I/R) injury. Interestingly, increasing glucose uptake in PP2Cm-KO mice through overexpression of an insulin independent glucose transporter GLUT1 rescues and improves the cardiac response to IR injury, suggesting that enhancing glucose utilization can compensate for defective BCAA catabolism.

**Methods:** We utilize a cardiac tissue-specific stable isotope tracer (SIT)-based metabolomics platform to identify regulatory mechanisms of cardiac metabolism in vivo. PP2Cm-KO and WT control hearts are perfused with mixed substrate buffer containing either uniformly labeled 13C-glucose or 13C-BCAAs. Metabolite content of heart tissue is extracted and analyzed using gas chromatography mass spectrometry (GCMS) or liquid chromatography mass spectrometry (LCMS). GCMS allows for the detection of 13C enrichment in TCA cycle intermediates and related amino acids. LCMS allows for the detection of 13C enrichment in sugar phosphates, CoAs and ketoacids. Pairing a cardiac tissue-specific mass spectrometry (MS) based approach with SIT metabolite profiling provides insights into the interplay between glucose and BCAAs in the PP2Cm-KO heart.

**Discussion:** Preliminary findings have shown a decrease in mean 13C enrichment in the perfused hearts of PP2Cm-KO mice in the presence and absence of BCAAs. TCA cycle and glycolytic intermediates showed decreased levels of C13 incorporation on GCMS and LCMS. We predict that labeling experiments involving 13C labeled BCAAs will show interaction and utilization in noncannonical pathways.

**Keywords:** BCAA, glucose, metabolomics, PP2Cm
80: High Glucose Suppresses Branched-chain Amino Acid Catabolism through downregulation of KLF15
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Background: The branched-chain amino acids (BCAAs), including leucine, isoleucine and valine, are essential amino acids for mammals. Emerging evidence suggested that BCAAs catabolism was significantly changed during the development of cardiovascular diseases, however the mechanism involved in the regulation of BCAA catabolism is unknown.

Methods: Transgenic mice with cardiac specific overexpression of glucose transporter 1 and neonatal cardiomyocytes are used in this study.

Results: In mouse hearts with increased glucose uptake and utilization due to overexpression of glucose transporter 1 (GLUT1-TG) we observed a 2-fold downregulation of Kruppel-Like Factor 15 (KLF15) and its target genes involved in BCAA catabolism by microarray analysis. Quantitative PCR results further confirmed that the mRNA of KLF15 was significantly decreased in GLUT1-TG (-44%, p<0.05 vs. WT, N=6) as well as its targets encoding multiple BCAA catabolism enzymes e.g. branched chain amino-acid transaminase 2 (BCAT2) (-62%), branched-chain alpha-keto acid dehydrogenase complex (BCKDHC) (-54%), mitochondrial protein phosphatase 2C (PP2Cm) (-63%) (p<0.05, N=6). Targeted LC-MS analysis of the GLUT1-TG hearts found higher intracellular BCAAs levels than WT (by 40%, p<0.05) while the BCAA metabolites, e.g. α-keto-β-methylvalerate (KMV) and α-ketoisocaproate (KIC) were decreased (-37%). These observations led us to hypothesis that high glucose suppressed BCAA catabolism through downregulation of KLF15. To test our hypothesis, neonatal cardiomyocytes (CM) were incubated with normal glucose (NG, 5.5 mM) or high glucose (HG, 25mM) medium. HG treatment significantly decreased the expression of BCAT2 (-58%), BCKDHC (-34%) and PP2Cm (-61%) (p<0.05, N=3), accompanied by downregulation of KLF15 (-47%, p<0.05, N=3) compared to NG. In order to examine whether KLF15 was responsible for glucose induced suppression of BCAA catabolism, CM was infected with adenovirus harboring KLF15 to normalize the KLF15 expression under HG condition. We found that overexpression of KLF15 prevented the downregulation of BCAA catabolism genes in response to HG.

Discussion: Our results suggest that increased intracellular glucose negatively regulates BCAA catabolism through inhibition of KLF15. These findings have important implications for cardiac pathology associated with increased reliance on glucose, such as pathological hypertrophy and heart failure.

