#1: Transposon-based germ-line transformation of the Coffee Berry Borer, *Hypothenemus hampei*: Opportunities for functional genomics
Acevedo, Flor E.; Barrera, Carlos; Aluvihare, Channa; Harrell, Robert A.; Benavides, Pablo.

The coffee berry borer (CBB), *Hypothenemus hampei* (Coleoptera: Curculionidae), poses a serious threat to worldwide coffee production, causing annual losses of about $500 million. This invasive beetle is a very aggressive pest with ecological, behavioral and genetic characteristics that make effective control difficult to achieve. As an effort to improve our understanding of the genetic regulation of the insect's basic biology we developed a transposon-based germ-line transformation protocol. The location of presumptive germ cells as well as main embryological events was documented in CBB by observing alive embryos under a composed microscope. Embryogenesis of CBB is typical of long germ-band insects and took approximately 144 hours at 27C. The syncitial blastoderm stage was observed at approximately 8.5 hours after egg laying, and pole cells were visible at the posterior end of the embryo. To transform the insect's germ cells two sets of injections were performed: In the first set, a mixture of two vectors and two transposase expressing plasmids (pPB (piggyBac) 3xP3ECFP + PBhsÎ”SST & pMos (Mos) 3xP3DsRed + PKhsp82Mos) was applied. From 495 injected embryos, 50.7% G0 adults were recovered and one transgenic line containing pPB 3xP3ECFP was obtained. In the second set a different mixture of vectors and transposase-expressing plasmids was injected (pMinos (Minos) 3xP3DsRed + PHss6hs1LMi20 & pHermes (Hermes) Actin5CEGFP + pBCHsHH) resulting in 144 (82.7%) G0 adults. Three of these adults produced transgenic progeny containing pMinos 3xP3DsRed. These transgenic tools along with transcriptomic and genomic analysis would be useful in functional genomics studies and would potentially lead to the development of new insect control techniques.

#2: Low Resting Metabolic Rate in Exercise-Associated Amenorrhea is Not Due to a Reduced Proportion of Energetically Expensive Tissue Compartments
Heather C.M. Allaway, Karsten Koehler, Rebecca J. Mallinson, Emily A. Southmayd, Mary Jane De Souza, Nancy I. Williams

Energy deficiency in exercising women is associated with menstrual disturbances and a concomitant reduction in resting metabolic rate (RMR) when expressed relative to body size or lean mass. It remains unknown whether this apparent RMR suppression is a consequence of a reduction in metabolically active tissue compartments during energy deficiency or due to metabolic adaptations at the tissue level.

Purpose: To explore whether the reduced RMR in women with exercise-associated amenorrhea is explained by a lower proportion of energetically expensive tissue compartments or the result of metabolic adaptations.

Methods: RMR and metabolic tissue compartments were compared among exercising women with amenorrhea (AMEN, n=42) and eumenorrheic, ovulatory menstrual cycles (OV, n=37). RMR was measured using indirect calorimetry and predicted from metabolic tissue compartments as measured by dual-energy X-ray absorptiometry (DXA).

Results: Measured RMR was lower than DXA-predicted RMR in AMEN (1215Â±31 vs. 1327Â±18 kcal/d, p<0.001) but not in OV (1284Â±24 vs. 1252Â±17, p=0.16), resulting in a lower ratio of measured to DXA-predicted RMR in AMEN vs. OV (91Â±2% vs. 103Â±2%, p<0.001). Total triiodothyronine was also reduced in AMEN when compared with OV (80.1Â±3.4 vs. 92.4Â±2.1 ng/dL, p=0.003). Residual mass was greater (p<0.001) and adipose tissue was reduced (p=0.003) in AMEN when compared to OV. Brain, skeletal muscle, and bone mass were not different among groups.

Conclusion: Our findings suggest that RMR suppression in exercise-associated amenorrhea is not the result of a reduced size of energetically expensive tissue compartments but due to metabolic adaptations at the tissue level that are indicative of energy conservation.

#3: Ron receptor tyrosine kinase plays a protective role in diet induced obesity and associated steatohepatitis
Joselyn N. Allen, Limin Zhang, Shan Yu, Gayathri Balandaram, Jeffrey M. Peters, Andrew D. Patterson, Pamela A. Hankey-Giblin

Obesity is one of the leading causes of preventable death in the U.S and a well-established risk factor for several pathological conditions including steatohepatitis. The activation of inflammatory tissue resident macrophages has proven to play a key role in the progression of the metabolic syndrome. Consequently, mechanisms that manipulate macrophage phenotype has become of great interest for developing therapeutic strategies for combating obesity and its associated diseases. Our lab has shown that Ron tyrosine kinase, a receptor expressed on tissue resident macrophages, limits an inflammatory response while promoting tissue repair. In this study, we first investigate the impact of Ron signaling on metabolic features preceding steatohepatitis using high fat diet (HFD) fed wild type (WT) and Ron deficient (Ron KO) mice. Ron KO mice demonstrated more severe events associated with obesity such as increased adiposity, exacerbated hyperglycemia and trend toward increased liver weights when compared to WT animals. Altered Ron signaling resulted in a shift in the plasma lipoprotein distribution from primarily VLDL and LDL to predominantly HDL in the WT control mice. To then establish the role of Ron signaling in steatohepatitis, high cholesterol diet (HCD) fed ApoE KO and Ron KO ApoE KO mice (DKO) were evaluated. We showed that DKO mice experience more severe steatohepatitis which is attributed to an increased prevalence of activated inflammatory Kupffer cells. In vitro studies ascertain that Ron manipulation of macrophage phenotype is in part, a result of downstream AMPK activation. These data indicate the role of Ron in attenuating obesity and its associated diseases and identify a novel downstream pathway involved in this process.

#4: Looking at Odd and Branched-Chain Fatty Acids in Cow's Milk
Fatemeh Bahari, Paddy Ssentongo, Anna Robuccio, Steven J. Schiff, Bruce J. Gluckman

Milk fat depression (MFD) is a condition observed on dairy farms and is a decrease in milk fat yield and is commonly caused by feeding high grain and high unsaturated fat diets. Changes in milk fatty acid (FA) profile during MFD are well described, including increases in specific trans intermediates from ruminal microbial biohydrogenation of unsaturated FA. Odd-and branched chain FA (OBFA) found in milk fat originate predominantly from absorption of microbially

synthesized FA and previous work has established correlations between OBFA and changes in the rumen microbial population. The objective of the current experiment was to characterize the modification of OBFA during induction and recovery of diet-induced MFD. Samples were used from a study where nine Holstein cows were fed either a high fiber, low oil diet (control), or a low fiber, high oil diet (induction). The control diet always followed the induction diet. Milk samples were taken every other day during both induction of and recovery from MFD. Milk FA were extracted and methylated. Milk FA profile was determined by gas chromatography and the OBFA were identified using bacterial FA standard. Overall, the induction diet resulted in a rapid decrease in i14:0, i15:0, a15:0, 15:0, i16:0, a17:0 with a maximal reduction in total OBFA occurring on d 3 of induction period. The exceptions were i14:0 and i16:0 which did not differ until day 7 of the induction period. During recovery all OBFA increased and reached control values by day 3. This demonstrated that OBFA are changed during diet-induced MFD and that the microbial population within the rumen changes rapidly during both induction and recovery. Increasing OBFA is of interest for human health and this provides insight into dietary factors that can be used to increase their production.

#5: Evidence of Seizures and Sudden Death in a Post-Malarial Model of Epilepsy
Michel Baldin, Elizabeth Palmer, Daniel E. Rico, Kevin Harvatine

It is well established, though relatively unknown, that cerebral malaria (CM) leads to epilepsy in an estimated 300,000 children per year. Mortality rates among persons with epilepsy are 2-3 times that of in developed countries, and are reported as high as six times in regions where malaria is endemic. Sudden unexplained death in epilepsy (SUDEP) serves as a major mortality risk, and is now thought to involve the interaction of seizures and cardio-respiratory failure.

We investigated mice cured of CM for evidence of epilepsy and SUDEP to identify models to both investigate the mechanisms of epileptogenesis and complex epilepsy-related phenomena and to test intervention strategies.

We investigated four murine models of CM for evidence of post-malarial epilepsy by combining mouse strains (Swiss Webster (SW), C57BL/6, CBA) and two Plasmodium-berghei (Pb) parasites (NK65 and ANKA): SWPbNK65,

SW-PbANKA, C57BL/6-PbANKA, and CBA-PbANKA. Cohorts of three-week old littermates were inoculated with infected erythrocytes, then rescued with Artesunate when they demonstrated signs of advanced CM.Animals were implanted with EEG, EMG, and ECG electrodes 14 or more days post treatment, and video-EEG monitored continuously for 1-8 months per animal.

In all model combinations studied, recurrent spontaneous seizures were observed in a large fraction (50-90%) of the animals that survived to recording. Post treatment death rates prior to implant were observed in some mixtures, with the largest rate in SWPbANKA. In these cases, death was typically accompanied by a single seizure-like behavioral event followed either by immediate death or a severe decrement in health.

All epileptic mice with ECG recordings showed significant changes in cardiac activity associated with seizures. In 80% of the seizures, a transient preictal episode of tachycardia occurred followed by ictal and late-ictal bradycardia. AV node blocks were observed ictally and post-ictally in 66% of seizures.

We have developed models of post-malarial epilepsy (PoME). In long-term chronic recordings, we observe complex interactions between seizures, heart arrhythmias, and death. These observations are consistent with pathologies of the human condition of sudden unexplained death in epilepsy(SUDEP). These models, which are induced from infection not genetic mutation, therefore provide a unique platform for the study of the mechanisms of SUDEP and models to investigate the complex interactions between seizures, cardiac and respiratory dysfunction, and SUDEP.

#6: Transition Metals at Supported Lipid Bilayers, presented by Alexis Baxter
Alexis J. Baxter, Matthew F. Poyton, Anne Sendecki and Paul S. Cremer

In order to understand biological processes such as ion regulation, cell signaling, and lipid oxidation, the behavior of transition metals at biointerfaces must be explored. The Cremer Group investigates the association of transition metals like Zn^{2+}, Cu^{2+} and Fe^{2+} with lipids from cell membranes. We have developed a novel fluorescence quenching assay to measure the affinity of transition metals for specific lipids in bilayers. Using model membrane systems, we have discovered that transition metals can have high affinity for numerous physiologically relevant lipids, enhanced oxidation at membrane surfaces and cause domain formation.

#7: Orai1 Concatemers Reveal a Hexameric Orai1 Channel Assembly
Xiangyu Cai, Yandong Zhou, Robert M. Nwokonko, Natalia A Loktionova, Xianming Wang, and Donald L. Gill

The multimeric assembly of the CRAC channel has remained a contentious issue despite strong crystallographic evidence of a hexameric structure for both Drosophila and human Orai1. Recent studies suggested concatemeric tetramers of Orai1 mediate authentic CRAC current whereas equivalent hexameric concatemers form only non-selective cation channels. We expressed concatenated human Orai1 constructs with intervening 36-aa linkers. We observed that expression of dimers, trimers, tetramers, pentamers and hexamers of human Orai1 in HEK cells stably expressing STIM1-YFP (HEK STIM1-YFP), all gave rise to similar high levels of Ca^{2+} entry. All constructs were C-terminally tagged with tdTomato and all were observed to be exclusively PM-expressed. Each construct also gave similar Ca^{2+} entry when expressed in HEK Orai1 knockout cells stably expressing STIM1. Expressed in HEK STIM1-YFP cells, the Orai1 dimers, trimers, tetramers, pentamer and hexamers all gave rise to authentic CRAC channel activity with similar inward rectification and reversal potential. Substituting wt-Orai1 with the pore-inactive E106A mutant in tetramers, we observed that the initial two N-terminal units of the tetramer are crucial for channel activity. The remaining two C-terminal residues in tetramers are inconsequential for function. In hexamers, the position of inactive mutants in the concatemer are not specific. Substitution with a single E106A mutant monomer in the hexamer had the same effect in reducing channel function at each position. Substitution with two E106A residues resulted in little remaining channel function. We interpret these results to reveal that hexamers

are likely the true functional Orai1 channel unit. Tetramers are likely to feed dimers into a hexameric structure, with the two C-terminal residues outside the hexameric ring, explaining why these last two residues in the tetramer can be substituted for E106A mutants without loss of activity. Certainly, the hexameric concatemer gives a fully functional CRAC channel.

#8: A pilot study to test preschool children's intake of vegetables prepared with herbs and spices to create a variety of flavor options
Elizabeth Carney, Wendy Stein, Nicole Reigh, Kathleen Keller

American children consistently fail to meet the recommended daily servings of vegetables. Adequate vegetable consumption in children is associated with known health benefits, mainly decreased risk for developing diseases. One possible explanation for low vegetable consumption is that children have innate aversions to bitter compounds, which are found in many vegetables, particularly green vegetables. Previous research shows that children who can taste the bitterness of PROP, a compound used to test genetic bitter sensitivity, are more susceptible to low vegetable intake. Our current study (n = 40) will test the hypothesis that adding multiple herb and spice blends to vegetables to increase flavor variety within in a meal will increase liking and intake in children that are 3- to 5- years old. Previous research has shown that increased variety within a meal increases intake of food overall, but this has not yet been tested on vegetable consumption. To investigate the affect of flavor variety on vegetable intake, children attended 2 laboratory visits with their parents and consumed two randomized test meals of common foods: macaroni and cheese, applesauce, carrots, 2% milk, and water. Cooked carrots were used as a model system because they are generally familiar to and accepted by children of this age. On one visit, the meal included one serving each of carrots seasoned with cinnamon-nutmeg-ginger, black pepper-oregano-garlic, or cardamom-cumin-allspice (Variety Condition). On the other visit, the meal included three servings of carrots with the cinnamon-nutmeg-ginger blend (No Variety Condition). Children were given a max of 30 minutes to eat as much as they would like and intake was measured with pre- and post-meal weights. On their first visit, children's reaction to the bitter compound PROP was tested using a validated child-friendly forced choice design between yucky/bitter or tastes like water. Additionally, children rated their liking of carrot and broccoli samples prepared separately with each of the three spice blends. Liking was measured using a validated, child-friendly 5-point visual scale. While carrots are typically accepted by children, broccoli has more bitter attributes and may be rejected more frequently, especially by children with genetic sensitivities to bitter tastes. We hypothesize that children will consume more carrots during the meal with the variety condition than the meal with the no variety condition. In addition, we also hypothesize that those children with genetic sensitivity to PROP will have lower intake of carrots and lower liking of broccoli when compared to children who are not sensitive to PROP, however we still predict an increased intake of carrots when offered in the variety condition compared to the single condition. These simple spice additions to vegetables may help to increase intake in the home and school. Findings from this study may help children meet daily recommendations, leading to healthier lifestyles.

