rezoned. Both strategies would be utilized during the zoning change phase for property to be developed.

Posters

A COMPARATIVE ANALYSIS OF THE HEALTH OF THE NI RIVER AND MASSAPONAX CREEK IN SPOTSYLVANIA COUNTY. Dr. M. L. Bass, E. E. Stewart, A. T. Elliott & H. E. Richters, University of Mary Washington. The main focus of this research project was to determine the health of both the Ni River and Massaponax Creek in Spotsylvania County, VA. The two streams provide a good demonstration of the effects of urban development. There are four sample sites located on Massaponax Creek and three sites on the Ni River. At each site, macrobenthic samples were taken as bio-indicators of pollution. These samples were corroborated by water chemistry analyses, including conductivity, dissolved oxygen levels, fecal coliform, alkalinity, calcium hardness, phosphate and nitrate concentrations and total dissolved solids. All samples were taken within each season. An examination of the macrobenthic results indicate that throughout the summer, the %EPT of the Ni River was approximately 90% at most sites. The low was of 82.8% at the McEwan Farm site. At Massaponax Creek, the summer's %EPT ranged from 97.3% at the Wetland to a low of 62.7% at Route 208. These ranges are caused by variations in water chemistry. During autumn and winter, the %EPT's steadily fell, as the organisms completed their aquatic life cycles. It can also be noted that one particular site on Massaponax was afflicted by a high CFU count, as well as high spikes in alkalinity, phosphate and hardness in the winter. It has been determined that the Ni River is healthier than Massaponax Creek. This is represented by the higher EPT percentages and diversity of macrobenthic individuals within the Ni River. However, Massaponax Creek has maintained good health despite increased development within its watershed.

Medical Sciences

THIOREDOXIN-INTERACTING PROTEIN MEDIATES HCYS-INDUCED NLRP3 INFLAMMASOME ACTIVATION IN MOUSE PODOCYTES. Justine M. Abais, Krishna Boini, Min Xia, & Pin-Lan Li, Department of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond VA 23298. Our recent studies have demonstrated that NADPH oxidase-derived reactive oxygen species (ROS) activates NLRP3 inflammasomes causing homocysteine (Hcys)-induced podocyte and glomerular injury, however the precise mechanism regarding how ROS activates the inflammasome is still unknown. The current study explored whether thioredoxin-interacting protein (TXNIP) mediates Hcys-induced NLRP3 inflammasome activation in podocytes. TXNIP, the regulatory inhibitor of the antioxidant thioredoxin (TRX), is thought to dissociate from TRX in response to elevated levels of oxidative stress to bind to inflammasome protein NLRP3 and activate the inflammasome complex. Genetic or pharmacologic inhibition of TXNIP by small interfering RNA or verapamil prevented Hcys-induced NLRP3 inflammasome formation and activation both *in vitro* and *in vivo* by reducing colocalization of NLRP3 with ASC or caspase-1, blocking Hcys-induced coimmunoprecipitation of TXNIP with NLRP3, and diminishing

caspase-1 activity and IL-1β production. In addition, TXNIP inhibition protected podocytes from Hcys-induced injury as demonstrated by normal expression levels of podocyte markers podocin and desmin, and preservation of glomerular function by reduced proteinuria and albuminuria *in vivo*. These results concluded that TXNIP binding to inflammasome protein NLRP3 is a key signaling mechanism for Hcys-induced NLRP3 inflammasome formation and activation, and subsequent glomerular injury (supported by NIH grants DK54927 and 1F31AG043289).

THE BALANCE OF T-LYMPHOCYTE SPECIFYING TRANSCRIPTION FACTORS AND THE CYOTOKINE EXPRESSION IN OBESE PATIENTS WITH NAFLD. M. Keaton[2], R. Mehta[1,2], A. Neupane[2], & A. Baranova[1,2], [1]Betty and Guy Beatty Center for Integrated Research, Inova, Falls Church VA 22042 & [2]School of Systems Biology, George Mason Univ., Fairfax VA 22030. The accumulation of immune cells within visceral adipose tissue (VAT) in subjects with NAFLD has been poorly understood. The immune response in obesity is reported to involve the shift in ratio of subsets of T cells (T_h1, T_h2, T_h17) within VAT through the transcription factors, TBX21, GATA3 and RORγ, respectively. The aim was to assess the ratio of T-cell lineage transcription factors and T-cell associated cytokines in obese patients with NAFLD. Forty-five biopsy-proven NAFLD patients' VAT samples were collected. RNA was extracted, and then converted, to cDNA. RT-PCR profiling was performed using validated primers for T cell lineage specifying markers (CD3E - CD4+ cell marker; TBX21 - Th1; GATA3 - Th2; FOXP3 - Treg). ACTB was used for normalization. Group comparisons for ratios like TBX21/CD3E and TBX21/GATA3 were carried out amongst all possible cohorts. There were consistently higher ratios of GATA3/CD3E compared to TBX21/CD3E and FOXP3/CD3E within each histological cohort related to grade of steatosis, histologic NASH, and stage of fibrosis. Group comparison of cytokines showed TGF-B1 (FC=0.13, p=0.04) and IL-1B (FC=0.79, p=0.03) were down-regulated in patients with NASH. IL-1B (FC=0.25, p=0.007) was down-regulated in patients with advanced hepatic fibrosis. The immune imbalance that is seen in obese NAFLD patients does not seem to depend on the expression of transcription factors involved in T cell lineage determination in the adipose tissue.

