Abstract Booklet

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Diet Significantly Influences Physiological and Metabolic Outcomes of the Angiotensin II Mouse Model of Cardiorenal Injury

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There is an established relationship between diet and morbidity and mortality, especially in regards to cardiovascular disease. However, a majority of basic biomedical research in animal models routinely neglects or oversimplifies diet in their experimental design. Animal research plays a significant role in our understanding of disease processes and the development of novel treatment options, and it is possible that neglecting diet in experimental design may have drastic consequences for the translational potential of basic research. We have developed a novel rodent diet [“Americanized” diet (AD)] that includes modifications of several nutrients to match relative intake values reported for humans. The goal of the current study is to determine how diet influences several different outcomes of the common mouse model of cardiovascular and kidney injury [chronic angiotensin II (AngII) infusion]. Weanling male C57Bl/6 mice were purchased from the Jackson Laboratory and given 1-week to acclimate to pelleted chow diet. Then, mice were randomly assigned to receive 1 of 3 diets: standard rodent chow, a commercially available Western diet (WD) or our novel AD ad libitum. After 6 weeks, all mice underwent a brief surgery to implant miniosmotic pumps delivering AngII (700 ng/kg*min, S.C.). Blood pressure and food intake were recorded each week during the study. After 4-weeks, mice were euthanized and tissues were collected and processed for the identification of differentially expressed genes related to hepatic lipid metabolism and hypertension using PCR arrays (Qiagen). Mice fed chow had a slower development of hypertension in response to AngII as compared to mice fed WD or AD (P≤0.05 at week 2). As expected, there was a significant effect of diet on body weight (P<0.001) with mice fed WD having the greatest weight gain, followed by AD, and then chow. Interestingly, the administration of AngII significantly influenced weight gain in all mice regardless of diet (P<0.001) when data was compared to historical controls and adjusted for caloric intake (covariate). The expression of 84 genes related to hepatic lipid metabolism or hypertension were determined from representative liver and heart tissues (respectively) from each treatment group. In regards to hepatic lipid handling, 20% (16 out of 84) of the assayed genes had a greater than 2-fold difference when comparing the effect of consuming AD and WD whereas 45% (38 out of 84) of genes related to hypertension were affected. Taken together, our studies show that diet significantly influences physiological, metabolic, and molecular outcomes of the chronic AngII infusion model of cardiorenal injury. Additional studies are needed to confirm these results, identify the cellular mechanisms dictating these differences, and better understand the implications of these data as they pertain to the improvement of preclinical animal studies.
Determining the Physiological, Immunological, and Metabolic Consequences of Chronic Angiotensin II Infusion in Mice

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Cardiovascular and renal disease remain as significant medical burdens in the US, affecting millions of Americans each year. The chronic angiotensin II (AngII) infusion mouse model of cardiovascular and kidney injury remains to be a valuable tool to study disease pathogenesis and explore potential treatment options. However, there exists variability in the literature regarding both the establishment of the animal model as well as expected outcomes. Therefore, in order to utilize this model in our laboratory to study disease pathophysiology, we set out to characterize several parameters in mice with differing concentrations of AngII over a 4-week period. 8-week, male C57Bl/6 mice were purchased from the Jackson Laboratory and given 14-days to acclimate to the new environment and to collect baseline blood pressure measurements. Mice were then randomly assigned to receive 0 (saline vehicle), 400, 800, or 1200 ng/kg*min AngII via miniosmotic pump inserted into the subcutaneous space in the rear flank. Mouse systolic blood pressure and body weights were recorded each week for a 4-week period. During the 4th-week, mice were individually housed in metabolic cages to collect urine and assess food and water intake. Mice were then euthanized and kidney and liver tissues were collected. Total RNA was isolated from representative samples from the vehicle and 1200 dose and expression of genes related to renal inflammation and liver amino acid metabolism were quantified. As expected, dosage of AngII significantly influenced SBP (P=0.003), with the 800 and 1200 doses having a faster development of hypertension as compared to 400 and saline control. There was a similar effect on urinary output, with the 800 and 1200 doses have a greater 24-hour urinary output (P<0.04) as compared to mice with vehicle and 400 (regardless of water intake). Dosage of AngII significantly (P=0.014) influenced weight gain over the 4-week study, with dose being inversely related to weight gain despite a similar caloric intake (P=0.2) and accounting for caloric intake as a covariate. 84 genes related to renal inflammation or hepatic amino acid metabolism were assays by PCR array (Qiagen). 19 of the assayed genes (22%) related to renal inflammation were affected by AngII infusion, showing a greater than 2-fold change as compared to control treated mice. Several genes related to hepatic amino acid metabolism were also changed, however only 3 showed a greater than 2-fold change and were mostly associated with ketogenesis. In summary, there appears to be a strong dose-dependent response in this important animal model of cardio-renal injury. This has implications when interpreting the data existing in the literature using various dosages of AngII and lengths of exposure. Aside from the established effects of AngII infusion on cardio-renal injury and tissue inflammation, the metabolic effects are also intriguing and warrant further investigation to fully understand this important physiological axis and animal model of disease.
Diet Significantly Affects Outcomes of 2 Animal Models of Acute Kidney Injury