#9: Targeted delivery of molecular cargo into the mosquito germline
Duverney Chaverra-Rodriguez, Grant L. Hughes, Yasutsugu Suzuki, David Peterson, Jason L. Rasgon

Genetic manipulation is a powerful technique for addressing research questions in arthropods of medical importance. Current approaches rely upon delivering DNA or endonucleases to preblastoderm embryos via embryonic microinjection. However, embryonic microinjection is technically challenging, is limited to a small number of arthropod taxa, and is inefficient even in optimized species. As such, there is a critical need to develop methods for arthropod genetic manipulation that are simple, accessible for many researchers and generally compatible for a large variety of arthropod species. During oogenesis, insects transfer yolk protein precursors to developing oocytes by receptor-mediated endocytosis (RME). We are developing tools exploiting this phenomenon to specifically target DNA and protein cargo into the mosquito germline for heritable modification of the chromosomal genetic sequence. We will discuss progress in optimizing this technique and in transferring the technology to multiple mosquito species.

#10: Manipulation of chromatin configuration for suppression of host immunity by zinc finger effectors (MoZFEs) of rice blast pathogen
Yueying Chen, Wenhua Liu and Yinong Yang

Rice blast disease, caused by the fungal pathogen *Magnaporthe oryzae*, is one of the most devastating plant diseases in the world. The fungal pathogen is known to produce various protein effectors to target host proteins and facilitate pathogenesis and disease development. We have recently identified a family of *M. oryzae* zinc finger effectors (MoZFEs) and demonstrated their entry into rice cells during the fungal infection. Interestingly, transgenic rice lines expressing MoZFEs exhibited suppression of defense gene expression and increased susceptibility to rice blast disease. To elucidate the MoZFE-mediated virulence mechanism, nine putative host targets of MoZFEs were identified from rice by the yeast two-hybrid screening. One of them directly interacts with MoZFEs in plant cell nucleus and is a conserved histone chaperone important for chromatin remodeling. Based on the preliminary data, we hypothesize that MoZFEs target a histone chaperone and likely modify chromatin configuration to suppress defense gene expression and host immunity. Further testings of this hypothesis will help elucidate the molecular mechanism of the rice-*M. oryzae* interaction and facilitate the development of new strategies to improve rice disease resistance and food security.

#11: Fast and robust detection of ancestral positive selection from genomic data
Xiaoheng Cheng, Cheng Xu, Michael DeGiorgio

Natural selections leave unique genetic signatures on a population, and are greatly informative of their past biology and environment. Advances in whole-genome sequencing has dramatically increased the size and complexity of available genomic data, while powerful tool for detecting ancestral positive selections are still lacking. We therefore developed the Ancestral Branch Statistic (ABS) based on genetic distances among 4 sub-populations to detect sweeps on the internal branch. We prove ABS to have comparable power with the newly-published likelihood-based method 3P-CLR, with a much faster computing speed. Scanning genomic data of East Asians, Europeans and Africans reveal both expected and new candidate genes.

#12: Interferon-γ receptor (IFNγR) signaling is essential for the development of Toll like Receptor-7 mediated systemic lupus erythematosus

Sathi Babu Chodisetti, Phillip P. Domeier, Chetna Soni, Stephanie L. Schell, Melinda J. Elias, Ziaur S.M. Rahman

Systemic lupus erythematosus (SLE) is an autoimmune disorder characterized by the production of antibodies against self-antigens (autoantibodies). Spontaneously developed germinal center (Spt-GC) B cells and follicular helper T cells (Tfh) are involved in the generation of these autoantibodies. TLR7 plays a pivotal role in Spt-GC B cell and Tfh development. Autoimmune-prone B6.Sle1b mice carrying an extra copy of TLR7 in the Y-linked autoimmune accelerating (Yaa) locus (B6.Sle1b.Yaa mice) have significantly higher Spt-GC B cells and Tfh cells that strongly correlate with exacerbation of lupus disease. Here we report that IFNγR deficiency in B6.Sle1b mice that carry Yaa locus (B6.Sle1b.Yaa.IFNγR-/-) leads to significantly reduced Spt-GC and Tfh responses, resulting in decreased anti-nuclear antibody (ANA)-specific IgG antibody forming cells, serum autoantibody titers and renal pathology compared to control mice (B6.Sle1b.Yaa). These results are consistent with the data obtained from mice treated with TLR7 ligand. Further, in vitro results demonstrate that STAT1 dependent B cell-intrinsic IFNγR signaling is crucial for TLR7-induced IFNγ production by B cells. Together, these data describe the absolute requirement of IFNγR signaling in TLR7-induced autoimmunity. Future studies will involve deciphering the molecules that associate with TLR7:IFNγR signaling pathways and targeting them for therapeutic interventions to treat SLE.

#13: The Ron receptor tyrosine kinase regulates inflammatory response in an experimental model of multiple sclerosis
Adwitia Dey, Lindsay M. Snyder, Veronika Weaver, Margherita T. Cantorna, Pamela A. Hankey-Giblin

In the experimental autoimmune encephalitis (EAE) murine model of multiple sclerosis (MS), both central nervous system (CNS)-resident macrophages (microglia) and infiltrating monocyte-derived macrophages contribute to disease progression. The Ron receptor tyrosine kinase is expressed on tissue resident macrophages including microglia and is involved in regulating inflammatory responses. An in vivo deletion of Ron (Ron KO) promotes inflammatory macrophage activation (M1) and limits a reparative macrophage phenotype (M2). Herein we investigated whether Ron expression plays a critical role in regulating disease progression in EAE. Ron KO mice exhibit delayed onset of EAE (day 14) compared to Wild-Type (WT) (day 10), however Ron KO mice exhibit greater peak severity at onset. Ron KO mice display T-cell mediated peripheral inflammation, as demonstrated by the significant increase in the secretion of interferon gamma (IFNγ) but not IL-17 and IL-10. At day 14, cultured lymph node cells from Ron KO mice exhibit increased expression of M1-macrophage mediated biomarkers iNOS, COX-2, IL-6, IL12B, IL1Î² and TNF-Î±. A likely cause of this increased inflammatory response can in part be attributed to a T-cell mediated increase in IFNγ. Furthermore, CNS tissues from Ron KO mice have increased gene expression of hallmark inflammatory biomarkers, such as iNOS, IL12B and COX-2 at day 14. The results indicate an interplay between the innate and adaptive immune system, in fostering an M1-mediated inflammatory state as the underlying factor for the observed increase in disease severity in Ron KO mice. Maintenance of Ron expressing macrophages could then be a potential therapeutic approach to treating MS symptoms.

#14: Increases in perceived stress during energy deficiency is associated with lower follicular phase estrogen exposure and distinguishes anovulation from less severe exercise-related menstrual disturbances in young women

Clara V. Etter, Mary Jane DeSouza, Jay L. Lieberman, Nancy I. Williams

BACKGROUND: It is well documented that low energy availability and psychological stress can independently and in combination perturb reproductive function in mammalian species. Prolonged functional hypothalamic amenorrhea is associated with low and unchanging concentrations of estrogen and progesterone and alterations in stress and metabolic hormones. Clinical consequences include the development of the Female Athlete Triad, increased risk of injury, unfavorable changes in cardiovascular function, and transient infertility. While decades of research has documented the causal role of low energy availability, no studies have explored the possibility that psychological stress also contributes to reproductive dysfunction in exercising women. PURPOSE: To investigate the contribution of energy availability and psychological factors in the etiology of subclinical reproductive dysfunction induced by a three month exercise and caloric restriction intervention in sedentary, regularly menstruating women (n=36). METHODS: Women (age 18-24 yrs, BMI 21-29 kg/m2) were randomized to either an exercise only group or one of four groups with combinations of caloric restriction and exercise to induce varying levels of an energy deficit. The intervention occurred over the course of three menstrual cycles and included supervised exercise (5 d/wk, 50-85% VO2max, 20-75 min) with controlled feeding. Menstrual disturbances (luteal phase defects (LPD), oligomenorrhea, and anovulation (ANOV)) were detected using daily measures of urinary estrone-1-glucuronide (E1G), pregnanediol glucuronide (PdG), and mid-cycle luteinizing hormone, and menstrual calendars. Depressive symptoms were assessed at Baseline with the Beck Depression Inventory (BDI) (Beck, 1961), and Perceived Stress was measured every two weeks with the 14-item Perceived Stress Scale (Cohen, 1983). Subjects were divided into groups based on their change in perceived stress score from Baseline to the end of the intervention: Decrease in Perceived Stress -12.5 to -0.5; Low Increase in Perceived Stress 0.5 to 4.5; High Increase in Perceived Stress 5.0 to 36.5. The proportion of women in each Perceived Stress group who experienced at least one of each type of menstrual disturbance was determined along with effects of Perceived Stress group on urinary E1G and PdG. Logistic regression was used to detect associations between average energy deficit, Perceived Stress, and the induction of menstrual disturbances. Repeated measures ANOVA was performed to determine the effects of perceived stress on urinary E1G AUC and PdG AUC as calculated for each intervention cycle. RESULTS: The intervention caused moderate weight loss (0-4 kg), increases in fitness, declines in percent body fat and declines in luteal phase PdG exposure ($p<0.05$). Average percent energy deficit, changes in aerobic fitness, and changes in BMI across the intervention were not significantly different between the perceived stress groups. The average energy deficit, but not perceived stress, was associated with the incidence of a menstrual disturbance, particularly LPD ($p=.037$). The change in perceived stress, but not energy deficit, was predictive of those who experienced at least one anovulatory cycle ($p=0.021$). Specifically, High Increase in Perceived Stress was related to a higher incidence of ANOV vs. Decrease and Low Increase in Perceived Stress (High Increase, 58.3% vs. Low Increase, 14.3% and Decrease, 10.0%, $p< 0.013$). Low and High Increase in Perceived Stress were also associated with lower follicular phase E1G exposure ($p=.021$). BDI was not related to menstrual disturbances or urinary metabolites of reproductive hormones. CONCLUSION: Modest levels of caloric restriction and exercise induce subclinical luteal phase

defects whereas increased psychological stress is associated with more severe disturbances, i.e., anovulation and reduced estrogen exposure in the context of energy deficiency. These results have implications for understanding individual susceptibility to exercise related menstrual disturbances. Future studies should address the reproducibility of this finding in a field setting and the underlying mechanisms of psychosocial determinants of menstrual disturbances.

#15: Somatic Awareness and Self-Symptom Recognition in Advanced Heart Failure Patients
Michael Evans

Purpose: This study examined somatic awareness and self-symptom recognition in Stage D heart failure (HF) patients, comparing those patients who were newly diagnosed with HF to those patients with chronic HF.

Background: Heart failure is a chronic, debilitating disease that affects almost six million Americans. However, there is little known about how patients recognize and interpret symptoms of the disease. Two such phenomena lacking adequate attention are somatic awareness and self-symptom recognition. In addition, these concepts have not been studied in relation to the duration of time a patient has been diagnosed with HF.

Methods: The mixed methods design that guided this study was the concurrent triangulation design. Using this design, a prospective cross-sectional survey design was conducted to understand if a difference exists in somatic awareness between newly-diagnosed and chronic stage D HF patients. The newly-diagnosed group included patients with Stage D HF who had been diagnosed with HF for two years or less. The chronic HF group included patients diagnosed with HF for longer than two years. Somatic awareness was measured using the HF Somatic Perception Scale, v. 3, an 18- item Likert scale. In addition, explorative qualitative descriptive interviews were conducted to better understand self-symptom recognition in this sample. All qualitative data were coded using the items from the HF Somatic Perception Scale. Each yes answer to the items on the HF Somatic Perception Scale was analyzed to determine whether the participant perceived the symptom to be related to HF.

Results: The sample included newly-diagnosed Stage D HF patients (n=9) and chronic HF patients (n=11). Analysis indicated a difference between the newly-diagnosed and chronic groups, $t(18) = -2.45$, $p = 0.03$. The chronic group had a higher mean somatic awareness score, 28.82, compared to the newly-diagnosed group, 12.33. In addition, length of time from diagnosis was significantly correlated with the HF Somatic Perception total score ($rs = 0.53$, $p = 0.02$). Qualitative analysis found that none of the participants recognized their HF symptoms as being a result of HF.

Conclusions: While results showed higher somatic awareness scores for the chronic group than for the newly-diagnosed group, it is unclear whether the difference is related to length-of-time of living with HF or to other variables, including co-morbidities with symptoms similar to HF. However, while participants did not attribute their symptoms to HF, it is not possible to conclude that they had poor self-symptom recognition; instead, they may have been accurate in relating their symptoms to another co-morbid condition. Implications from this research nevertheless include that Stage D HF patients may not recognize symptoms of HF, which is a concern

because of the potential resulting delay in appropriate treatment. It follows that more effective education for advanced HF patients is needed to help them understand HF symptoms and so to participate more effectively in their treatment, thus potentially improving patient outcomes and decreasing the economic burden of HF overall. Future longitudinal research needs to be conducted on a larger sample to examine somatic awareness and self-symptom recognition before definitive conclusions can be reached.