PRECLINICAL ASSESSMENT AND SPECTROSCOPIC CHARACTERIZATION OF THE ABUSED SYNTHETIC CANNABINOID, CP47,497. K. L. Samano[1], L. E. Wise[1], J. L. Poklis[1], A. Poklis[1,2,3] & A. H. Lichtman[1], [1]Dept. of Pharmacology & Toxicology, [2]Dept. of Forensic Science & [3]Dept. of Pathology, Virginia Commonwealth University, Richmond, VA, 23298. The objective of the present study was to characterize the pharmacological consequences of the abused synthetic cannabinoid compound, CP47,497. Cannabimimetic effects were assessed using the well-established tetrad model that is sensitive to the chief psychoactive compound present in marijuana, THC, and consists of four outcome measures: catalepsy, antinociception (tail flick latency), hypothermia, and locomotor activity. CP47,497 was significantly more potent than THC in each measure. The CB_1 receptor antagonist, rimonabant, reversed all pharmacological effects of CP47,497. Consistent with these findings, CB_1 (-/-) mice were resistant to the cannabimimetic effects of CP47,497. Immediately following behavioral testing, mice were humanely euthanized and blood and tissue were harvested for CP47,497 quantification. Samples are currently being

analyzed on an Applied Biosystems Liquid Chromatograph/Tandem Mass Spectrometer (LC/MS/MS) interface utilizing electrospray ionization and selective ion monitoring, using acetonitrile liquid-liquid extraction. Additionally, ongoing research is examining tolerance and dependence liability, as well as CB_1 receptor adaptations following repeated administration of CP47,497. In conclusion, the results of the present study indicate that acute administration of CP47,497 elicits markedly more potent CB_1 receptor mediated effects (i.e. 5-9 fold) than THC.

PRESERVATION OF T CELL FUNCTION AS A NECESSARY COMPONENT IN CHEMOTHERAPEUTIC STRATEGIES IN TREATMENT OF MELANOMA TUMORS. Se W. Jeong & Timothy N. J. Bullock, Dept. of Pathology, University of Virginia, Charlottesville, VA 22908. Metastatic melanoma has a poor prognosis as patients are only expected to live six to twelve months after diagnosis. PLX4720 is a specific inhibitor that targets a B-Raf gene mutation that is characterized by a V to E substitution at position 600. This has been highly anticipated as more than half of the patients present this mutation. While PLX4720 has had dramatic short-term success, the tumors escaped the B-Raf inhibition through other proliferative pathways. Therefore, six additional inhibitors were identified to have synergistic cytotoxicity with PLX4720 against melanoma tumors. This project probed how these combinations of chemotherapies, that have proven synthetic lethality against melanoma, impacted upon T cell function and cytokine expression. Ultimately, the goal was to define the least detrimental combinations of drugs to T cells for combined chemotherapy/immunotherapy approaches. Although most inhibitor combinations were not deleterious, only one combination, PLX4720 and Masitinib, a platelet-derived growth factor receptor inhibitor, was detrimental to T cell effector function. Therefore, our data indicates that it is possible to select multi-model therapy using combinations of chemotherapies and immunotherapy.

hOCTS: NOVEL TARGET FOR ANTIDEPRESSANT THERAPY. Kavita Iyer[1], Xiaolei Pan[2], Osama Alwassil[1], Douglas Sweet[2], & Malgorzata Dukat[1], [1]Department of Medicinal Chemistry & [2]Department of Pharmaceutics, School of Pharmacy, Virginia Commonwealth University, Richmond VA 23298. Drugs that target norepinephrine and serotonin transporters (NET and SERT, respectively) are commonly utilized to treat depression, however they have certain drawbacks associated with them. There is an acknowledged need to identify novel antidepressants. Human organic cation transporters (hOCTs) are proteins that modify the transport of endogenous neurotransmitters such as serotonin and norepinephrine. Recently, antidepressants have been shown to interact with hOCTs and may be a potential target for drug development. Our laboratory has synthesized a series of quinazoline analogs that are constrained analogs of *m*-chlorophenylguanidine (MD-354), a $5\text{-}HT_3$ receptor partial agonist. Two quinazoline analogs (KEO-099 and KEO-147) were found to be $5\text{-}HT_3$ receptor antagonists and tested positive in the mouse tail- suspension test. However, the two analogs differed in their ability to bind to $5\text{-}HT_3$ receptors as well as NET and SERT. It was concluded that neither NET nor SERT are able to adequately explain the antidepressant effects of the quinazoline analogs. This led us to examine these analogs at hOCTs to determine if their action at hOCTs could contribute to their

antidepressant effects. Results from the preliminary tests indicate that quinazolines inhibit the transport of 1-methyl-4-phenylpyridine (MPP^+), a model substrate for hOCTs. Moreover, we have established a rudimentary structure-activity relationship (SAR), which identifies substituents seemingly important for both binding as well as selectivity for hOCT subtypes.