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Diet has a well-established influence on health and disease in humans worldwide. However, the precise mechanisms responsible for this relationship are still unclear. Recently, we have developed a novel rodent diet that embodies many of the dietary inadequacies reported in Western cultures [termed Americanized diet (AD)]. Interestingly, many of the dietary manipulations made have the potential for specific negative effects on kidney health and function in addition to traditional cardiovascular and hepatic risks. To further build upon the potential impact of inadequate dietary quality and renal health, we set out to determine how diet influences outcomes of animal models of acute kidney injury (AKI).

Weanling (3-week old) male C57Bl/6 mice were purchased from the Jackson Laboratory and given 1-week to acclimate to the new environment and a pelleted chow diet. Mice were then randomly assigned to receive 1 of 3 diets ad libitum: a standard laboratory rodent chow, a commercially available Western diet (WD), and our novel AD. After 6-weeks, mice underwent 1 of 2 different models of acute kidney injury: unilateral ischemia reperfusion injury (uIRI) or folic acid-induced nephropathy (FA). Mice were euthanized 24-48 hours after uIRI or FA, respectively and tissues were collected for histological analysis (H&E) or quantification of gene expression by conventional RT-PCR or identification of genes related to nephrotoxicity using a PCR array (Qiagen). Histology showed successful induction of AKI by both methods, with rampant tubular cast formation and acute tubular necrosis evident in ischemic tissues, whereas tubular dilation and moderate tubular cast formation was the key feature in tissues from FA-treated mice. Although there were no discernable difference in tissue histology amongst the treatment groups, there were differences observed in the expression of genes related to inflammation and tissue remodeling. In the FA-model, renal collagen 3 mRNA expression was significantly greater in mice fed WD (P=0.03) and a-smooth muscle actin also tended to have a greater expression (P=0.09) as compared to mice fed chow or AD. Similarly, preliminary studies from the uIRI group also show a numerical, but drastic increase in renal IL-6 and TGF-b in mice fed WD and AD as compared to mice fed chow. Renal tissues from the FA study identified several (29, 35%) of the 84 assayed genes related to nephrotoxicity were significantly influenced by FA treatment and differed in mice treated with WD or AD, suggesting that the pathological processes may not be similar despite both being considered “caloric-dense.” Taken together, our data strongly suggests that diet significantly influences the immunopathology of kidney disease in animal models. Diet appears to influence the inflammatory response and subsequent tissue remodeling following AKI, suggesting diet impacts long-term consequences following AKI. Furthermore, the apparent difference in expression of genes related to nephrotoxicity suggest diet may significantly influence the cellular basis for kidney injury and highlights the need for more research in this area.
Thermal Ultrasound Induction of an Abdominal Heat-Shock Response Prevents Postoperative Ileus