#16: Studies of postural synergies: A sensitive tool for early Parkinson's disease
Ali Falaki, Xuemei Huang, Mechelle M. Lewis, Mark L. Latash

One of the cardinal features of Parkinson's disease (PD) is postural instability or balance impairment, which becomes more prevalent with disease severity. This disabling symptom of PD represents a fundamental change in disease progression signifying the transition from Hoehn and Yahr (HY) stage-II to HY stage-III. Although many studies have investigated the effect of PD on motor functions, there is no standardized technique to measure balance instability. We hypothesized that multi-muscle synergies may be used as a biomarker sensitive to changes in motor coordination, even in PD patients with no clinical symptoms of postural instability. We also explored the sensitivity of indices of synergic control to dopamine-replacement treatment. These studies were done under the framework of the uncontrolled manifold (UCM) hypothesis. Eleven HY stage-II PD patients and eleven age-matched healthy controls stood on a force-platform and performed three main tasks: voluntary cyclic body sway, quick discrete body sway forward, and a quick load release from extended arms resulting in a postural perturbation. Four muscle groups with parallel modulation in activation levels (M-modes) were identified from surface electromyographic (EMG) activity of 13 leg and trunk muscles on the right side of the body, using principal component analysis with factor extraction and Varimax rotation. Multiple linear regression was used to link small changes in M-modes to shifts of the center of pressure in the anterior-posterior direction (COPAP) the Jacobian matrix. Further, a synergy index was computed reflecting the relative amount of inter-trial M-mode variance within the null-space of the Jacobian. Compared to healthy controls, PD patients showed: (1) a lower amount of variance accounted for by the identified M-modes, (2) lower synergy indices during steady-state standing, and (3) delayed and reduced anticipatory synergy adjustments (ASAs: a drop in preparation to a quick action). In a follow-up study, five HY stage-II and five HY stage-III PD patients were tested both off and on their PD medications. Off-drug, the differences between PD patients and controls were larger, due in part to the lower amount of the M-mode variance preserving the COPAP coordinate. These results support the idea of impaired multi-muscle synergic control of posture in PD patients without clinically identifiable postural problems. It allows quantifying three PD-associated problems in postural control: (1) The less consistent organization of muscles into groups (M-modes); (2) Lower postural stability during steady-state tasks; and (3) Impaired ability to prepare to a quick action. The third one may be causally related to episodes of freezing, which represent one of the most disabling symptoms of PD. The findings highlight the importance of balanced interaction among brain loops involving the basal ganglia and cerebellum.

#17: Deletion of CTNNB1 in inhibitory circuitry contributes to autism-associated behavioral defects
Fengping Dong, Joanna Jiang, Colleen McSweeney, Donghua Zou, Long Liu, Yingwei Mao

Mutations in Î²-catenin (CTNNB1) have been implicated in cancer and mental disorders. Recently, loss-of-function mutations of CTNNB1 were linked to intellectual disabilities (IDs), and rare mutations were identified in patients with autism spectrum disorders (ASD). As a key regulator of the canonical Wnt pathway, CTNNB1 has an essential role in neurodevelopment. However, the function of CTNNB1 in specific neuronal subtypes is unclear. To understand how CTNNB1 deficiency contributes to ASD, we generated CTNNB1 conditional knockout (cKO) mice in parvalbumin interneurons. cKO mice had increased anxiety, but had no overall change in motor function. Interestingly, CTNNB1 cKO in PV-interneurons significantly impaired object recognition and social interactions and elevated repetitive behaviors, which mimic the core symptoms of patients with ASD. Surprisingly, deleting CTNNB1 in parvalbumin-interneurons enhanced spatial memory. To determine the effect of CTNNB1 KO in overall neuronal activity, we found that c-fos was significantly reduced in the cortex, but not in the dentate gyrus and the amygdala. Our findings revealed a cell type specific role of CTNNB1 gene in regulation of cognitive and autistic-like behaviors. Thus, this study has important implications for development of therapies for ASDs carrying the CTNNB1 mutation or other ASDs that are associated with mutations in the Wnt pathway. In addition, our study contributes to a broader understanding of the regulation of the inhibitory circuitry.

#18: A genetics approach to understanding the host-pathogen parasitic relationship of apple and *Erwinia amylovora*

Melissa Finley, Tim McNellis

Fire blight, caused by the bacterium *Erwinia amylovora*, is a destructive disease affecting apples and pears. This study seeks to elucidate the ability of auxotrophic *E. amylovora* mutants, which are unable to synthesize one or more of the biological molecules required for growth, to effectively induce disease, in comparison to the prototrophic wild type, which is fully capable of synthesizing all of the molecules required for growth. This comparison will reveal more about the parasitic relationship of *E. amylovora* to its host, specifically what types of biological molecules the bacteria are capable of scavenging from host tissues. In this work, over 5000 mutants were obtained via Tn5 mutagenesis of a Pennsylvania wild type strain, 6P1, and tested for auxotrophy on M9 minimal media plates and confirmed as auxotrophs in a secondary liquid media assay. Of the 5000 tested, approximately 115 were auxotrophic. Confirmed auxotrophic mutants were then inoculated in immature Gala apple fruits and monitored for fire blight symptom development. DNA was then isolated from each mutant in order to analyze the segments bearing the Tn5 mutated gene sequence and deduce the affected genes and their products. Future work will include inoculation of selected pathogenic auxotrophic mutants in 2 year old apple trees to compare disease development to that which occurs in immature fruits. This study will contribute to the understanding of *E. amylovora* parasitic nutrient acquisition by comparing biosynthetic pathways affected in pathogenic and non-pathogenic mutants, which should identify host molecules needed for bacterial growth and disease development.

#19: Presence of three antibiotics in wheat plants and groundwater at The Living Filter: a wastewater reuse site

Alison Franklin, Clinton Williams, Danielle Andrews, Emily Woodward, John (Jack) Watson

With rising demands on water supplies, wastewater treatment plant (WWTP) effluent is often reused to irrigate agricultural lands. Emerging contaminants, like antibiotics, are frequently found in effluent due to limited removal during WWTP processes. With incidences of antibiotic resistant bacteria in human and animal medicine increasing, concern has arisen about the environmental fate of antibiotic compounds when WWTP effluent is released into the environment. This study's aim was to analyze the presence of three antibiotics, sulfamethoxazole (SMX), trimethoprim (TMP), and ofloxacin (OFL), in wheat plants (*Triticum aestivum*) and groundwater at The Living Filter, an agricultural site where WWTP effluent is spray irrigated year round. Water samples were collected three times throughout the year (Spring, Summer, and Fall). Wheat plants were collected during the summer prior to and during harvest and, then, separated into grain and straw for subsequent analysis. Plant and water samples were analyzed by solid phase extraction cleanup and liquid chromatography tandem mass spectrometry. Plant tissues required an additional liquid-solid extraction step. Sulfamethoxazole and OFL were quantifiable in groundwater samples with concentration ranges of 1.9-660 ng/L and 0.14-67 ng/L, respectively. Trimethoprim was typically only detectable in groundwater, but had a high concentration of 22 ng/L in the summer. For wheat plants, residues of each compound were present on most plant surfaces. Ofloxacin was found throughout the plant in straw (10.2 Â± 7.05 ng/g) and grain (2.28 Â± 0.89 ng/g). Trimethoprim was found only on grain or straw surfaces, while SMZ were concentrated within the grain (0.64 Â± 0.37 ng/g). Overall, these findings demonstrate that when WWTP effluent is spray irrigated antibiotic compounds can be found in groundwater and plant tissues as well as adhere to plant surfaces. While overt toxicity is typically not a concern, these low levels of antibiotics found in groundwater and associated with plants used as food sources raise questions about potential long-term risks for human, animal, and ecological health.

#20: What Can the Domestic Hen Teach Us About Ovarian Function?

Kahina Ghanem, Alan Johnson

The domestic hen is an ideal model organism to study vertebrate ovarian function, due in large part to a single ovary that contains an orderly arrangement of follicles at different stages of development. Accordingly, the single largest follicle is ovulated on an approximate daily basis. The rate-limiting step in maintaining this process is the highly regulated selection of a prehierarchal follicle from a pool of undifferentiated, 6-8 mm follicles, referred to as prehierarchal follicles. The single, selected prehierarchal follicle rapidly undergoes changes that enable it to grow and accumulate yolk at a rate of ~2g/day. At a cellular level the granulosa cell (GC) monolayer surrounding the oocyte becomes responsive to follicle stimulating hormone (FSH) and initiates progesterone production. Although, some of the cellular changes that promote this transformation have been characterized, the most proximal event that leads to the selection of one follicle over the others remains to be identified. In this study we hypothesized that the selection of a single follicle occurs as a result of a comparatively higher sensitivity of the GC layer to FSH. Groups of age matched laying hens, 60-70 weeks of age (N=5 per treatment), were injected with phosphate buffered saline or 30 IU, 75 IU, 100 IU, 300 IU of pregnant mare serum gonadotropin (PMSG), or 25 IU FSH. Ovaries were collected 29 h post-injection. Follicles 1mm in diameter were dissected out and weighed. The selection status of a follicle was established based on a weight

of 0.24 g. The granulosa layer was collected from the most recently selected (9-12mm) follicles, the largest 6-8 mm prehierarchal follicle, and three smaller 6-8 mm follicles then incubated with 10 ng FSH in 1 ml complete DMEM medium for 3 h. The expression of mRNA encoding steroidogenic acute regulatory protein (STAR) and P450 side-chain cleavage enzyme (CYP11A1) was measured using quantitative real time PCR. Here we report that each dose of PMSG induced the selection of multiple follicles (2 to 13) in a dose-dependent manner, and this was confirmed by elevated STAR and CYP11A expression. PMSG was confirmed to act through FSH bioactivity as FSH injections also resulted in multiple (14 to 18) selected follicles. From these results we conclude that increasing the concentration of FSH bioactivity activates FSH receptor signaling and promotes the selection of multiple follicles in a dose-dependent manner.

#21: A New Unbiased Estimator of Gene Diversity for Samples Containing Related, Inbred, and Non-Diploid Individuals with Improved Variance
Alexandre M. Harris, Michael DeGiorgio

Gene diversity, or expected heterozygosity, is a common statistic for assessing genetic variation within populations. The accurate estimation of this statistic depends on factors including sample size and independence among allele copies in the sample. Dependence among allele copies can arise when individuals in the sample are related to one another, or when individuals are inbred. The original unbiased estimator (H^), which uses sample proportions as estimates of allele frequencies, was first introduced by Nei, and underestimates the true diversity of the population in samples containing relatives. DeGiorgio et al. previously developed a generalized version (H~) of this estimator to handle related and inbred individuals, and derived its exact variance. Though unbiased with relatives, H~ has an increase in variance relative to the Nei estimate. To address this, we introduce a new unbiased estimator of gene diversity (H~_new) in samples containing related or inbred individuals, which employs the best linear unbiased estimator (BLUE) of allele frequencies rather than the sample proportion. The BLUE has the advantage over the maximum likelihood estimator of allele frequencies in terms of computational efficiency, while still providing smaller variance than other linear unbiased estimators, such as sample proportion. H~_new retains the unbiased properties of H~ in samples with relatives, but has smaller variance and therefore smaller mean squared error. Interestingly, the theoretical variance for H~_new takes the same form as the variance of H~, except that individual measurements are weighted differently. We examine the properties of H~_new relative to six alternative estimators using both theory and simulations, and apply our estimator to a global human microsatellite dataset of 5,795 individuals at 645 loci. H~_new outperforms other estimators in simulation-based and theoretical comparisons, yielding the lowest mean squared error for gene diversity measurements, and similarly surpasses the others when applied to empirical data. We additionally developed an R package to compute this estimator from genomic and pedigree data, providing researchers with the ability to obtain accurate and precise estimates of genetic diversity in any type of sample.

#22: Zinc deficiency impairs fertility and oogenesis in *C. elegans*
James Hester, Wendy Hanna-Rose, Francisco Diaz.

Zinc is an essential micronutrient that is vital for successful reproduction. Zinc plays a role in successful spermatogenesis, oogenesis, fertilization, embryo development, and epigenetic programming. Given the ubiquitous role of zinc in reproduction, investigation into the cellular and molecular dynamics of zinc in reproductive tissues is an important area of research in order to understand infertility, epigenetic inheritance, and improve artificial reproductive technologies. New techniques and models are necessary to fully elucidate zinc's role in this context. The nematode *C. elegans* offers distinct advantages for reproductive research. The majority of *C. elegans* are self-fertilizing hermaphrodites, in which sperm and oocyte arise from a common gonad. This physiological arrangement allows researchers to observe and analyze oocytes, sperm, and developing embryos from the same subject. The short generation time, established genetic sequence, and conserved molecular mechanisms between *C. elegans* and vertebrates make this an intriguing model for reproductive research. We therefore sought to evaluate *C. elegans* as a model for zinc deficiency and test the hypotheses that zinc restriction would 1) lower fertility in *C. elegans* hermaphrodites; and 2) impair oocyte development as seen in mammalian models. We therefore placed developing worms onto culture plates treated with the zinc chelator N,N,N',N'-tetrakis(2-pyridylmethyl)ethane-1,2-diamine (TPEN) in order to restrict zinc. Fertility was then quantified by counting the number of offspring produced by control (n=5) or TPEN-treated (n=4) hermaphrodites throughout their reproductive lifespan. Zinc restriction significantly reduced the average number of progeny produced from 171.8 offspring per control worm to 41.75 progeny in TPEN treated worms ($p<0.001$). Subsequent imaging studies showed fewer maturing oocytes (control=8.4, TPEN=5.3; $p<0.001$) and fewer developing embryos (control=11.4, TPEN=7.5; $p<0.001$) in the reproductive tracts of TPEN treated subjects. DAPI staining also revealed altered chromosomal dynamics in developing oocytes, characterized by an expanded region of pachytene-stage oocytes (control=0.29 pachytene oocytes, TPEN=6.25; $p<0.001$). Supplementing TPEN-treated worms with zinc returned the number of developing oocytes and embryos to control levels and restored chromosomal dynamics. A lower number of unfertilized, developing oocytes in zinc-deficient worms confirms the hypothesis that at least a portion of impaired fertility resulting from zinc restriction is due to impaired oogenesis. Altered chromosomal dynamics also point to oocyte meiosis as a process vulnerable to perturbations in zinc levels. Possible deficits in sperm production, fertilization rate, or embryo development resulting from zinc deficiency remain to be investigated and quantified in this model. These results indicate that *C. elegans* are vulnerable to reproductive deficits as a result of zinc restriction, similar to vertebrate species. Our group has characterized a novel *C. elegans* phenotype that will be a valuable tool in investigating the role of zinc in reproduction. This research was supported in part by NIH Grant T32GM108563 and by the Huck Institutes of the Life Sciences through a J. Lloyd Huck Dissertation Research Grant.