NLRPs IN VISCERAL ADIPOSE TISSUE OF MORBIDLY OBESE PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD). Arpan Neupane[2], Rohini Mehta[1,2], Ancha Baranova[1,2], Arian Afendy[1], Zachary Goodman[1], & Zobair Younossi[1],[1]Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church, VA & [2]School of Systems Biology, George Mason University, Fairfax, VA. Inflammasome-associated Nucleotide-binding oligomerization domain, Leucine-rich Repeat and Pyrin domain containing (NLRP) family proteins serve as intracellular sensors of PAMPs (toxins, whole pathogens, etc.) and DAMPs (dsDNA, cellular debris, etc.). Inflammasome mediated activation of caspase-1 leads to cleavage of pro-IL1B and pro-IL18 and in turn induce expression of a number of cytokine genes triggering a cascade of inflammatory and immune responses. Obesity is a state of low-grade chronic inflammation and inflammation has been shown to play an important role in obesity-associated non-alcoholic fatty liver disease (NAFLD). NAFLD is strongly associated with visceral obesity. In this study we investigated the expression of 14 NLRPs genes in visceral adipose tissue of morbidly obese subjects (N=46) with NAFLD. Expression of 11 NLRPs members was not detected ($Ct \geq 35$). Interestingly, NLRP1 and NLRP3 (the most well studied NLRPs) were found be downregulated in morbidly obese subjects with severe NAFLD. This study for the first time determines the expression of NLRPs in visceral adipose tissue of morbidly obese subjects with NAFLD. The results show downregulation of inflammasome sensors with increasing severity of obesity-associated NAFLD. Further studies are needed to dissect the mechanism of NLRP downregulation.

COMBINED ADMINISTRATION OF THE SELECTIVE FAAH INHIBITOR PF-3845 AND COX INHIBITOR DICLOFENAC: INVESTIGATION IN A MURINE MODEL OF NERUPPATHIC PAIN. T. W. Grim[1], S. O'Neal[1], S. J. Kinsey[2], J. M. Pearson[3] & A. H. Lichtman[1], [1]Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA, [2]Department of Psychology, West Virginia University, Morgantown, VA, & [3]Ironwood Pharmaceutics, Inc. Cambridge, MA. Fatty acid amide hydrolase (FAAH), the primary degradative enzyme for the endogenous cannabinoid anandamide, offers a novel target to attenuate neuropathic pain, a condition refractory to existing analgesics. In the present study, we employed the chronic constriction injury model of neuropathic pain (CCI) to assess whether combined administration of the FAAH inhibitor, PF-3845, and the nonselective cyclooxygenase inhibitor (COX) inhibitor, diclofenac, would produce augmented antinociceptive effects. Dose response studies were conducted for each compound and subthreshold doses were given in combination to test for augmented antinociceptive effects in the CCI model. The co-administration of 5 mg/kg PF-3845 and 30 mg/kg diclofenac produced significant antinociception compared with either drug given alone. Metabolomic analysis found that PF-3845 elevated brain anandamide levels and

diclofenac reduced prostaglandin brain levels, regardless of whether the compounds were given separately or in combination.

PHOSPHORYLATION OF RAPTOR FOLLOWING PEMETREXED ACTIVATION OF AMPK OVERCOMES TSC2 DOWN REGULATION AND mTORC1 ACTIVITY. Stuti Agarwal, Catherine Bell, & Richard Moran, Deptartment of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond VA 23298. Pemetrexed is a highly unusual antifolate in that it has significant therapeutic utility against non-small cell lung cancers (NSCLC) and mesothelioma. We have shown that pemetrexed has a second mechanism, in addition to inhibition of thymidylate synthase, which leads to robust activation of AMP-dependent kinase (AMPK), and have suggested that this effect is causal of the activity of this drug against lung cancers. The most frequently mutated gene in NSCLC is p53 and, yet, the effects of pemetrexed are retained in p53-null carcinoma cells. Like K-Ras mutation and EGFR alterations, p53 loss causes hyperactivity of mTORC1; this is due primarily to loss of TSC2 function. The effects of pemetrexed also result in a functional loss of transcriptional activation by p53, with resultant loss of TSC2 function and concomitant expansion of RhebGTP. However, the robust stimulation of AMPK phosphorylation of Raptor in pemetrexed-treated cells overwhelms these effects and causes suppression of mTORC1.