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Postoperative ileus (POI) is an obligatory feature of surgery costing in excess of $1B/yr in the US. POI is caused by the iatrogenic induction of an intense gut muscularis molecular inflammatory response, which leads to the massive recruitment of leukocytes into the intestinal muscularis externa collectively causing ileus (2–15). Currently, there is no therapeutic intervention to avert POI. Existing literature demonstrates that a preconditioning heat shock response is extremely protective via its modulation of the immune response to a subsequent injury, such as ischemia-reperfusion injury. The clinical utility of the protective heat shock response has been hampered due to the previously used uncomfortable “whole-body hyperthermic” induction of heat shock. To our knowledge, a focal, organ specific induction of the heat shock response has not been previously investigated in any experimental model.

In our novel project, we utilized a preconditioning, focused hyperthermic ultrasound (HUS) treatment to induce an organ specific protective heat shock response. Thus, we hypothesized that the administration of a pre-operative abdominal HUS intervention will prevent the development of postoperative ileus (POI). We further hypothesized that the protective immune modulating mechanisms triggered by HUS will be mediated by the molecular activation of specific transcription regulators such as heat shock protein-70 (HSP70) and nuclear factor erythroid 2–related factor 2 (Nrf2) along with the subsequent induction of downstream anti-inflammatory mediators (such as, HO-1 and IL-10). Our previously published works have clearly demonstrated a significant role for both HO-1 and IL 10 in ameliorating postoperative ileus. However, to date no study has ever explored the endogenous role of HSPs in the context of ileus to our knowledge.

The data from our study clearly demonstrates the therapeutic potential of targeted HUS to induce a pre-operative heat shock response, which prevents the development of postoperative ileus in a murine model.

Bibliography
Peripheral Tissue Injury and Endotoxin Induce a Systemic Inflammatory Response with a Perfuse Intestinal Microvascular Leak that Triggers a Neurogenic Disruption in Gastrointestinal Motility

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Introduction/Background: Both traumatic peripheral tissue injury and lipopolysaccharide (LPS) induced sepsis leads to a systemic inflammatory response syndrome (SIRS) derived from tissue damage-associated molecular pattern molecules (DAMPs), pathogen-associated molecular pattern molecules (PAMPs), gut cytokines, and gut bacterial translocation leading to multiple organ dysfunction syndrome (MODS). Intestinal stasis contributes to SIRS and MODS by permitting intestinal bacterial overgrowth and translocation. Previous studies have revealed that DAMPs and PAMPs trigger inflammatory signaling causing ileus via a MyD88-dependent mechanism on both hematopoietic and non-hematopoietic cell types. We sought to develop a novel confocal imaging technique to elucidate a unique pathophysiological mechanism of trauma and endotoxin induced ileus and, additionally, explore therapeutic interventions.

Methods: The murine trauma model consisted of the subcutaneous implantation of a devitalized syngeneic tissue bone matrix (TBX = long bones and overlying skeletal muscle in a 2.5% penicillin/streptomycin) normalized to % of body weight into a cutaneous slit at the nape of an anesthetized recipient C57Bl/6. The endotoxemia model consisted of the intraperitoneal injection of varying dosages of LPS normalized according to body weight. Gastrointestinal motility in vivo was measured by the oral administration of a non-digestible, non-absorbable FITC-labeled dextran (FD70). The aboral distribution of FD70 after 80 minutes was measured in 15 gut segments and a geometric center (GC) was calculated. Myeloperoxidase+ neutrophils were quantified in muscularis externa whole-mounts. Jejunal and colonic circular muscle contractions were measured in a standard organ bath. Ammonium sulfate precipitation (35%-45%), centrifugation sizing (>100 kD) and SE-HPLC was used to isolate specific serum proteins.