#23: Lipid Emulsions, Rich in n-3 or n-9 Fatty Acids, Reverse Hepatic Steatosis in Lean Mice, After its Induction by Feeding Parenteral Nutrition Formula

Kuan-Hsun Huang, Limin Zhang, Philip Smith, Andrew Patterson, and A. Catharine Ross

Hepatic steatosis occurs in the early stage of the parenteral nutrition (PN)-associated liver disease. Previously we reported that Intralipid®, a lipid emulsion (LE) rich in n-6 fatty acids (FA), reduced hepatic triacylglycerol (TG) accumulation and markers of inflammation, once lipid accumulation has begun. However, it remains unclear whether other LEs (Omegaven®, rich in n-3 FA, and ClinOleic®, rich in n-9 FA), can reverse hepatic steatosis. Here, we compared 3

LE –Intralipid®, Omegaven® and ClinOleic® --for their ability to reverse hepatic TG accumulation after the onset of steatosis. Male C57BL/6 mice, n=8-9/group, were fed chow for 5 weeks (reference group) or a PN diet (Clinimix-E® with vitamins and minerals, and 3% Intralipid® for sufficient essential FA) for 2.5 weeks and tissues were collected from the PN2.5 group to establish that hepatic steatosis had developed. The remaining mice were then randomized into 4 groups: continuation of PN alone (PN5), or change to PN with 13.5% (en-%) of either Omegaven® (fish oil LE, FOLE), ClinOleic® (olive oil LE, OOLE), or Intralipid® (soybean oil LE, SOLE) and fed for another 2.5 weeks (end of wk 5). Transcripts of genes associated with lipogenesis and lipid mobilization, and liver total FA composition, were analyzed. FOLE and OOLE reduced TG vs. PN2.5 and PN5 ($P<0.001$); SOLE lowered hepatic TG vs. PN5 ($P<0.01$). FOLE mice had the lowest hepatic TG, not different from chow-fed mice, and the lowest transcripts for lipogenesis-associated genes and Srepbf, Ppar-α, and -γ ($P<0.001$). FOLE, OOLE, and SOLE lowered hepatic palmitic, palmitoleic, and vaccenic acids, while FOLE resulted in the lowest concentration of arachidonic acid but highest concentration of docosahexaenoic acid and eicosapentaenoic acid vs. PN2.5 and PN5 mice ($P<0.001$). Genes associated with lipid mobilization were also reduced by FOLE, including Acox1, Cpt-1, Acad1, and Mttp, all $P<0.0001$, while OOLE and SOLE lowered Acox1 ($P<0.001$). Overall, the inclusion of lipid in the form of 13.5 en-% LE, especially as FOLE, into the PN formula reversed the progression of hepatic lipid accumulation in mice with preexisting PN diet-induced hepatic steatosis.

#24: Viral directed microRNA manipulation within *Anopheles gambiae*
Rebecca Johnson and Dr. Jason Rasgon

Anopheles gambiae is the major vector of the deadliest human malaria parasite, Plasmodium falciparum, in sub-Saharan Africa. In recent years, this mosquito vector has been found to express over 66 unique microRNA (miRNAs). These short (~22 nucleotide) segments of noncoding RNA have been found to alter gene expression levels through post-transcriptional regulation processes known as RNA activation and RNA interference. Differential expression of miRNAs has been reported in various mosquito vectors during important biological processes such as malaria infection, egg development, and sugar absorption, yet the role of these endogenous miRNAs in *An. gambiae* has not been extensively investigated. Manipulation of specific miRNAs within *An. gambiae* may identify novel targets for control strategies and further our understanding of mosquito gene regulation processes. Transgenic viral agents such as the recently discovered *An. gambiae* densovirus (AgDNV) have the potential to stably infect *An. gambiae* and alter miRNA levels in vivo. AgDNV is species specific and has been shown to have nominal effects on the *An. gambiae* transcriptome. We have developed an AgDNV co-transfection system to manipulate *An. gambiae* miRNA levels by expressing endogenous pre-miRNA sequences or developed miRNA sponge sequences from a created viral intronic region. In vitro expression of mature *An. gambiae* miR-375 has been validated via qPCR, indicating proper pre-miRNA recognition and processing. AgDNV-based expression of miR-375 in vitro led to a decrease in mRNA transcripts encoding REL1, an important mosquito immune gene and a predicted target of miR-375. While other miRNAs have yet to be fully tested, this AgDNV system represents a novel molecular tool with which the role of endogenous *An. gambiae* miRNAs can be studied. Future studies using transgenic AgDNV may lead to innovative vector control methods and aid in basic An. gambiae miRNA function investigations.

#25: Efficacy of Chewing Gum as a Delivery System for Water and Fat Soluble Vitamins
Weslie Khoo, Joshua Lambert

Chewing gum is a popular product among consumers. Some commercially-available chewing gums contain functional ingredients including botanical extracts and vitamins. In the present study, we examined the release kinetics and plasma bioavailability of a panel of water-soluble and fat-soluble vitamins from two commercially available vitamin supplemented gums. We recruited 15 healthy subjects (age: 22-52 yrs, 60% women) and employed a single-blind, placebo-controlled cross-over study design. Time-dependent changes in saliva and plasma levels of vitamins were determined by high-performance liquid chromatography using both ultraviolet light and electrochemical detection methods. In general, the water- and fat-soluble vitamins in the gums were released from the gum matrix into the saliva, and these levels in the plasma were elevated compared to levels following the placebo. We found that there was release of water-soluble and fat-soluble vitamins from the gum into the saliva during chewing. In the plasma, ascorbic acid and niacinamide levels were increased by 78.8-82.8% and 215.1-273.4%, respectively, in vitamin-supplemented chewing gums compared to baseline. Levels of plasma fat-soluble retinol and dl-alpha-tocopherol were increased by 5.93-6.89% and 11.33-20.80%, respectively, in vitamin-supplemented chewing gums compared to baseline. To the best of our knowledge, this is the first report of the efficacy of chewing gum to deliver vitamins to human subjects. Further studies in deficient subjects are needed to determine whether chronic use of vitamin-supplemented chewing gum is an effective approach to correct deficiency.

#26: Mucosal resident Macrophages drive Adaptive Immunity against Entero-Invasive Infection
Balazs Koscso, Kavitha Gowda, Gaurav Manohar Rajani, Chetna Soni, Milena Bogunovic

Intestinal mucosa represents the largest surface of the body exposed to the outer environment. Mucosal mononuclear phagocytes are an essential element of the intestinal immune system. To combat a constant exposure to dietary and microbial antigens, mucosal mononuclear phagocytes, which consist of dendritic cells (DCs) and macrophages, are able to induce immune responses against gut pathogens while maintaining tolerance to dietary and commensal antigens. However, the mechanisms behind these regulations are only starting to be unraveled. Mucosal macrophages share an antigen-presenting phenotype with DCs, but develop through a distinct pathway and acquire unique functions when compared to DCs. Mucosal macrophages are essential for maintaining tissue homeostasis but, in contrast to DCs, their role in inducing adaptive immunity, particularly against intracellular pathogens such as *Salmonella enterica* (SL), has never been examined. Thus, the goal of our study was to explore the role of macrophages in shaping adaptive immune responses to enteric infection. By combining mouse models of selective DC or macrophage depletion with entero-invasive SL infection, we found that macrophages, but not DCs, prevent mortality from SL. Accordingly, macrophage depletion accelerated systemic spread of SL, prevented production of SL-specific mucosal IgA and compromised SL clearance from the gut lumen. Induction of adaptive immune responses against SL was dependent on the antigen-presenting molecule MHC Class II (MHCII) expressed by macrophages, underlying their role in antigen-presentation. Development of mucosal tertiary lymphoid tissue (TLT), which mainly consists of B cells and fewer macrophages and T follicular helper cells (Tfh), is the hallmark of SL infection. We found that TLT development was

attenuated by macrophage depletion or MHCII depletion in macrophages. Furthermore, infection drastically increased the number of macrophages in the large bowel. The enlarged pool of macrophages in the inflamed gut can be separated into three functionally distinct subsets - Nos2+ (bactericidal), Ccr7+ (MLN-migratory) and Il10+Tnfhi (resident). Intriguingly, only mucosal resident macrophages upregulated expression of Cxcl13 (encodes CXCL13, which organizes B cells in follicles) and Tnfsf13b (encodes B cell activating factor, BAFF). We thus conclude that mucosal macrophages are a driving force of SL-specific immunity, achieved through three complementary mechanisms: 1) bactericidal activity (bactericidal macrophages); 2) migrations to the MLNs followed by induction of SL-specific T cells and systemic antibody responses (MLN-migratory macrophages); and 3) induction of local protection via assembly of mucosal TLT and production of mucosal SL-specific IgA (resident macrophages).

#27: Viral DNA-dependent Induction of Innate Immune Response to Hepatitis B Virus in Immortalized Mouse Hepatocytes
Xiuji Cui, Daniel N. Clark, Kuancheng Liu, and Jianming Hu

Hepatitis B virus (HBV) infects hundreds of millions of people worldwide and causes acute and chronic hepatitis, cirrhosis and hepatocellular carcinoma. HBV is an enveloped virus with a relaxed circular (RC) DNA genome. In the nuclei of infected human hepatocytes, conversion of RC DNA from the incoming virion or cytoplasmic mature nucleocapsid (NC) to the covalently closed circular (CCC) DNA, which serves as the template for producing all viral transcripts, is essential to establish and sustain viral replication. A prerequisite for CCC DNA formation is the uncoating (disassembly) of NCs to expose their RC DNA content for conversion to CCC DNA. We report here that in an immortalized mouse hepatocyte cell line, AML12HBV10, in which NC uncoating is enhanced, the exposed viral DNA could trigger an innate immune response that was able to modulate viral gene expression and replication. When viral gene expression and replication were low, the innate response initially stimulated these processes but subsequently acted to shut off viral gene expression and replication after they reached peak levels. Inhibition of viral DNA synthesis or cellular DNA sensing and innate immune signaling diminished the innate response. These results indicate that HBV DNA, when exposed in the host cell cytoplasm, can function to trigger an innate immune response, which, in turn, modulates viral gene expression and replication.

#28: Analyzing Somatic Embryogenesis Gene Expression in Response to Tissue Culture Enhancer PLA1 Protein
Tina Lai and Wayne R. Curtis

Advances in molecular biology and bioinformatics are now unraveling the basis for why 'conditioned media' aids tissue culture regeneration of plants. Extracellular Phytocyanin-Like-Arabinogalactan-1 or PLA1 protein has been shown to improve somatic embryogenesis (SE) in cotton (*Gossypium hirsutum*); Poon et al. (2012) applied GhPLA1 proteins to tissue culture media for a two-fold increase in embryogenic calli production. Our work focuses on production of orthologous PLA proteins and investigation of their respective efficacies as a media additive towards enhanced regeneration of recalcitrant plants through SE. Specifically, we are evaluating PLA1 protein based on 1) observed effects on embryo production in *Theobroma cacao* SE tissue culture and 2) the effects of protein treatment on SE marker genes such as BBM, LEC1, LEC2,

AGL15, and FUS3. Understanding how PLA1 interacts with SE genes can give insight into the complex orchestration of gene expression during plant embryo development. The generality of many elements surrounding the SE tissue culture process should allow improvement across all plants. GhPla1 protein was applied to *T. cacao* by dripping protein solution as a way to use less protein than media addition, while achieving the same enhancement effects. This is consistent with our goal to implement exposure in temporary immersion bioreactor propagation systems. Preliminary results showed embryos were produced earlier in protein treated tissue and induced expression of SE transcription factor: BBM compared to non-treated tissue. Gene expression of BBM and other SE genes were analyzed via qPCR. In addition to GhPla1, we are developing PLA1 proteins from *Theobroma cacao*, *Oryza sativa*, and *Dioscorea rotundata* using recombinant *E. coli* protein expression. PLA proteins may prove to be a valuable addition to the in vitro toolbox (beyond plant hormones) for plant species that are difficult to transform or propagate.

#29: Autophagy facilitates malignant transformation but not progression of acute myeloid leukemia in an MLL-AF9-driven mouse model
Qiang Liu, Longgui Chen, David F. Claxton, Hong-Gang Wang

Acute myeloid leukemia (AML) is a hierarchical hematopoietic malignancy originating from leukemic stem cells (LSCs). Autophagy is a lysosomal degradation pathway that is hypothesized to be important for the maintenance of AML as well as contribute to chemotherapy response. Here, we employ a mouse model of AML expressing the fusion oncogene MLL-AF9 and explore the effects of Atg5 deletion, a key autophagy protein, on the malignant transformation and progression of AML. The in vivo deletion of Atg5 during primary transplantation delayed the malignant transformation of MLL-AF9-transduced bone marrow cells to AML. However, Atg5 deletion in transformed AML cells during secondary transplantation did not affect the survival of leukemic mice. Surprisingly, deletion of Atg5 in malignant AML did not affect chemotherapy response or LSC viability. In contrast, autophagy was found to be involved in the survival of differentiated myeloid cells originating from MLL-AF9-driven LSCs. Taken together, our data suggests that autophagy contributes to MLL-AF9-driven malignant transformation of hematopoietic progenitors but is dispensable for the maintenance of LSCs.

#30: Strain and plastic composite support (PCS) selection for Vitamin K (Menaquinone-7) production in Biofilm Reactors
Ehsan Mahdinia, Ali Demirci, Aydin Berenjian

Vitamin K, especially menaquinone-7 (MK-7), has received significant attention recently. MK-7 can be produced by microorganisms via fermentation process. However, the production levels are still low. Therefore, this study is undertaken to improve MK-7 fermentation by using biofilm reactors. In this phase of the study, strain selection by evaluating various Bacillus species such as *Bacillus subtilis* natto, *Bacillus licheniformis* and *Bacillus amyloliquifaciens* and plastic composite support (PCS) was investigated by using conventional medium (tryptic soy broth supplemented with 0.8% yeast extract) (TSB) and synthetic medium (SM) suggested by the literature, which is composed of 5% glycerol, 5% yeast extract, 18.9% soy peptone and 0.06% potassium phosphate dibasic. Furthermore, four different types of PCS (SF, SFY, SFYB, SFYR)

were evaluated for selected strains in both media in terms of Vitamin K production and biofilm formation on PCS types. In the end, the combination of *Bacillus subtilis* natto NF1, SFY in TSB medium was selected as most potent for MK-7 production. MK-7 concentration was observed as high as 35.5 mg/L.