IL28B GENOTYPE ASSOCIATIONS WITH METABOLIC SYNDROME. Leah Byars[2], Ancha Baranova[1,2], James M. Estep[1,3] & Zobair Younossi[1,2,3], [1]Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church VA, [2]Center for the Study of Chronic Metabolic Diseases, School of Systems Biology, George Mason University, Fairfax, VA & [3]Center for Liver Diseases, Inova Fairfax Hospital. Hepatitis C Virus (HCV) is a viral infectious disease that affects an estimated 130-170 million people. In a number of recent studies, the *IL28B* gene has been shown to play an important role in the outcome of HCV treatment. Metabolic syndrome (MetS) is a group of medical disorders usually associated with obesity. Recent, but not conclusive, evidence suggests that *IL28B* genotypes may be associated with metabolic confounders of HCV. In HCV patients, there is a significant correlation between the presence of CC genotype and several metabolic factors. In non-HCV patients with NAFLD, no such correlation was found. The metabolic profiles of the CC and Non-CC groups of NAFLD patients were also not different from each other. The beneficial effects of CC genotype on metabolic profiles may only be conferred after incidental infection with HCV, or after the treatment with antiviral therapy. *IL28B* genotyping in larger groups of non-HCV patients is warranted to determine the true effect of *IL28B* genotypes on metabolic outcomes.

ACTION OF DECONSTRUCTED MDPV ANALOGS AT hDAT. F. T. Sakloth[1], R. Kolanos[1], E. Solis[2], L. De Felice[2] & R. A. Glennon[1], [1]Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond VA 23298 & [2]Department of Physiology and Biophysics, Virginia Commonwealth University, Richmond VA 980551. The number of cases reported on the abuse of psychoactive "bath salts" has intensified in the past few years. These "synthetic cathinones" are β-keto analogs of amphetamine and were scheduled as of October 21, 2011. Methyenedioxypyrovalerone

(MDPV), a major constituent of bath salts, is the first synthetic cathinone known to act as a dopamine re-uptake inhibitor, i.e. to show a hyperpolarizing current. The purpose of this project was to understand which structural features contribute to its hyperpolarizing action, and determine what is it about MDPV that converts it from a depolarizing to a hyperpolarizing agent? MDPV was deconstructed into seven analogs, making only one structural change at a time. All the analogs, as with MDPV, showed a hyperpolarizing current in electrophysiological studies using frog oocytes transfected with hDAT. The IC_{50} values were measured and they ranged over a 200-fold span. Amongst the structural features that contribute to affinity, the tertiary amine and/or the extended side chain are optimal for affinity, but the extended side chain seems critical for affinity. However, the carbonyl oxygen atom and the methylenedioxy ring are not major contributors to affinity and not required for the hyperpolarizing action. In addition to understanding the structural contribution of MDPV to its hyperpolarizing action we are the first to report on the mechanism of newly confiscated MDPV analogs. [Supported by NIH grant DA 5R01DA033930.]

ENDOCANNABINOID METABOLIC INHIBITORS FOR THE TREATMENT OF AFFECTIVE ASPECTS OF MORPHINE WITHDRAWAL. T. F. Gamage & A. H. Lichtman, Dept. of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond VA 23298. Inhibition of the endocannabinoid catabolic enzymes fatty acid amide hydrolase (FAAH) with PF3845 or monoacylglycerol lipase (MAGL) with JZL184 reduces morphine withdrawal signs. It is uncertain if these anti-withdrawal effects extend to aspects related to the affective components of withdrawal. In the present study, we tested whether FAAH or MAGL inhibition would block the acquisition of conditioned place avoidance (CPA) to morphine withdrawal. While morphine (30 mg/kg) blocked acquisition of CPA and reduced jumping, neither JZL184 (4 or 40 mg/kg), PF3845 (1, 3, or 10 mg/kg), nor clonidine (0.03-1 mg/kg) affected acquisition of naloxone-precipitated morphine withdrawal-induced CPA. Additionally, delta-9-tetrahydrocannabinol (1, 3, or 10 mg/kg) did not affect withdrawal CPA. However, both JZL184 and THC significantly reduced the percentage of mice that exhibited jumping behavior. These data suggest that while inhibition of endocannabinoid metabolism reduces overt behavioral withdrawal signs, it is ineffective at reducing the aversive aspects of withdrawal. Thus, these drugs only reduce a subset of aspects related to opioid withdrawal. Future studies will examine the effects of combination treatment of dual FAAH/MAGL inhibition on acquisition of withdrawal CPA as well as other affective-related withdrawal behaviors.