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Keywords: Glucose, BCAAs, transcriptional regulation
**Title:** Defective branched-chain amino acids catabolism induces metabolic remodeling in heart  

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**Background:** The branched-chain amino acids (BCAA), i.e. leucine, isoleucine and valine, are essential amino acids required for protein homeostasis, energy balance, and nutrient signaling. BCAA catabolism in mitochondria is regulated by the branched-chain keto acid dehydrogenase (BCKDH) complex. A mitochondria localized phosphatase 2C (PP2Cm) is a key regulator of BCKDH activity. We previously found that PP2Cm deficiency resulted in defect BCAA catabolism, increased oxidative stress, and in zebrafish caused cardiac dysfunction.

**Methods and Results:** In the present study, the PP2Cm knock-out (KO) mice showed normal cardiac function, assessed by echocardiography at 2 months (FS: 44±3 and 43±2% for KO and WT, P=ns). Myocardial high energy phosphate content and the isovolumic contractile function assessed by 31P NMR spectroscopy in isolated perfused hearts, were also normal (PCr/ATP=1.90±0.03 (KO) vs. 1.81±0.05 (WT), P =ns). However, 13C NMR isotopomer analysis revealed a significant decrease in the relative contribution of glucose to oxidative metabolism (16±3 vs. 26±2% for KO and WT, respectively, P=0.018) in the KO accompanied by an increase in fatty acid oxidation (51±4 vs. 39±3% for KO and WT, respectively, P=0.020). Glycogen content in the KO hearts was also reduced by > 50% (4.4±0.5 vs. 10.9±1.8 µmol glucose/g, P=0.000). As anticipated, 31P NMR spectroscopy for hearts perfused with nontracer 2-deoxyglucose indicated that the rate of insulin-stimulated glucose uptake in the KO heart was decreased by > 20% (0.47±0.007 vs. 0.60±0.02 2-DG-P/ATP/g/min for KO and WT, respectively, P=0.0007). The pyruvate dehydrogenase (PDH) flux estimated from [4-13C] glutamate/[3-13C] alanine enrichment was also significantly suppressed (8.16±0.96 vs. 12.19±1.15 % for KO and WT, P=0.034). These findings collectively suggest a metabolic remodeling in the KO. When subjected to 25 minutes low-flow ischemia (1% of baseline) and 40 minutes reperfusion, cardiac function recovered to 51±11% in WT (n=7) but only 8±3% in KO (n=11) (P=0.002). The recovery of PCr, ATP, and Pi during reperfusion in the KO also failed to reach the level of WT hearts. Increasing glucose uptake and utilization in the KO by overexpressing insulin-independent glucose transporter GLUT1 (TG) rescued the exacerbated I/R injury. Cardiac function recovered to 49±9% in KO-TG (n=6).

**Conclusion:** In conclusion, our results suggest that defective BCAA catabolism induces cardiac metabolic remodeling by suppression of glucose entry, which contributes to exacerbated I/R injury.

**Keywords:** Branched-chain amino acids; Metabolism; Ischemia/reperfusion injury; Nuclear magnetic resonance
82: Increased Myocardial Fatty Acid Oxidation Protects Against Hypertrophy and Diastolic Dysfunction
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Background: Our previous work demonstrated that increasing myocardial fatty acid oxidation (FAO) by 50%, via deletion of acetyl CoA carboxylase 2 (ACC2), did not cause cardiac dysfunction under non-stressed conditions. Moreover, cardiac dysfunction was preserved and cardiac hypertrophy was attenuated during chronic pressure-overload. Therefore, we further tested the cardioprotective effect of enhanced myocardial FAO using a cardiac-specific inducible deletion of ACC2 (iKO) in mice fed a high fat diet (HFD) or during chronic administration of angiotensin II (AngII).

Methods: Male iKO and control (CON) mice were randomly assigned to HFD (60% kcal/fat) or control diet (CD, 17% kcal/fat) for 12wks. In a separate cohort of iKO and CON mice, AngII or vehicle (saline) was delivered for 4wks by osmotic mini-pumps. Echocardiography and tissue Doppler imaging measured systolic and diastolic function, respectively. Substrate oxidation was assessed in tissue extracts from isolated perfused hearts via 13C NMR spectroscopy.