#31: Genome-wide association analysis identifies genetic loci associated with resistance to multiple antimalarials in *Plasmodium falciparum* from China-Myanmar border
Zenglei Wang, Mynthia Cabrera, Xiaoying Liang, Karen Kemirembe, Sony Shrestha, Awtum Brashear, Xiaolian Li, Jun Miao, Liwang Cui

Drug resistance has been one of the greatest challenges for malaria control, and the recent emergence of resistance in *Plasmodium falciparum* to artemisinin family drugs is concerning. To identify genetic markers potentially associated with antimalarial drug resistance, we performed genome-wide association analysis to assess the associations of single nucleotide polymorphisms (SNPs) from a high-density SNP array with in vitro sensitives to 10 commonly used antimalarial drugs in 94 parasite isolates from the China-Myanmar border area, where artemisinins have the longest history of deployment. Among the numerous SNPs identified to be associated with reduced in vitro drug sensitivities, a SNP located in the autophagy-related protein 18 (ATG18, PF3D7_1012900) was associated with decreased IC50 values to dihydroartemisinin, artemether and piperaquine, which have all been used extensively in this region. Being an artemisinin-interacting protein and a putative phosphatidylinositol-3-phosphate binding protein, the identification of ATG18 suggests a potential involvement of autophagy in artemisinin resistance.

#32: Objective, but not Subjective, Sleepiness is Associated with Inflammation in Sleep Apnea
Yun Li, MD, Alexandros Vgontzas, MD, Julio Fernandez-Mendoza, PhD, Ilia Kritikou, MD, Maria Basta, MD, Slobodanka Pejovic, MD, Jordan Gaines, BA and Edward O Bixler, PhD

Background
Daytime sleepiness is common in patients with obstructive sleep apnea (OSA), and an important criterion for diagnosis and treatment of OSA. The prevalence of EDS in OSA is 16-22% in epidemiologic samples, and is the most common complaint in clinical samples.
The Multiple Sleep Latency Test (MSLT) is considered the gold standard method for the objective measure of daytime sleepiness, whereas the Epworth Sleepiness Scale (ESS) is the most widely used self-report questionnaire for the assessment of subjective daytime sleepiness in clinical settings. Objective and subjective measures of excessive daytime sleepiness (EDS) are only weakly associated. However, no study has examined whether these two measures differ in terms of underlying mechanisms and/or prognostic value. It has been suggested that pro-inflammatory cytokines, such as interleukin-6 (IL-6), promote sleepiness/fatigue, whereas, cortisol, the end product of the hypothalamic-pituitary-adrenal (HPA) axis, promotes vigilance and hyperarousal. We have previously hypothesized that sleepiness is associated with higher levels of pro-inflammatory cytokines and lower levels of cortisol.

Objective
In the current study, our overall objective was to examine whether the underlying pathophysiologic mechanisms between objective vs. subjective sleepiness differ in a population

of patients with OSA. Specifically, we hypothesized that objective, but not subjective, EDS is associated with higher IL-6 levels and lower cortisol levels in patients with OSA.

Methods

We studied 58 OSA patients (mean age 53.73 ± 7.02 years, and 63.8% were male gender) who underwent 8-hour in-lab polysomnography for four consecutive nights. Sleep variables were calculated based on the mean values from nights 2 and 3, so that we controlled for first night effect as well as the sleep-disturbing effect of blood drawing (night 4). A thorough medical assessment, including physical examination, routine laboratory tests and sleep history was completed for each subject. Objective EDS was evaluated using MSLT and subjective EDS was evaluated using on the fourth day. A clinical cut-off point of MSLT values ≤ 8 minutes and ESS scores > 10 were defined as objective and subjective EDS, respectively. 7,8 24-hour serial blood samples were collected every 60 minutes and IL-6 and cortisol levels were assessed on the fourth day and night in the sleep laboratory.

Results

OSA with objective EDS was associated with 1) significantly elevated mean 24-hour (Odds Ratio (OR) =1.56, 95% confidence interval (CI) 1.04-2.34, p=0.03) and daytime (OR =1.79, 95% CI 1.08-2.96, p=0.03) IL-6 levels, and marginally significantly elevated nighttime (OR= 1.26, 95% CI 0.96-1.65, p=0.10) IL-6 levels; and 2) significantly decreased 24-hour (OR= 0.38, 95% CI 0.16- 0.92, p= 0.03) and daytime (OR=0.37, 95% CI 0.16-0.84, p= 0.02) cortisol levels as compared to OSA without objective EDS. In contrast, subjective EDS was not associated either with elevated IL-6 levels or decreased cortisol levels.

Conclusion

Our findings suggest that OSA with objective EDS is the more severe phenotype of the disorder associated with low-grade inflammation, a link to cardiometabolic morbidity and mortality. Objective EDS compared to subjective EDS is a stronger predictor of OSA severity and may be useful in the clinical management of the disorder.

#33: Induction of tolerogenic mediators in uterine immune cells during early pregnancy in dairy heifers

MM Kamat, S Vasudevan, JL Pate, TL Ott

Problem: Embryo loss during early pregnancy contributes to infertility. A portion of these losses are hypothesized to be immune mediated but little is known about the immune cell responses to the presence of the embryo. This study tested the hypothesis that endometrial resident immune cells are induced to a tolerogenic phenotype by signals emanating from the developing conceptus.

Method of Study: Uterine tissue was collected from Holstein dairy heifers on Day 17 of the estrous cycle and Days 17 and 20 of early pregnancy. Tissues were labeled with antibodies against CD47 and Indoleamine 2,3-dioxygenase (IDO) and labeling intensity was analyzed using ImageJ software (n=5 heifers/status/Day). In addition, abundance of mRNA for CD172a and CD47 were analyzed in RNA extracted from whole endometrium by quantitative PCR (n=7-9

Day 17 cyclic, n=6 Day 17 pregnant and n=5 Day 20 pregnant). Results were analyzed using MIXED procedures of SAS using preplanned orthogonal comparisons.

Results: Endometrial labeling intensity for CD47 was greater ($p=0.05$) in endometrium from pregnant compared to cyclic heifers. Differences in labeling intensity were greatest in the shallow stroma and shallow glands ($p<0.05$). Labeling intensity for IDO staining was greater ($p<0.05$) in endometrium from Day17 pregnant heifers compared to Day17 cyclic heifers. Among pregnant heifers, labeling intensity was almost 3 fold greater at Day 17 versus Day 20 ($p<0.01$). Differences in IDO labeling intensity were greatest in the luminal epithelium, but were also detected in the shallow glands, deep glands and myometrium. No differences were observed in total endometrial mRNA abundance for CD47 and CD172a.

Conclusions: Results presented here support the hypothesis that conceptus signals affect resident immune cells at very early stages of pregnancy. We hypothesize that increased CD47 expression interacts with its receptor, CD172a, to induce inhibitory signals via Immunoreceptor Tyrosine based Inhibitory Motifs (ITIM). This interaction is known to bring about cell-cell adhesion and T cell inactivation. Increased IDO expression may induce production of kynurenine, a ligand for the Aryl Hydrocarbon Receptor (AhR) and is involved in Regulatory T cell generation. Thus, during early pregnancy in cattle, embryonic signals, including IFN tau, may promote development of tolerogenic immune phenotype for successful establishment of pregnancy.

#34: Pain Management of Substance using Trauma Patients and the Impact on Nurses: A Systematic Review

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Background: WHO and DHHS have identified traumatic injuries, pain management, and substance use as major public health crises. Trauma patients with a history of substance use undergoing pain management create a nexus that challenge nursing care. The aim of this systematic review was to determine the current state-of-the-science related to nurses' responses to the unique needs of this trauma patient population.

Methods: PubMed, CINAHL, ProQuest Nursing and Allied Health Source, PsychINFO, Web of Science, Academic Search Complete, The Cochrane Library, and Science Direct were searched for articles published between 1990 and 2015. Search terms were trauma OR injury; pain OR pain management; substance abuse OR substance use OR drug abuse; nurses' attitudes OR perceptions. Inclusion criteria were English language and peer-reviewed journal articles. Exclusion criteria included neonates, children, mental illness, and PTSD. Methodological screening used PRISMA, COREQ, and STROBE criteria. Quantitative and qualitative literature were included for review. Full text review of 58 articles resulted in 45 articles for systematic review. Metasynthesis techniques were used to interpret and analyze the findings.

Results: Study populations included mixed health professionals (physicians, medical residents, nurses, nurse's aids), nurses and physicians, nurses only, and nursing students. The studies were primarily descriptive, cross-sectional surveys (n= 18), qualitative (n=9), quasi-experimental studies (n=3), literature reviews (n=14), and one case study. Overall, the synthesis highlights that

nurses feel responsible for providing adequate care to substance-using patients, however, they perceive these patients are under-assessed and under-medicated. In addition, caring for trauma patients results in nurse burnout and emotional exhaustion. No studies included all three of the search terms in one study.

Conclusion: Caring for the complex needs of trauma patients can be physically, intellectually, and emotionally burdensome for nurses. Combining the negative aspects of caring for substance-using patients with the already difficult task associated with caring for traumatic injuries presents nurses with a challenge.

#35: De Novo Synthesis from Tryptophan in the Absence of a QPRTase Homolog Contributes to NAD+ Biosynthesis in *C. elegans*
Melanie R. McReynolds, Wenqing Wang, Lauren Holleran and Wendy Hanna-Rose

NAD+ biosynthesis has proven to be an attractive and promising therapeutic target for influencing health-span and obesity-related phenotypes as well as tumor growth. It's a necessity to elucidate exactly how manipulating NAD+ biosynthetic pathways can lead to therapeutic benefits to fully utilize this target for drug discovery. The goal of my research is to understand how NAD+ homeostasis is maintained to support its core metabolic roles and its signaling and regulatory roles involving NAD+ consumers. It's been reported in the literature that *C. elegans* lack the de novo NAD+ biosynthetic pathway because quinolinic acid phosphoribosyltransferase (QPRTase) is not present in the genome. However, all genes coding for the key enzymes required for production of quinolinic acid (QA) from tryptophan are present in the *C. elegans* genome. Therefore, we hypothesized that de novo synthesis from tryptophan in *C. elegans* contributes to NAD+ biosynthesis. In order to investigate if de novo synthesis is indeed active in the absence of the QPRTase homolog, we first asked if QA gets incorporated into NAD+ in *C. elegans*. Using stable isotope/mass spectrometric flux analysis, I observed that label incorporated into QA was subsequently incorporated into NAD+ as well; supporting the notion de novo NAD+ synthesis is active. I further hypothesized that if QA were an endogenous precursor for NAD biosynthesis, then blocking this pathway would compromise NAD+ levels in vivo. Target metabolomics revealed a decrease in NAD+ and QA levels when this pathway was blocked. This data supports the hypothesis that this pathway is active and required for maintenance of global NAD+ levels. Next, I investigated if supplementation with QA could reverse a phenotype associated with NAD+ deficiency. Consistent with the previous results, supplementation with QA rescued the developmental phenotype and increased NAD+ levels in mutants that lack salvage NAD+ biosynthesis. This evidence suggests that boosting this pathway can lead to homeostatic mechanisms when salvage synthesis is blocked, further supporting its core role in maintaining NAD+ homeostasis. Finally, we've identified key candidate enzymes that may play the role of the missing QPRTase in *C. elegans*. Using genetics, I am currently investigating the role of these potential enzymes in NAD+ de novo synthesis. This data will outline the mechanisms by which tryptophan de novo synthesis is functioning in *C. elegans*. This work has implications for efforts to therapeutically target individual NAD+ biosynthetic pathways providing key insight into compensatory homeostatic mechanisms.

#36: Effects of differential cannabinoid agonists on development of antinociceptive tolerance
Caitlin Nealon, Aaron Kline, Nathan DeTurk, Michael Zee, and Daniel Morgan

A major barrier to the clinical utility of cannabinoid agonists is the potential for rapid development of tolerance at therapeutic doses. Tolerance to G protein-coupled receptor (GPCR) agonists, including cannabinoids, has classically been thought to occur through GPCR kinases (GRK) and arrestin-mediated receptor desensitization and down-regulation. We have previously shown that tolerance to Δ9-THC occurs in part via desensitization of the cannabinoid receptor 1 (CB1). Mice expressing a desensitization-resistant form of the CB1 receptor (S426A/S430A) exhibit delayed tolerance to the antinociceptive effects of Δ9-THC. However, in contrast with many synthetic cannabinoid agonists, Δ9-THC is a strongly desensitizing, weakly internalizing, partial agonist for CB1. Therefore, the purpose of this study was to investigate the extent to which tolerance for strongly internalizing full CB1 agonists CP55,940 and WIN55,212-2 were also mediated by this mechanism. Tolerance to the antinociceptive, cataleptic, and hypothermic effects of daily 30 mg/kg Δ9-THC, 0.3 mg/kg CP55,940, and 10 mg/kg WIN55,212-2 was assessed in wild type and S426A/S430A desensitization-resistant mutant mice. Mice were also assessed for tolerance to Δ9-THC, CP55,940, and WIN55,212-2 via shifts in the cumulative dose responses curves following seven days of once daily treatment with each agonist. Antinociceptive tolerance to CP55,940 was modest in both wild type and S426A/S430A mice, while tolerance to 10 mg/kg WIN55,212-2 was partially prevented in desensitization-resistant mice. Although S426A/S430A mice demonstrated significant tolerance to the antinociceptive effects of 30 mg/kg Δ9-THC, this tolerance was reduced compared to wild type mice. Our results demonstrate that different mechanisms may mediate tolerance to various cannabinoid agonists acting at CB1. In particular, our results suggest that while GRK and arrestin-mediated signaling may be partially involved in tolerance to the antinociceptive effects of all three agonists it is the most crucial for tolerance to WIN55,212-2. These studies explore the differences in agonist-specific effects in cannabinoid tolerance and offer potential avenues to better understand development of tolerance to their antinociceptive effects on acute pain.