Posters

ADIPONECTIN PREDICTOR OF LONG-TERM SUSCEPTIBILITY TO OBESITY. E. Jenkie[1], J. Bahamonde[2], B. Brenseke[2], M. Friedman[1], L. Mitchell[1] & R. Prater[1,2], [1]EVCOM; [2]VMRCVM. In 2009-2010, 35% of adult Americans were obese and at high risk for metabolic disease due to sedentary behavior and chronic overindulgence of fat and carbohydrates. Adiponectin is a protein hormone secreted by adipose tissue and is inversely correlated with body fat percentage in adults. It aids in the suppression of metabolic derangements that may result in obesity, type 2 diabetes, atherosclerosis and metabolic syndrome. Our laboratory proposed that maternal malnutrition, represented

as high fat diet (HFD), high sugar diet (HSD) or ethanol consumption (EtOH) throughout pregnancy, would elevate long-term risk of obesity in mouse offspring and that adiponectin in offspring from dams exposed to these diets could be measured in order to predict long-term susceptibility to obesity. Liver adiponectin was measured in control, HFD, HSD and EtOH groups at postnatal day 1, 21 and 42 in C57BL/6 mice using a mouse adiponectin ELISA. Serum insulin levels were measured, and histopathology reports and x-ray microtomography images were obtained for disease detection. Adiponectin decreased with age in all groups. The treatment groups had lower adiponectin levels (HFD group was lowest), compared to controls. Additionally, HFD offspring were found to have higher insulin levels than controls, and their femurs displayed fat accumulation and lesions resembling osteoporosis. Findings suggest that prenatal malnutrition can lead to diminished adiponectin levels and that measuring adiponectin may help to predict future risk of obesity and related disorders. This biomarker may then be manipulated in novel ways (e.g., nutritional interventions) in order to improve long-term health.

THE EFFECTS OF GLIAL CELL MODULATORS ON METHAMPHETAMINE (METH) INTRAVENOUS SELF-ADMINISTRATION AND FOOD-MAINTAINED BEHAVIOR IN RATS. S. E. Snider[1], E. S. Hendrick[1] & P. M. Beardsley[1,2,3], [1] Department of Pharmacology and Toxicology, [2] Institute for Drug and Alcohol Studies, [3] Center for Biomarker Research and Personalized Medicine, School of Pharmacy, Virginia Commonwealth University, Richmond Virginia. Methamphetamine (METH) administration activates glial cells in the CNS and increases pro-inflammatory cytokine production and release. Glial cell activation and pro-inflammation have been linked to drug abuse-related behavior. Ibudilast (AV411; 3-isobutyryl-2-isopropylpyrazolo-[1,5-a]pyridine), which inhibits phosphodiesterase (PDE) and glial-mediated pro-inflammatory activity, AV1013, an amino analog of ibudilast with similar glial but limited PDE activity, and minocycline, a tetracycline derivative with similar glial attenuating capabilities, all attenuate METH (0.03 mg/kg/inf) self-administration. The effects of ibudilast (10 mg/kg) and minocycline (30 & 60 mg/kg) on food-maintained responding were also examined using a behavioral economic approach. Demand curves for both METH (0.03 mg/kg/inf) and food-maintained behavior were obtained by increasing the FR value daily using the following progression: 1, 3, 6, 9, 13, 19, 26, 35, 47, 62, 82, 108. After matching the essential values (α levels) of METH and food pellets, twice daily ibudilast or once daily minocycline, was administered for three days under FR1 conditions. Ibudilast and minocycline both significantly ($p<0.05$) reduced both METH- and food-maintained responding. These results indicate that ibudilast and minocycline have complex effects potentially able to attenuate behavior maintained by multiple events.

BLOCKADE OF THE NMDA RECEPTOR ION CHANNEL ENHANCES THE DISCRIMINATIVE STIMULUS EFFECTS OF NITROUS OXIDE. Kellianne J. Richardson & Keith L. Shelton, Department of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond VA 23284-2006. The abuse-related CNS effects of nitrous oxide (N_2O) are poorly understood. *In vitro* data suggests that N_2O alters the function of NMDA and $GABA_A$ receptors, amongst others. Our goal was to

assess the neurotransmitter systems responsible for producing the intoxicating effects of N_2O using drug discrimination. Twenty-four mice were trained to discriminate 10 min of exposure to 60% N_2O/40% O_2 versus 100% O_2 in daily 5 min operant sessions. Mice acquired the discrimination in an average of 38 days. N_2O produced concentration-dependent substitution for the training concentration. Full substitution required 7 min of 60% N_2O exposure but the offset of stimulus effects N_2O following the cessation of exposure was more rapid. In substitution studies the NMDA channel blocker MK-801 produced up to 49% N_2O lever-selection at 0.56 mg/kg. While MK-801 failed to produce more than partial substitution, an intermediate dose of 0.17 mg/kg MK-801 significantly shifted the N_2O concentration-effect curve to the left. The competitive NMDA antagonist CGS 19755 and the positive $GABA_A$ modulator midazolam failed to substitute for N_2O. The stimulus effects of nitrous oxide also partially overlap with volatile solvents and anesthetics. Taken together these data suggest the subjective stimulus properties of N_2O may be partially mediated by NMDA antagonism. Other receptor systems not yet examined are probably also involved. Supported by NIDA grant RO1-DA020553.