Results: HFD resulted in obesity and glucose intolerance in both genotypes. HFD and iKO each resulted in a 60% increase in FAO, while FAO was increased 2.5-fold in iKO-HFD vs CON-CD. Systolic and diastolic function was unaltered in iKO and/or HFD hearts. Heart weight to tibia length ratio (HW:TL) increased 20% in CON-HFD but not in iKO-HFD mice (p<0.05). With AngII administration, HW:TL increased 30% and fibrosis increased 3.5-fold in CON (p<0.05 vs CON-vehicle) while both of these parameters were attenuated 50% in iKO (p<0.05 vs CON-AngII). E'/A' and E/E' ratios were significantly altered in CON-AngII while iKO-AngII were similar to CON-vehicle. FAO decreased in CON-AngII but iKO-AngII remained similar to CON-vehicle.

Conclusion: These data suggest that increased cardiac FAO via inducible deletion of ACC2 does not exacerbate cardiac dysfunction during HFD. Furthermore, AngII-induced adverse cardiac remodeling, evidenced by myocardial hypertrophy, fibrosis, and diastolic dysfunction, is attenuated in ACC2-iKO hearts.

Acknowledgements: This work has been supported by funding from the American Heart Association and the National Institutes of Health.

Keywords: heart; lipid metabolism; obesity; acetyl CoA carboxylase 2

83: A Novel Signalosome in the Alpha1a-Adrenergic Receptor and its Genetic Variant Signaling Pathway
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Introduction: Human α1-adrenergic receptors (AR), members of G protein-coupled receptor superfamily (GPCR), regulate stress-facilitated changes in the
cardiovascular system and mediate human blood pressure via smooth muscle cell (SMC) proliferation and vasoconstriction. α1aAR subtype is unique in its ability for agonist-induced constitutive cycling and signaling. We have shown that human α1aAR-G247R (247R) genetic variant, identified in a hypertensive patient, constitutively couples to β-arrestin1/MMP/EGFR transactivation pathway leading to hyperproliferation in fibroblasts(1), cardiomyoblasts(2), and SMCs. Spinophilin (SPL), a large scaffolding protein, binds to 3rd intracellular loop of some GPCRs, including α1ARs. SPL alters α1bAR signaling by recruiting RGS2 (negative Regulators of G-protein Signaling) to the receptor. We hypothesized that differential interaction of α1aAR-WT (WT) or 247R with SPL/RGS2/β-arrestin signalosome mediate unique signaling of WT and 247R in different cardiovascular cells. In this study we examined the direct interaction of SPL and RGS2 with WT and 247R, and their role in cell proliferation and signaling pathways.

**Methods:** To determine receptor-protein interactions, co-immunoprecipitations were performed using HEK293 cells expressing HA-tagged α1ARs and either full length Myc-SPL or its fragments or Flag-RGS2. Protein expression was analyzed by Western blot. SPL knockdown or RGS2 overexpression was performed by transfecting cells with SPL-specific or scrambled siRNA or Flag-RGS2 and cell proliferation was determined by cell counting.

**Results:** Our results demonstrate a distinct interaction of α1ARs with SPL and RGS2. While WT has stronger interaction with SPL compared with 247R or α1b, 247R interaction with RGS2 is stronger. RGS2 overexpression in SMCs inhibited 247R-triggered hyperproliferation, but not in WT cells. 247R expression in SMCs or cardiomyoblasts also induces upregulation of endogenous SPL levels compared with control or WT cells. SPL knockdown inhibited hyperproliferation, while co-overexpression of RGS2 and SPL restored the hyperproliferation of 247R cells.

**Discussion:** In this study we reveal that SPL regulates α1aAR signaling by its differential binding to WT or 247R in different cardiovascular cells by recruiting RGS2 protein to the receptors. Due to its strong interaction with WT, SPL recruits RGS2 and inhibits receptor signaling, while weak interaction of SPL with 247R diminishes the inhibitory effect of RGS2 thus permitting hyperproliferation of 247R cells. Because SPL is a major docking site for many regulatory proteins, including RGS2, its knockdown inhibits 247R-triggered hyperproliferation by allowing RGS2 to bind directly to 247R as observed in response to RGS2 overexpression. Overexpression of SPL together with RGS2 partially restores hyperproliferation suggesting that in 247R cells SPL binds RGS2 preventing RGS2-247R interaction. These novel findings are unique as they unravel critical role of SPL and RGS2 in regulating α1AR signaling, as well as identifying SPL as a potential novel target for treatment of α1AR-mediated cardiovascular disorders.


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**Keywords:** GPCR, Spinophilin, RGS2, transactivation