#37: Soar1 dimer binding to the c-terminus of Orai1 induces clustering
Robert Nwokonko, Yandong Zhou, Xiangyu Cai, Xizhuo Wang, Xiangming Wang, Natalia Loktionova, Donald Gill

Store-operated calcium entry (SOCE) is a ubiquitous signaling mechanism in eukaryotic cells crucial for mediating longer term Ca2+ signals and restoring endoplasmic/sarcoplasmic reticulum Ca2+ after ligand induced depletion. The key operators in SOCE are the Ca2+ selective PM Orai1-3 channels and the ER/SR resident, single pass transmembrane calcium sensors STIM1 and STIM2. STIM1 is activated when ER/SR luminal Ca2+ is depleted, inducing it to unfold and bind to Orai1 channels in the PM. Active Orai1 channels create discrete microdomains of high Ca2+ within ER-PM junctions that contain roughly 100-fold greater Ca2+ concentrations than resting cytoplasmic levels. Simulations of Stim-Orai Ca2+ microdomains by Samanta K. et al. reveal that physically coupled Orai1 channels generate more saturated Ca2+ domains at the apposing ER membrane and laterally extend the concentrated domain farther from the channel cluster along the PM surface compared to randomly spaced Orai1 channels within an ER-PM junction. Cryo-EM by Perni S. et al. reveal Orai1 channels within an ER-PM junction are spaced (9-13 nm), roughly the distance between c-terminal binding regions of concatemerized peptides of the Stim-Orai Activating Region (SOAR) of Stim1. Clustering of Orai channels is critical for generating Ca2+ saturated microdomains,

however the mechanism of clustering is vague. We have discovered that Orai1 clustering is dependent on the presence of at least two function c-terminal binding domains on Soar concatemer dimers. In HEK Orai1-His cells expressing wildtype Soar-Soar (S-S) concatemers, we observe an increase in puncta formation at the plasma membrane. When one subunit within a Soar concatemer is mutated to F394H (SH-S or S-SH), a residue critical for high-affinity c-terminal binding to Orai1, there is a dramatic decrease in puncta. We also observe that linking a large fluorescent protein, CFP, to the c-terminus of Orai1 can sterically hinder clustering. HEK Orai1-CFP stable cells do not form puncta, regardless of the presence of two functional c-terminal binding domains on Soar concatemers. There is also a functional difference in ICRAC magnitude in HEK Orai1-His cells that is dependent on the presence of two functional Soar subunits. Intriguingly, this dependence is absent in sterically hindered cells. Our data demonstrates that clustering of Orai1 channels is dependent on the presence of two functional Soar subunits, and supports a unimolecular coupling mechanism between Orai and Stim that promotes full channel activity and the generation of Ca2+ saturated microdomains at ER-PM junctions. The Stim2 splice variant Stim2.1 has an additional 8 amino acid insertion within the c-terminal binding domain of the Soar2 region, and concatemerized S-S2.1 peptides behave similar to SH-S or S-SH. This suggests that eukaryotes may have evolved a mechanism mediated through regulation of Stim2 splicing that can affect the clustering dynamics of Orai ER-PM junctions in cells.

#38: Population dynamics of tortrix moths in Pennsylvania fruit orchards
Damie Pak, Ottar Bjornstad, David J. Biddinger

The cyclic fluctuations of insect species can provide critical insights on how life-history traits can affect population dynamics and community structure. Interannual and generational cycles can scale up and lead to interesting patterns of interspecific synchrony with important consequences for co-existence in specious arthropod guilds. We explored how the varying voltinism, feeding behaviors, and spring emergence of five economically important moth pests in Pennsylvania fruit orchards shape their community dynamics. Additionally, we analyzed the synchrony between the moth species to better understand how it relates to continued co-existence of species sharing similar resources.
For this study, we analyzed the abundance data of moths collected and identified at the Fruit Research and Extension Center (Bigglerville, Pennsylvania) from 2000 to 2013. Species abundance was averaged across sites and continuous wavelet transformation was used to analyze the periodicity of population fluctuations. For analyzing interspecific dynamics, we utilize cross-wavelet analysis to locate periods of high correlation and phase relationships among the moth species.
Wavelet analysis of the moth species showed significant periodicity at both the generational and annual scale. Certain species such as the obliquebanded leafroller (*Choristoneura rosaceana*) had significant intra-year periodicities for only certain years (2000-2008). Other species like the tufted apple bud moth (*Platynota idaeusalis*) showed a consistent and significant periodicity for all years. For all species however, there are changes in the amplitude of fluctuations which we hypothesize may be due to variation in yearly temperature.
The cross-wavelet analysis between different species indicated complex phase-relationship at different scales. We found that the three bivoltine species are generally in-phase at the generational scale but at the annual scale, our analysis suggested that phase-relationship can

vary. It is possible that changes in the phase-relationship can be attributed to changes in spring emergence of moths after diapause.
Our results show that population fluctuations of the moth species can vary over time and at different scales which can lead to significant changes on community dynamics.

#39: Systemic, rather than local, macrophages limit virus spread following an intradermal infection
Nick J. Parekh, Michael L. Davies, Irene E. Reider, Tracy E. Krouse, Chetna Soni, Lauren W. Kaminsky, Matthew A. Fischer, Nico van Rooijen, Ziaur SM Rahman, Chris C. Norbury

Following a peripheral virus infection, the immune system activates a robust response in an effort to limit virus dissemination and prevent or reduce systemic spread. It is the initial actions of the innate immune response, matched against the pace of virus replication, that are important for mediating a successful outcome for the host. The poxvirus, Vaccinia virus (VACV), is restricted from causing systemic disease, but the precise mechanism of preventing systemic virus dissemination following a peripheral infection remains poorly understood. It is critical to understand the mechanisms that prevent systemic dissemination of viruses that spread via the lympho-hematogenous route after a peripheral infection, such as; measles, mumps, dengue, and many others of importance to human health.
Uninhibited, poxviruses spread from the site of infection through the draining lymph node to the spleen and liver, and from these tissues virus particles further spread systemically through the blood. Immune cells recruited to the site of infection or cells resident within the draining lymph node are widely considered to restrict the spread of many viruses. However, our recent work suggests that systemic, rather than local, populations of macrophages are responsible for preventing widespread dissemination of replicating VACV following intradermal infection. Utilizing various cell-depletion methods, we found that systemic depletion of macrophages allows for VACV spread to the ovaries, an assay for unrestricted spread. Further, when depletion of macrophages was limited to the site of infection or the draining lymph node, VACV was unable to reach the ovaries, suggesting that cells within these tissues are not required to prevent virus spread. However, systemic macrophage depletion was no longer sufficient to allow virus spread when depleted more than 4 days after infection, indicating that after 4 days, it is likely that a recruited cell type is able to prevent spread of VACV from the peripheral site of infection. Our data indicate that two populations of marginal zone macrophages are primary targets of VACV infection in the spleen, and depletion of these cells allows widespread virus dissemination. Therefore, we show that the level of virus control is not the site of infection or draining lymph node, but is likely via infection of macrophage populations in the spleen that do not allow productive replication of VACV and may trigger further innate and adaptive immune responses.

#40: Optimal Control of Unimanual and Bimanual Actions: An Inverse Optimization Approach
Behnoosh Parsa, Satyajit Ambike, Mark L. Latash

Mathematically studying human motor control like all other complex systems deals with redundancy of possible outputs. And the way our central nervous system chooses the most appropriate set of system variables involving in a task, among other infinite possible options, has remained an open question. To address this problem mathematically, using forward optimization

techniques has become a popular approach. However, although this mathematical solution alleviated the burden of studying motor control, the basis on which its objective criteria is chosen, has been always a central concern for researchers. Recently, the idea of using inverse optimization techniques (IOPTs) has pervaded in the area of studying human motor control. That is, for example, by collecting the preferable fingers' force pattern during a force sharing task, one can approximate the objective criterion applying IOPTs. In almost all studies using IOPT, the experimental data were collected during an adequate number of trials each had different static external condition. Using a dynamic external moment production during each trials we collect numerous data points rather than just one set of fingers' force combination. We investigate how this method of data collection influence IOPT; moreover, study the differences in approximated objective criterions derived for right, left, and both hand grasping task.

#41: Chlamydia and gonorrhea acquisition among adolescent and young adults in Pennsylvania: A rural and urban comparison
Casey Pinto, MS, CRNP, Lorah Dorn, PhD, Vernon Chinchilli, PhD, Ping Du, MD, PHD

Background: American adolescents and young adults between the ages of 15-24, account for 50% of all sexually transmitted diseases (STDs) annually. Rural populations in this age group remain understudied, yet are more likely to engage in high-risk behaviors that may increase their chances of acquiring an STD.

Objective: To compare adolescent and young adult STD rates in rural versus urban populations of Pennsylvania for the years 2004-2014.

Methods: This study is an exploratory analysis using Pennsylvania STD surveillance data and the US Census to estimate rate ratios using negative binomial regressions within the framework of generalized estimated equations. The analyses were conducted at the school district-level and controlled for poverty, gender, and year.

Results: Compared to urban communities, rural communities had higher rates of chlamydia in 18-19 year-olds (2010-2014), 20 year-olds (2011-2014), 21 year-olds (2011-2014), and 22-24 year-olds (2014) when controlled for poverty and gender ($p<.05$). Higher rates of gonorrhea were noted in rural 18-19 year-olds only (2014, $p<.05$).

Conclusions: In Pennsylvania, adolescents and young adults, aged 15-24 years living in rural populations, are more likely to acquire STDs in recent years than their urban counterparts. The higher rates of STDs is concerning since literature indicates many of these rural youth may not seek care since they are asymptomatic, lack sex education, and fear negative community perception related to lack of anonymity. Additional research is needed to further explore potential causes of this shift of STD acquisition from urban to rural populations.

#42: Developing a Tet-On 3G and PiggyBac system for tunable and temporal gene expression in human pluripotent stem cells
Lauren Randolph, Xiaoping Bao, Xiaojun Lian

As highly proliferative cells with the potential to become any cell type, human pluripotent stem cells (hPSCs) provide an in vitro platform to study development and disease at the cellular and molecular level. The discovery of induced pluripotent stems cells (iPSCs) in 2006 opened new avenues for probing development and disease. With tight regulation and controlled expression of a gene in question, hPSCs provide an interesting clinical model towards understanding the relationship between genetic elements and disease progression or manifestation. An accurate, disease specific, cellular model could be used for high throughput drug screening. Additionally, patient derived iPSCs could provide an opportunity create personalized therapy and drug regimens. In order to achieve disease modeling that accurately incorporates the temporal expression of the genes involved in a specific disease, a strategy for tunable and temporal genetic regulation is needed. We are developing a system incorporating both Tet-on 3G and PiggyBac elements for tunable and temporal regulation of gene expression of hPSCs and their derivatives. We have fully integrated the plasmid system into the genome of hPSCs and will demonstrate tight temporal regulation with the addition and removal of doxycycline to the media. The expression level will be modulated with direct proportionality to the concentration of doxycycline administered, and expression will be maintained in differentiated cell types. This all-in-one system for gene expression regulation has extensive applications in medical genetics to further understanding of gene expression in developmental and disease models.

#43: Turnabout Is Fair Play: Herbivory-Induced Plant Chitinases Excreted in Fall Armyworm Frass Suppress Herbivore Defenses in Maize
Swayamjit Ray, Patrick C.M.S. Alves, Imtiaz Ahmad, Iffa Gaffoor, Flor E. Acevedo, Michelle Peiffer,Shan Jin, Yang Han, Samina Shakeel, Gary W. Felton, and Dawn S. Luthe.

The perception of herbivory by plants is known to be triggered by the deposition of insect-derived factors such as saliva and oral secretions, oviposition materials, and even feces. Such insect-derived materials harbor chemical cues that may elicit herbivore and/or pathogen-induced defenses in plants. Several insect-derived molecules that trigger herbivore-induced defenses in plants are known; however, insect-derived molecules suppressing them are largely unknown. In this study, we identified two plant chitinases from fall armyworm (*Spodoptera frugiperda*) larval frass that suppress herbivore defenses while simultaneously inducing pathogen defenses in maize (*Zea mays*). Fall armyworm larvae feed in enclosed whorls of maize plants, where frass accumulates over extended periods of time in close proximity to damaged leaf tissue. Our study shows that maize chitinases, Pr4 and Endochitinase A, are induced during herbivory and subsequently deposited on the host in the feces. These plant chitinases mediate the suppression of herbivore-induced defenses, thereby increasing the performance of the insect on the host. Pr4 and Endochitinase A also trigger the antagonistic pathogen defense pathway in maize and suppress fungal pathogen growth on maize leaves. Frass-induced suppression of herbivore defenses by deposition of the plant-derived chitinases Pr4 and Endochitinase A is a unique way an insect can co-opt the plant's defense proteins for its own benefit. It is also a phenomenon unlike the induction of herbivore defenses by insect oral secretions in most host-herbivore systems.

#44: Damage and Damage-Control: Invertebrates within Sustainable Cropping Systems
Karly Regan, Christina Mullen, Mary Barbercheck

Reducing synthetic chemical inputs and tillage can have numerous benefits in agroecosystems, such as building soil health, promoting biodiversity, and reducing non-target effects of pesticides. In addition to these benefits, low-disturbance cropping systems may also enhance predator communities and biological control potential. While tillage can kill or disrupt invertebrates, planting a winter cover crop may help sustain invertebrate communities by providing habitat and nutritional resources. We investigated soil and cover crop management practices on arthropods within a reduced-tillage organic cropping systems experiment. We measured crop damage from invertebrates and predation rate of insects in maize plots undergoing four different crop management strategies. In 2015, herbivore damage did not differ among the four cropping systems. Larvae of European corn borer (*Ostrinia nubilalis*), a key pest for maize in Pennsylvania, were more prevalent in treatment plots preceded by a red clover (*Trifolium pratense*) and timothy (*Phleum pretense*) winter cover crop than by hairy vetch (*Vicia villosa*) and triticale (*Triticale hexaploide*) but there were no differences in damage from this pest. In baited predation assays, predation rates of sentinel prey were higher in treatment plots preceded by hairy vetch and triticale before the cover crop was terminated, but did not differ after maize emergence. No difference among tilled maize plots and non-tilled maize plots were measured for any of the invertebrate parameters measured. This data will continue to be collected for two more years to further understand effects of cover crop species and tillage on invertebrates within organic maize plots.