THE EFFECTS OF WESTERN-STYLE DIET ON ATTENTIONAL SET SHIFTING PERFORMANCE. S. Marwitz, L. Woodie & S. Blythe, Dept. of Biol., Washington & Lee University, Lexington, VA 24450. The rate of obesity in the human population has risen to alarming levels in recent years. Obesity has been linked with a number of maladies such as insulin resistance, leptin resistance and diabetes. However, there is now growing evidence that obesity may even be linked to hippocampal damage. We conducted a pilot study to find if an attentional set shifting task could detect damage done to the hippocampus from a high fat diet. We used two groups of rats, one maintained on a high fat diet (n=19) and the other maintained on a control diet (n=19). The diets continued for 11 weeks when we performed the attentional set shifting task. Before the attention task the rats were maintained on limited versions of their respective diet so that they reached 85% of their body weight. In the task the rats were presented with two identical glass cups filled with a different material and scent. Rats were required to remember which scent or material was baited, with tasks increasing in difficulty. We found that diet did not have a significant effect on performance in the task ($p=0.177$, $p=0.751$). However, there was significant decline in performance as the tasks progressed and became more difficult ($p=0.052$, $p=0.033$). With this data and further research into other attention tasks, we hope to establish an appropriate test to shed light on the effects of a high fat diet on the brain. (Supported by: Howard Hughes Medical Institute Fund, Levy Foundation Grant, The Virginia Academy of Sciences and Washington & Lee University.)

STATISTICAL ANALYSIS OF ENVIRONMENTAL RISK FACTORS OF BREAST CANCER. Amirhossein Shamsaddini[1,2], Ancha Baranova[1,2], Aybike Birerdinc [1,2], [1]School of Systems Biology, College of Science, George Mason University & [2]Betty and Guy Beatty Obesity and Liver Program, Inova Health System, Falls Church, VA. Breast cancer is the most frequently diagnosed cancer among women, accounting for about 30% of all new cancer cases each year. In the early 1990s, it was suggested that exposure to some environmental chemicals such as organochlorine compounds (OC) may play a causal role in the etiology of breast cancer through estrogen-related

pathways. We used 1999–2009 National Health and Nutrition Examination Survey data to examine associations between serum concentrations of OC pesticides and breast cancers. Statistical analysis (*Logistic Regression*) was performed with SPSS v.20 on these databases after carefully screening for any inconsistency. Although further study is necessary to confirm these findings, these statistical results suggest that OC pesticide exposures may have a significant effect on breast cancer risk.

HUMAN LEUKOCYTE ANTIGEN (HLA) CLASS I COMPLEX: CLINICAL SIGNIFICANCE AS AN INDEPENDENT PREDICTOR OF NON-ALCOHOLIC FATTY LIVER DISEASE. Ali Moosvi[1,3], Azza Karrar[2,3], Zachary Goodman[2], Ancha Baranova[1,2,3] & Zobair Younossi[1,2], [1] Center for the Study of Chronic Metabolic Diseases, George Mason University, Fairfax, VA, [2]Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church, VA, & [3]School of Systems Biology, College of Science, George Mason University, Fairfax, VA. The overall aim of our study is to assess the association between the Human Leukocyte Antigen (HLA) Class I Antigens polymorphism and hepatic steatosis, which is an independent predictor of cardiovascular disease. Patients with biopsy-proven Non-Alcoholic Fatty Liver Disease (NAFLD) (n =199) were genotyped with the PCR Sequence Specific Oligonucleotides (PCR-SSO) for the HLA-A,-B, and –C. NAFLD patients were grouped to steatohepatitis (NASH) and Non-NASH NAFLD. Univariate and multivariate analyses were performed to determine significant correlations. HLA –A11, -B51 were found to be strongly associated to high-grade hepatic steatosis. In multivariate analysis approach, our data demonstrated an independent association between HLA-A11 and lower risk of high-grade hepatic steatosis, in addition to an independent association between HLA-A31, -B64, -B57 and histologic NASH. Notable differences were seen within the HLA-C loci, where Cw7 correlated to a protective effect against Advanced Fibrosis, while Cw8 had a predisposing effect. Identified risk haplotypes will aid in management of NAFLD by stratifying patients into groups with increased risk in developing not only NASH or Advanced Fibrosis, but also cardiovascular diseases.

MATRICELLULAR PROTEINS AND THEIR SPLICE VARIANTS IN OBESE NAFLD PATIENTS. Z. Asif[1,3], R. Mehta[1,2] & A. Baranova[1,2], [1]Center for the Study of Chronic Metabolic Diseases, George Mason University, Fairfax, VA, [2]Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church, VA, & [3]Biology Department, College of Science, George Mason University, Fairfax, VA. Matricellular proteins (MPs) are extracellular proteins that modulate cell-matrix interactions and cell function. MPs are doing this by remodeling the extracellular matrix. Obesity is a chronic disease that has reached epidemic proportions worldwide. Obesity is accompanied by excessive storage of fat in adipose tissue and ectopic sites like liver. Obesity is associated with many chronic diseases, notably nonalcoholic fatty liver disease (NAFLD). During obesity, the adipose tissue undergoes extensive remodeling (hyperplasia and hypertrophy) to accommodate excessive fat deposition. This extracellular matrix remodeling is, in turn, associated with altered expression of MPs. Some of the MPs have alternate isoforms whose expression and roles have not been examined in the context of obesity and associated NAFLD. These isoforms may be released into circulation and may affect distant sites such as liver. The aim was to

conduct a literature survey and shortlist MPs implicated in obesity and fatty acid metabolism. A literature survey was done on MPs using PUBMED and Google Scholar. MPs of interest were shortlisted and primers were designed for the different variants. Among the MPs, primers were designed for osteopontin (OPN), angiopoietin-like 4 (ANGPTL4), and autotaxin (ATX). Their expression in obese patients with NAFLD was detected. Future work: To design primers specific to each mRNA isoform and to detect their expression in obese patients with NAFLD.

THE RELATIONSHIP BETWEEN DEPRESSION AND THE FATTY LIVER INDEX. Nayeem Hossain[1,2], Michael Estep[1,2,3], & Ancha Baranova[1,2], [1]Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church, VA, [2]Center for the Study of Chronic Metabolic Diseases, School of Systems Biology, George Mason University, Fairfax, VA, & [3]Center for Liver Diseases, Inova Fairfax Hospital, Falls Church, VA. The fatty liver index (FLI) uses various chemical and physical measurements. This study observed the effect of depression's chemical environment on these values, focusing on triglyceride level and body mass index (BMI). Mann-Whitney tests compared triglyceride levels and BMI of those with depression to those not depressed in the overall population; subpopulations diagnosed with NASH were compared for further analysis. Triglyceride levels of depressed individuals were significantly higher than those not depressed in the overall population ($P < 0.0232$). The triglyceride value of individuals with both NASH and depression was not significantly higher ($P < 0.1738$) than those with only NASH. The average BMI was not significantly higher in depressed individuals than those not depressed in both overall population ($P < 0.3371$) and NASH subpopulation ($P < 0.4122$). Elevated triglyceride levels cause the FLI of depressed individuals without NASH to be higher than those of individuals without depression or NASH. Greater waist circumference, attributed to elevated triglyceride levels, further affects FLI values.

THE GENE EXPRESSION PATTERNS IN THE ADIPOSE TISSUE OF METABOLIC SYNDROME PATIENTS IN THE CONTEXT OF GENDER. Ashley Greer[1,2], Brianda Beverley [1,2,3], Micheal Estep[1,2,3], [1]Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church VA, [2]CSCMD, GMU, Fairfax, VA, & [3]NVCC, Annandale, VA. Adipose tissue metabolism differs between men and women. Study of these differences may shed light into pathologies such as metabolic syndromes that affect men and women differently. The aim of this study is to examine mRNA and miRNA expression from visceral adipose tissue and circulating orexigenic hormones with respect to gender and obesity associated disease. Visceral adipose tissue (VAT) was collected from biopsy-proven NAFLD patients who were undergoing bariatric surgery (N=24, Male=6, Female=18). MiRNA expression data from the VAT of NAFLD patients were obtained. Differences in circulating concentration or expression was considered significant between men and women if the absolute value of the change was >1.5 and a P-value of <0.05 was obtained. Regarding the cohort in which miRNA expression was measured, of the males in this group, 33% of patients have a diagnosis of diabetes, and 50% have a diagnosis of non-alcoholic steatohepatitis (NASH). Many of the differentially expressed miRNA correlate significantly with clinical and laboratory data in women. The variation in the expression patterns between men and women support that there is a gender-dependent difference in morbid obesity.

Further analysis on the expressions and functions of these molecules is necessary. Further study of this gene could express a difference in growth regulation in men and women. Better understanding of the functions for these molecules may help to develop gender-specific screenings or treatments for morbidly obese men and women.

PARADOXICAL EFFECTS OF MORPHINE WITHDRAWAL IN MOUSE MODELS OF ANXIETY-LIKE BEHAVIOR. J. P. Crowley III, T. F. Gamage, & A. H. Lichtman, Dept. of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond VA 23298. Opioid dependence presents a serious threat to society and affects approximately 2.4 million individuals in the United States. Cessation of drug taking leads to a withdrawal syndrome consisting of somatic signs, flu-like symptoms, and anxiety, which contribute to relapse. Typical models of anxiety have a lack of face validity in terms of expression of anxiety-like behavior during opioid withdrawal. It's been shown that mice going through morphine withdrawal display an increased open-arm time in the elevated plus maze, a behavior typically interpreted as anxiolytic. We assessed morphine withdrawal in two assays typically used to assess anxiety, the light/dark box (LD box), a conflict task similar to the EPM, and novelty-induced hypophagia (NIH), which measures consumption of palatable food in the home cage and a novel environment. In this assay a decrease in consumption and/or increase in latency is interpreted as anxiogenic behavior. Male ICR mice were implanted with either 75 mg morphine or placebo pellets and tested 48 h later receiving either naloxone (0.03-0.1 mg/kg) to precipitate withdrawal or saline. Morphine withdrawal increased the time spent in the light side of the LD box and reduced the latency to consume food in the NIH test – effects typically interpreted as anxiolytic. The increased time in the light side might reflect a shift in defensive behaviors toward escaping. Additionally, naloxone may be blocking morphine's suppressive effects on food consumption thus manifesting as a decrease in latency to consume. Further studies will test the effects of withdrawal in assays with greater face validity for escape behavior.