#45: Heparan sulfate proteoglycans regulate autophagy in Drosophila
Claire Reynolds, Na Zhou, Jie Xu, Jielin Xu, Taryn Serman, and Scott Selleck

Heparan sulfate-modified proteoglycans (HSPGs) are important regulators of signaling and molecular recognition at the cell surface and in the extracellular space. Disruption of HSPG core proteins, HS-modification, or HS-degradation can have profound effects on growth, patterning, and cell survival. We provide evidence that HSPGs are critical regulators of autophagy in Drosophila. Inhibiting HS synthesis in the muscle produces loss of mitochondria and disruption of a postsynaptic membrane specialization, the subsynaptic reticulum, at the neuromuscular junction. These phenotypes were accompanied by increased autophagy and were rescued by reducing the function of a key autophagy component. In the fat body, the central energy storage and nutritional sensing organ in Drosophila, compromising HS-biosynthesis also produced increased levels of autophagy markers. Lethality resulting from defective HS biosynthesis was rescued by reducing the function of a key autophagy component, showing that regulation of autophagy is a critical activity of HSPGs during development. These findings demonstrate that HS biosynthesis has important regulatory effects on autophagy and that autophagy is critical for determining the complexity of postsynaptic membrane specializations.

#46: Spectroscopic Characterization of Ions at Biointerfaces
Bradley Rogers, Saranya Pullanchery, Seung-Yi Lee, Tinglu Yang, and Paul Cremer

In order to understand biological processes such as ion regulation, cell signaling, and lipid oxidation, the behavior of ions at biointerfaces must be explored. Investigations at the molecular level showed that these processes are dominated by ion-biomolecule interactions. First discovered in 1888, the Hofmeister series ranks cations and anions in order of their ability to influence protein solubility in aqueous solutions. The ions in both of these series play crucial

roles in many biological processes. Cremer research group aims to understand the molecular-level details of ion behavior at biointerfaces by using MCR-Raman, FT-IR, etc.

#47: Evolution of nonpathogenic persistent plant viruses in pepper
Maliheh Safari, Marilyn J. Roossinck

Plant viruses can have different lifestyles. Most well studied viruses are acute viruses that cause disease in their host by rapid replication. Acute viruses can infect hosts systemically and are transmitted vertically and horizontally. On the other hand, plant persistent viruses are widespread in both commercially relevant crops and wild plants, replicating in their hosts for many generations without causing any visible disease. Movement between plant cells and transmission through grafting has not been observed in persistent viruses. They are distributed to all host cells through host cell division, and vertically transmitted via the gametes to seeds at rates close to 100%. The roles of plant persistent viruses have not been studied thoroughly, while their very long-term relationships with their hosts, and their vertical transmission suggest beneficial interactions with their hosts. Peppers are perennial plants, native to South America, and as domesticated plants human selection accelerated their evolution. Two persistent viruses, Bell pepper endornavirus and Pepper cryptic virus 1, have been reported from peppers. Studies on plant acute viruses shown that plant virus populations are genetically heterogeneous, and the distribution of these variants in the population may change over time and in different hosts. Since plant persistent viruses are in their host for a long time, understanding their evolution can shed light on the evolution of viruses. To investigate the evolution of these persistent viruses in peppers, dsRNA was extracted from over one hundred peppers leaves, including different cultivars of *C. annuum*, *C. chacoense*, *C.chinense*, and *C.bacccutum*. The presence of these viruses was tested by RT-PCR using the specific primers for their RNA dependent RNA polymerase. The nucleotide sequence of the RT-PCR products determined and the phylogeny of these two viruses in different peppers have been analyzed.

#48: Immune response of Tobacco Hornworms (*Manduca sexta*) following consumption of Horsenettle (*Solanum carolinemse*)
Alex Serpi

Botanists have at least 18 words to describe plant hairs, including trichomes. These common features of leaves are divided into two distinct categories: glandular trichomes contain defensive compounds that have been shown to deter herbivores, while non-glandular trichomes do not possess specialized structures for storage of defensive chemicals. Non-glandular leaf hairs have been shown to play a variety of roles, however their role in plant defense is only implicated. Some evidence shows that insects develop more slowly on plants with trichomes when compared to conspecifics that have reduced trichome numbers, but this could be due to other differences in leaf defensive chemistry or leaf microbiome. This study seeks to define the isolated function of stellate trichomes of horsenettle in anti-herbivore defenses. Specifically, we predicted and found that trichomes adversely affect larval growth and survival of *Manduca sexta* (Sphingidae). These effects were determined by measuring consumption rates, growth rates, and survivorship after feeding on artificial diet with and without (control) trichomes. Additionally, larval hemolymph was analyzed using disk diffusion assays and spectroanalysis to determine antibiotic strength, total protein, and phenyloxidase immune response. Larvae consuming a trichome-laced

diet consumed less and grew less than larvae on the control diet. Moreover, we found that trichomes damaged the peritrophic membrane of the guts of 4th instar larvae using SEM imaging. Consequently, we hypothesize that a diet with horsenettle trichomes causes internal gut damage and poses a serious challenge to the immune system of the moth larvae.

#49: Poliovirus 3C protein is a phosphatidylinositol-phosphate-binding protein
Djoshkun Shengjuler, Simou Sun, Yan Mei Chan, Joseph M. Moran, Akira Uchida, Ibrahim M. Moustafa, Jamie J. Arnold, Paul S. Cremer, David D. Boehr, and Craig E. Cameron

One of the most exciting advances in virology over the past few years has been the realization that phosphatidylinositol 4-phosphate (PI4P) is an essential lipid component of positive-strand RNA virus replication organelles. In the cell, phosphoinositides are used as markers of specific membranes that proteins and enzymes use to ensure appropriate localization and/or activation. Cellular proteins recognize phosphoinositides using one of a few structural scaffolds, the most famous of which is the Pleckstrin homology (PH) domain. We observed that poliovirus 3C protein binds to several phosphoinositides, including PI4P. Hints of the location of the binding site were obtained by molecular docking experiments and confirmed by using nuclear magnetic resonance spectroscopy. To measure the affinity, we used supported lipid bilayer binding experiments in the context of a microfluidic platform. This is a label-free, fluorescence method, which allowed us to test the interaction in a physiologically relevant manner. Together, these data reveal a new structural scaffold competent for phosphoinositide binding that may represent a tractable target for the design of antiviral agents.

#50: Mechanisms of apoptotic cell-mediated dysregulation of germinal center tolerance checkpoint: Implications in autoimmunity
Chetna Soni, Stephanie L. Schell, Dr. Ziaur S.M. Rahman

High-affinity antibody-secreting B cells are generated through somatic hypermutations (SHM) in their immunoglobulin genes. SHM occurs in B cells within germinal centers (GCs), in secondary lymphoid organs. Owing to its random nature, SHM generates autoreactive B cells as by-products, which are deleted by GC-specific peripheral tolerance mechanisms. Loss of GC tolerance leads to positive selection and expansion of autoreactive B cells causing autoantibody production, as in systemic lupus erythematosus (SLE). One contributing factor in murine and human SLE is inefficient clearance of apoptotic cells. Patients with SLE usually show a deficiency in clearing apoptotic cells. TAM (Tyro-3, Axl and Mer) receptor tyrosine kinases (TAM-RTKs) along with their ligands PROS-1 and/ Gas6 are involved in the efficient clearance of ACs in mice and humans and signal to inhibit innate immune responses. TAM-RTKs are expressed on myeloid cells. Their deficiency or aberrant function can lead to autoimmunity. Polymorphisms in Mer tyrosine kinase (MerTK) gene are associated with human SLE and multiple sclerosis. In previous studies form our lab we demonstrated that (MerTK) deficiency results in apoptotic cell accumulation in GCs, loss of B cell tolerance, Th1 cytokine induction, and autoimmunity in mice. To delineate the mechanism(s) of MerTK-dependent dysregulation of germinal center responses, we reasoned that MerTK signaling could directly affect antigen-presentation by macrophages and other APCs; possibly also resulting in increased self-antigen presentation. To test the hypothesis, we analyzed OT-II T cell proliferation in MerTK deficient (Mer-/-) vs WT mice after OVA-immunization. We observed increased proliferation of OT-II T

cells in Mer-/- mice compared to WT mice. To further delineate which APCs in the GC environment could contribute to increased Ag-presentation we used in-vitro OVA antigen-presentation system. B cells, bone marrow derived DCs (BMDCs) or bone marrow derived macrophages (BMM) from Mer-/- or WT mice were used as APCs. We observed more proliferation of OT-II T cells when cultured with B-APC and DC-APC from Mer-/- mice compared to WT mice. However, very interestingly, MHC-II dependent antigen-presentation by BMM only occurred in the presence of apoptotic cells.

We propose that defective MerTK signaling causes enhanced antigen presentation by APCs especially in the presence of apoptotic cells, which promotes activation of T cells even with low affinity for self-antigens. Therefore, in the presence of ACs, even low affinity activated autoreactive BCRs find help from T cells to get positively selected in the GCs leading to autoantibody production. Further studies are underway to confirm these observations.

#51: Investigating and Detecting Small Molecules at Lipid Membranes
Simou Sun, Tinglu Yang, Paul S. Cremer

A pH modulation biosensor has been designed in our laboratory to detect ligand-receptor interactions on supported lipid bilayers. This technique has been successfully applied to study viral protein-lipid binding, protein-ion binding pairs, anti-microbial peptide-lipid interactions and drug-molecule membrane interactions.

#52: Understanding Farmers' Perspectives on Climate Change
Kaila Thorn, Dan Tobin, Rama Radhakrishna

In the past century global temperatures have increased by nearly 2°F, leading to increases in pests, extreme precipitation, and warmer winters. In the Northeast United States (U.S.) the agriculture and forestry fields play a major role in the economy and land management with land in the Northeast being 21% farmland and 62% timberland, the agriculture industry alone brings in $21 billion in commodity sales annually. Understanding producers needs and perceptions about climate change is essential in helping this industry handle the impacts it will be faced with as a result of changes in the climate.
The USDA Northeast Regional Hub has partnered with Pennsylvania State University and Cornell University to research climate change in agriculture, forestry, and the natural resources. Currently the team is developing focus group interviews with agricultural producers from the top commodities shared between New York and Pennsylvania. The interview questions look at producer perspectives on climate change and who they communicate with when impacted by extreme weather events. Previous work done by the team was the development of a capacity report, which determined the current focus of climate change activities and priorities for extension agents, specialists, and researchers at the land-grant universities in the Northeast. This poster will blend the previous capacity report work with the first impressions of the Pennsylvania producer focus group sessions, as well as next steps in producer focus groups.

#53: Determining the Essential Features of an Exceptional Curvature-Sensing Amphipathic Helix

Erin Tyndall, Kumaran Ramamurthi, Fang Tian

Cellular function requires many processes that organize, deform and curve the membrane, including autophagy and endocytosis. Recently it has been shown that the membrane curvature alone is responsible as a regulator of activity and localization of proteins such as ArfGap1 and SpoVM. The small peptide SpoVM is responsible for beginning the process of coating the forespore in *Bacillus subtilis*, which it does by localizing exclusively to that slightly curved convex membrane structure. It is the first peptide found to recognize shallow curvature. Recently we have determined 3D structures of this 26 amino acid protein and one of its non-functional mutants. This has provided some structural and mechanistic insights into SpoVM's curvature sensing properties.

The SpoVM structure consists of 3-turn α-helix coupled to a flexible N-terminal loop, as compared to the standard straight helix of other curvature sensing helices. Additionally SpoVM's helix has an unbalanced non-polar face, with a very small polar side featuring only one charged residue. We have found that both the single charge, and relative length of helix and N-terminal tail play a loop in helping SpoVM localize to the correct subcellular location. We will present both in vivo and in vitro data demonstrating the impact of these features on the structure and function of SpoVM.

#54: Comparative Analysis of the Y Chromosome Genomes of Great Apes

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The female genomes of the three hominines - human, chimpanzee and gorilla - have diverged from each other by less than 3%. Yet, the human and chimpanzee male-specific regions of the Y chromosomes (MSYs), the only two sequenced hominine MSYs, are highly divergent with more than 30% of non-homologous sequences. Moreover, we have previously demonstrated that Y-chromosomal X-degenerate genes are better conserved between human and gorilla than between human and chimpanzee. In this study, we are focusing on the comparative analysis of the male-specific regions (MSYs) of the hominine Y chromosomes.

The gorilla Y chromosome sequence has been the missing piece for a thorough investigation of the hominine Y chromosomes. Here, we sequence the whole genome amplified flow-sorted gorilla Y chromosome DNA with both short-read (Illumina) and long-read (PacBio) technologies. Combining existing tools with new methods for extracting and assembling Y-chromosome specific sequences established in our lab, we were able to generate the first draft assembly of gorilla Y chromosome. As a result of our analysis, we estimate the divergence level, gene content, and detect rearrangements among hominine Y chromosomes. Additionally, we study the polymorphism of the Y chromosome in hominine populations by analyzing male-specific microsatellites and copy number variations of ampliconic genes. These insights are important for conservation genetics.

#55: Vitamin A supplementation only transiently increases retinol concentrations in extrahepatic organs of neonatal rats raised under vitamin A-marginal conditions
J. Kalina Urbanek, Libo Tan, Michael H. Green, A. Catharine Ross

Vitamin A (VA, retinol) supplementation is recommended for children > 6 mo old in countries with high rates of malnutrition, based on the positive results of clinical trials conducted in the 1990s. However, the results of studies on the benefits of supplementing neonates < 5 mo old are inconsistent. The objective of this study was to determine the body distribution of VA in neonatal rats raised under VA-marginal conditions (control group) and the effect of VA supplementation on the concentration and mass of retinol in plasma, liver, and lungs, as well as several understudied extrahepatic organs, such as brain, brown adipose tissue, and skin. Methods: Neonatal rats (n = 103), nursed by mothers fed a VA-marginal diet, were randomized and treated on postnatal day 4 (P4) with an oral dose of either VA (6 μg retinyl palmitate/g body weight) or canola oil as control. Subsequently, pups (n = 4/group/time) were euthanized at 13 time points from 30 min to 24 d after dosing and the following organs were collected: plasma, liver, lungs, kidneys, stomach, intestine, brain, white and brown adipose tissue, skin, and the remaining carcass. The total retinol concentration and mass in each organ was measured with ultra-performance liquid chromatography. Results: Control pups maintained a marginal plasma VA concentration, while the VA concentration in the liver was deficient. Despite its deficient status, the liver contained most (~77%) of the whole-body VA mass, similarly as in previous studies of adult rats with an adequate liver VA concentration. White adipose tissue, which was nearly absent prior to P12, contained only ~1% of the whole-body VA mass, compared to 10-20% reported in adult rats. The remaining extrahepatic, non-digestive organs together stored <10% of the whole-body retinol mass. VA supplementation significantly increased total retinol concentrations in all organs. However, this increase lasted for only 1 d in most extrahepatic organs, with the exception of white adipose tissue, where it lasted for 18 d. Conclusions: Our findings suggest that extrahepatic organs in neonatal rats may not be sufficiently developed to store VA at the adult capacity and that the scarcity of adipose tissue may predispose neonates to a low VA status. Moreover, given the transient effect of VA supplementation on extrahepatic organs, a more frequent supplementation schedule, along with other nutrition interventions, may be necessary to maintain a steady supply of retinol to the rapidly developing extrahepatic organs.
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#56: Changes in uterine immune regulatory factors and effects of pregnancy associated glycoproteins on uterine immune cells during early pregnancy in dairy heifers
Sreelakshmi Vasudevan, Manasi M Kamat, Joy L Pate, Troy L Ott

The bovine uterus has an abundance of lymphoid and myeloid lineage immune cells during early pregnancy. We hypothesized that these cells play roles in tissue remodeling and maintenance of tolerance. To assess tissue remodeling we analyzed expression of vascular endothelial growth factor (VEGF) and transforming growth factor beta (TGFB) by immunofluorescence (IF) labeling. For immune regulatory factors we analyzed the inhibitory proteins lymphocyte-activation gene 3 (LAG3), programmed death-ligand 1 (PD-L1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA4). Endometrium was collected from Holstein dairy heifers (n=4-5/group) on Day 17 of the estrous cycle (D17C) and Day 17 (D17P) and 20 (D20P) of

pregnancy, prepared for IF of immune regulatory proteins and RNA was extracted for qPCR for mRNA abundance. For IF, images from five different areas of the uterine wall (UW): luminal epithelium (LE), shallow stroma (SS), shallow glands (SG), deep glands (DG) and myometrium (Myo) were captured and percent area labeled was quantified using ImageJ. Abundant VEGF was observed in tissue collected on D17P with less VEGF expressed ($p<0.05$) on D20P across the entire UW. A similar pattern was detected for TGFB ($p<0.05$). The decline in VEGF and TGFB protein expression between D17P and D20P led us to determine if immune regulatory molecules changed during this same period. Abundance of mRNA of PDL1 was18-fold greater ($p<0.05$) in D17P compared to D17C endometrium, followed by a reduction ($p<0.05$) in D20P. There was a tendency ($p=0.067$) for LAG3 mRNA to be greater in pregnant than cyclic animals. Immunofluorescence analysis for CTLA4 revealed an increase ($p<0.05$) in percent area labeled across UW in pregnant compared to D17C endometrium. We next determined if pregnancy associated glycoproteins (PAG) could affect immune cell function during this time. Results indicated a 200-fold increase in PAG concentrations in uterine flushes between D17P (n=5) and D20P (n=4) from 17 ng/mL to 3.2 Âµg/mL. To determine effects of PAG on immune cells, CD45+ immune cells from D17C endometrium (n=4) and blood (n=5) were labeled with carboxyfluorescein succinimidyl ester (CFSE) and proliferation in response to concanavalin A (ConA; 40 Âµg/mL) was assessed. The cells were treated with PAG (2 µg/mL, 20 µg/mL), interferon tau (IFNT; 10000 U/mL) or their combination for 72 hours. After culture, cells were labeled with CD3 antibody to determine the proliferation response of the T cell subset. Proliferation of CD45+ uterine immune cells and the T cell subset was reduced ($p<0.05$) in response to 2 and 20 µg/mL PAG compared to untreated controls. There was no effect of PAG or IFNT on uterine cell proliferation in the absence of stimulation. The effect of PAG on immune cell proliferation was specific to uterine CD45+ cells and was not detected in peripheral blood CD45+ cells from these heifers. In summary, the endometrium undergoes changes in immune regulatory proteins during early pregnancy. Some of these changes may be mediated by conceptus-secreted PAG, which can modulate immune cell proliferation.

#57: Fz, Goa, and Axin Recruit Microtubule Nucleation Sites to Dendrite Branch Points
Alexis Weiner, Dylan Seebold, Nick Michael, Michelle Guignet, Chris Kozlowski, Dylan Barbera, Melissa Rolls

In Drosophila neurons, dendrite branch points act as hubs for microtubule organization. We have identified two microtubule control mechanisms that operate at branch points: microtubule steering and regulated nucleation. Apc2 is a component of the steering machinery and g-tubulin is the central nucleation protein. GFP-tagged versions of Apc2 and g-tubulin localize to branch points. To understand how these key regulators are concentrated at their site of action, we performed candidate screens using the very clear localization pattern of Apc2-GFP as a readout. We identified several groups of proteins from the initial screen including proteins that regulate actin polymerization through the Arp2/3 complex, the scaffold ankyrin2 and its membrane protein partner neuroglian, frizzleds, heterotrimeric G proteins and axin. Importantly, several of the proteins identified in the screen themselves localized to dendrite branch points; these included axin and ankyrin2. To determine whether the same suite of proteins is used to position g-tubulin, we knocked down representatives from each group and assayed g-tubulin-GFP at dendrite branch points. We found frizzleds, heterotrimeric G proteins and axin were critical for g-tubulin recruitment. Furthermore, we showed the proteins that were required for g-tubulin were

also necessary for proper microtubule polarity in dendrites. We also tested a functional requirement for frizzleds and heterotrimeric G proteins in nucleation by assaying the nucleation-dependent increase in microtubule dynamics after axon injury. These proteins were required for the increase in nucleation in dendrites, confirming their role as critical regulators of g-tubulin localization outside the cell body. The evidence thus far suggests that there exists a partial overlap between pathways responsible for Apc2 and g-tubulin localization. We show here that frizzleds, heterotrimeric G-proteins, and axin act as master regulators through this process to manage microtubule nucleation and steering.

#58: Risk Perception for Contracting HIV: A Principle-Based Concept Analysis
Rachel Wion, MS, RN

Aim: Human Immunodeficiency Virus (HIV) has been in the public eye for three decades. The yearly rate of new infections is 50,000. Low risk perception for acquiring HIV contributes to these infections. The aim of this principle-based concept analysis was to determine the state of the science of risk perception in the context of contracting HIV.
Methods: This concept analysis used the principle-based method. PubMed and CINAHL were searched with the terms "risk perception" AND "HIV." Forty-eight articles were included in the final analysis. The articles were read, after which a table was made to aid in analysis. Then the articles were reread and appropriate bundles of meaning were placed within categories in the table. The columns were then individually summarized.
Results: Risk perception for contracting HIV lacks conceptual clarity. The epistemological principle has not been met; therefore the other principles are compromised as well. A tentative definition was made. Risk perception for contracting HIV is awareness about the possibility of acquiring the disease. This awareness can lead to behavior changes. Risk perception shifts over time. Not all who are at high risk for contracting HIV have a matching high risk perception. Others negotiate or rationalize their risks for contracting HIV.
Conclusions: The concept of risk perception for contracting HIV is a dynamic yet immature concept. Conceptual clarity must be obtained prior to any measurement of the concept. Instruments should be developed to provide a common and consistent measure of HIV risk perception and then tailored interventions can be implemented.

#59: Antisense oligonucleotide-mediated knockdown of Mpzl3 protects from the metabolic consequences of a high-fat, energy-dense diet in mice
Beth Worley, Tom Auen, Sadie Dierschke, Traci Czyzyk

In recent decades, obesity has become a major health concern in the United States and many other parts of the world. It is associated with a variety of medical conditions including heart disease, high blood pressure, diabetes, respiratory deficiencies, and stroke. In the United States from 2011-2014, the prevalence of obesity was just over 36% in adults and 17% in adolescents. These values continue to rise despite public health programs and initiatives to promote a healthier lifestyle. Body weight regulatory mechanisms are also poorly understood, contributing to a lack of clinically efficient pharmacological agents for weight loss. Thus, there is an unmet medical need to identify metabolic pathways regulating body weight. It was previously shown

that mice deficient in the gene encoding myelin protein zero-like 3 (Mpzl3-KO) had reduced body weight and fat mass, increased energy expenditure, improved glycemic control, and reduced hepatic lipid synthesis (Czyzyk et al. 2013. AJPEM 305(2):E282-92). In this study, we examined the effects of antisense oligonucleotide (ASO)-mediated knockdown of Mpzl3. Male six-week-old C57BL6/N mice were placed on a high-fat, energy-dense diet for seven weeks, followed by placement into scrambled control or Mpzl3 ASO treatment groups via block randomization by body weight. ASOs were administered IP twice weekly at a dose of 50mg/kg. Measurement of food intake after two weeks treatment showed no difference in consumption. After three weeks of treatment, mice receiving Mpzl3 ASO injections showed significantly reduced body weight ($p<0.05$) and fat mass ($p<0.01$) compared to control animals. Serum analyses at this time point revealed reductions in circulating cholesterol levels by 17% ($p<0.05$) and triglycerides by 43% ($p<0.001$). Indirect calorimetry experiments after four weeks treatment showed a marked increase in activity levels in Mpzl3 ASO treated mice compared to controls, but no change in energy expenditure. Furthermore, the respiratory exchange ratio (a measure of CO2/O2 production) was significantly reduced in the Mpzl3 ASO group indicating an increase in whole body fat metabolism ($p<0.01$) in these mice. Additional serum collected at the time of sacrifice (seven weeks treatment) showed further reductions in circulating cholesterol by 38% ($p<0.0001$) and triglycerides by 24.5% ($p<0.001$). Tissue was harvested from liver, brown (BAT) and white adipose (WAT), intestine, skeletal muscle, and hypothalamus. RT-PCR analysis revealed significant reductions in relative mRNA levels of Mpzl3 in the metabolically active tissues: liver, BAT, intestine, and skeletal muscle; WAT trended towards a reduction. Knockdown was not seen in the hypothalamus. We have therefore shown that ASO-mediated knockdown of Mpzl3 in mice lowered body weight and fat mass, increased whole body fat oxidation leading to reduction in circulating cholesterol and triglycerides, and increased activity level. Furthermore, these data suggest that reduction of MPZL3 might have therapeutic potential for obesity and comorbid conditions.

#60: Innate immune protein lipocalin-2 and iron mitigate EGCG-mediated inhibition of myeloperoxidase

Beng San Yeoh, Rodrigo Aguilera Olvera, Vishal Singh, Xia Xiao, Mary J. Kennett, Bina Joe, Joshua D. Lambert, Matam Vijay-Kumar

Green tea *(Camellia sinensis*, Theaceae) contains a plethora of antioxidant polyphenols that may promote health. Amongst these polyphenols, the (-)-epigallocatechin-3-gallate (EGCG) is extensively studied for its potential therapeutic properties in models of inflammatory bowel disease (IBD), although its molecular mechanism is not completely understood. Here, we demonstrate that EGCG can inhibit the activity of myeloperoxidase (MPO; a pro-oxidant neutrophil enzyme associated with IBD flares) in a dose-dependent manner in vitro. By using spectral analysis, we show that EGCG prevents the MPO-catalyzed reaction by reverting the reactive peroxidase heme back to its native inactive ferric state. Oral administration of EGCG to dextran sodium sulfate (DSS)-induced colitic mice significantly reduced the colonic MPO activity and alleviated pro-inflammatory mediators associated with gut inflammation, confirming that our findings extend to in vivo conditions as well. In light of previous reports that EGCG can complex with innate immune protein lipocalin-2 and iron, we next tested whether these factors can affect the bioactivity of EGCG. Intriguingly, the presence of lipocalin-2 or iron significantly

mitigate the ability of EGCG to inhibit MPO in vitro. Further, the efficacy of EGCG against DSS-induced gut inflammation is diminished when orally co-administered with iron. These findings indicate that EGCG-mediated inhibition of MPO could be one of the mechanisms by which EGCG exerts its mucoprotective effects. However, counter-regulatory factors such as host lipocalin-2 and dietary iron could explain why EGCG does not ameliorate gut inflammation in some IBD patients.

#61: Molecular Mechanisms of Hepatitis B Virus Covalently Closed Circular DNA Formation
Jun Luo, Lu Gao and Jianming Hu

Hepatitis B virus (HBV) remains a major human pathogen causing acute and chronic viral hepatitis, liver cirrhosis, and cancer. As a prototypic hepadnavirus, HBV contains a small, partially double-stranded, relaxed circular (RC) DNA genome. Early during viral infection, RC DNA from the incoming virion is converted to the covalently closed circular (CCC) DNA, which can also be formed from progeny RC DNA synthesized de novo in cytoplasmic viral nucleocapsids (NCs). CCC DNA functions as the only viral template capable of coding for all the viral RNA species and is thus essential to initiate and sustain viral replication. Current antiviral therapies can suppress viral replication but cannot eliminate CCC DNA, the persistence of which remains a major obstacle toward curing chronic HBV infection. Currently, little is known about how CCC DNA is formed due to the lack of convenient experimental systems that can support efficient CCC DNA formation. For CCC DNA formation, RC DNA must be released from viral nucleocapsids into the host cell nucleus. The disassembly process of nucleocapsids (i.e., uncoating) required for RC DNA release is not understood.

We have recently shown that mutations of the viral capsid protein can lead to enhanced CCC DNA formation by stimulating NC uncoating. Furthermore, we have identified host cells with enhanced CCC DNA formation also via the stimulation of NC uncoating. These capsid mutants and host cells now provide native RC DNA substrates readily accessible for CCC DNA formation, which is being attempted under cell-free conditions with the help of cell extracts. As the viral polymerase protein remains covalently attached to RC DNA as a result of protein-primed initiation of viral DNA synthesis, its removal constitutes an essential step in the conversion of RC to CCC DNA. We and others have recently shown that a host cell DNA repair enzyme, the tyrosyl DNA phosphodiesterase 2 (Tdp2), is able to specifically cleave the polymerase-RC DNA linkage in vitro. However, the role of Tdp2 in CCC DNA formation in vivo remains controversial and seems to be opposite for HBV vs. its avian counterpart, the duck HBV (DHBV).

We are examining further the role of Tdp2 in RC DNA deproteination and CCC DNA formation in vivo, as well as the role of other host factors in this and other steps of CCC DNA formation. In order to elucidate the pathway(s) of CCC DNA formation from RC DNA, we are also attempting to isolate intermediate DNA species during the conversion process.