LIPID, GLUCOSE AND LIVER ENZYMES PROFILING OF OBESE NAFLD PATIENTS. Sweta Sedhai[1,2], Katherine Doyle[1,2], Amir Shamsad[1,2], Aybike Birerdinc[1,2] & Ancha Baranova[1,2], [1]Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church, VA & [2]School of Systems Biology, College of Science, George Mason University, Fairfax, VA. Insulin resistance is a major and very common issue in chronic diseases such as obesity, type 2 diabetes, cancer and cardiovascular diseases. It is also a key feature of Nonalcoholic Fatty Liver Disease (NAFLD). The main aim of this study is to quantify the insulin levels as well as glucose, ALT and AST levels in the serum of obese NAFLD patients. The data obtained in this experiment will be used in future studies for sample stratification. The Cholestech LDX diagnostic tool was used in this experiment. Lipid Profile GLU and ALT.AST (GPT.GOT) cassettes were placed in the Alere Cholestech LDX system. The correlation analysis was performed for 70 serum samples of obese NAFLD patients in which 54 serum samples were obtained from ELISA technique and 16 serum samples were done in Cholestech LDX system. The significant values for TRG was 0.001 with correlation coefficient (0.397), HDL was 0.018 with correlation coefficient (-2.90), LDL was 0.107 with correlation coefficient (0.210), Non-HDL was 0.020 with

correlation coefficient (.285), TC/HDL was 0.021 with correlation coefficient (0.283) and GLU was 0.00 with correlation coefficient (0.499). The data obtained through correlation analysis and linear regression supports the main idea of this study. The increased level of IL-8, IL-6, TNFa and C-peptide insulin are found in the patients with higher glucose, ALT and AST levels.

Natural History and Biodiversity

FREE-LIVING SOIL NEMATODE POPULATION DIVERSITY DYNAMICS AT AN *ASIMINA TILOBA* SITE IN VIRGINIA. Sarah R. Marzec & Theresa M. Grana, Department of Biological Sciences, Univ. of Mary Washington, Fredericksburg VA, 22401. Nematodes are microscopic roundworms that are highly successful in many environments. The model organism *Caenorhabditis elegans* is specifically a free-living nematode which can be found in soil but has been mostly isolated from anthropogenic habitats. Little is known about the environmental factors that affect *C. elegans* and where it is proliferate in nature. Information on ecological factors affecting *Caenorhabditis* species will be useful in identifying selective pressures that can influence genomic changes. The goals of this study are to find *C. elegans* and other *Caenorhabditis* species and shed light on relationships between the ecological factors and proliferating populations of nematodes. An *Asimina tiloba* site provides a natural Virginian habitat with a food source for *Caenorhabditis* species. This site is sampled every two weeks and any relevant ecological factors are recorded for the duration of a year. Nematodes are isolated from each soil sample and are separated based on morphology. The life stages of the nematodes from each sample are recorded and then the nematodes are sequenced for species identification. At present, five samples have been collected and several strains of nematodes have been isolated among and within the samples. All nematodes have shown to be in the dauer life-stage, a non-feeding migratory stage, showing that there are no established colonies. This is most likely due to the cold weather and we expect future results taken between the months of May through October to show proliferating populations.

INVENTORY AND CONSERVATION ASSESSMENT OF THE MOTH FAUNA OF VIRGINIA (LEPIDOPTERA). Steven M. Roble, Virginia Department of Conservation and Recreation, Division of Natural Heritage, Richmond, VA 23219. The insect order Lepidoptera includes the butterflies, skippers, and moths. Virginia's butterfly and skipper fauna of some 170 species has been well-studied for more than a century and continues to attract interest from biologists and amateur naturalists alike. In contrast, the much larger moth fauna of the Commonwealth remains poorly studied with the exception of a few pest species such as the Gypsy moth (*Lymantria dispar*). No formal compilation of the Virginia moth fauna exists, although survey efforts by staff of the Virginia Department of Conservation and Recreation, Division of Natural Heritage (DCR-DNH), during the past quarter century have begun to elucidate the composition, distribution, and conservation status of the "macro-moth" component of this fauna. Nearly 1,200 species of macromoths have been documented in Virginia, with a comparable number of "micro-moths" also expected to occur in the state, for a total