Results: The TBX trauma data demonstrated that saline and Protein S resuscitation (250µl at 0.5, 4 and 12 hrs) dramatically decreased mortality and averted TBX induced ileus compared to no fluid resuscitation. Endotoxin via an early endothelial cell dependent mechanism caused a dose-dependent increase in ileus severity, which was marginally improved with the Rho kinase inhibitor fasudil (5 mg/kg, i.p.). Both models of ileus appeared to initiate an intestinal muscularis vascular leak even in the absence of a myeloperoxidase+ leukocytic cellular inflammatory response. Therefore, we developed a novel confocal microscopic technique to image the entire microvascular network in a gut segment using intravenous injection of FITC-dextran (FD70 kD) and IB4-568 λ labeled microvessels. Imaging confirmed the existence of a massive vascular leak in both models. The intraperitoneal injection of serum (250µl) caused an immediate delay in transit GCs compared to lactate-Ringers (5.1±0.63 vs. 9.9±0.53, respectively). Exposure of jejunal and colonic circular muscles to serum (0.5, 1.0 and 2.5%) caused a dose-dependent inhibition of jejunal and colonic spontaneous clustered contractions (45.8±9.46%, 68.6±2.69%, and 78.8±3.26%, respectively), which was partially blocked by LNA. Serial serum ammonium sulfate precipitations (35%-45%), centrifugation sizing (>100 kD) and SE-HPLC isolated the ELF to specific serum proteins.

Conclusion: A confocal microscopic imaging technique was developed to obtain images of live tissue with microvascular leak. Peripheral tissue injury and endotoxin induced inflammatory mediators triggering a perfuse intestinal microvascular leak of a specific serum ELF within the gut muscularis that potently activates nitrergic and NANC motor neurons to dramatically alter contractility. Fluid resuscitation was shown to reduce the SIRS response on the gastrointestinal tract, as well as increase survivability.
The Effect of Anti-Retroviral Drugs on HERV Protein Expression in Cancerous and Non-Cancerous Cells

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Human endogenous retroviruses (HERV) are genomic sequences of retroviral origin which were thought to be integrated into germline chromosomes millions of years ago. The HERV-K class includes the most intact HERV elements. We have investigated whether anti-retroviral drugs, which are currently used to treat infection by the human immunodeficiency virus, are effective in inhibiting the expression of HERV-K in breast cancer cells, and whether inhibition of HERV-K is correlated to inhibition of cell proliferation. The anti-retroviral drugs included raltegravir (integrase inhibitor), dolutegravir (integrase inhibitor), darunavir (protease inhibitor), and nelfinavir (protease inhibitor). We used these drugs to treat in vitro cultures of three human breast cancer cell lines (BT-20, T47D, and MDA-MB-453), an immortalized epithelial cell line derived from the human mammary gland (MCF-12A), primary human mammary epithelial cells, and a human cervical cancer cell line (HeLa). Our data indicated that anti-retroviral drugs inhibited expression of HERV-K on both the RNA and protein levels in breast cancer cells. Interestingly, the anti-retroviral drugs generally had greater inhibitory effects on the proliferation of non-cancerous cells than on cancer cells. We hypothesize that HERV-K plays a role in the normal cell cycle and overexpression of HERV-K elements helps cancer cells to resist antiretroviral drugs.
Regulation of MicroRNA-214 Expression in the Mammalian Unfolded Protein Response

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The transcription factor X-box binding protein 1 (XBP1) plays critical roles in the immune system. For example, XBP1 is required for plasma cell development and can disrupt proper function of tumor-associated dendritic cells. A number of post-transcriptional mechanisms regulate XBP1, but a complete understanding of the processes that govern its expression and activity in distinct situations is lacking. The active form of XBP1, termed XBP1s, is dependent upon cytoplasmic splicing of Xbp1 mRNA by IRE1, a transmembrane endoribonuclease/kinase positioned in the endoplasmic reticulum (ER) membrane. IRE1 is a key signal transducer in the cellular response to ER stress, also known as the unfolded protein response (UPR). The IRE1-XBP1 pathway coordinates a broad program of gene transcription that promotes functions of the secretory pathway and alters various aspects of lipid homeostasis under conditions that perturb the ER environment and/or increase demand on its protein folding capacity. ER stress also activates the UPR signal transducer PERK, an ER transmembrane kinase that mediates efficient repression of protein synthesis. Other laboratories have shown that microRNA(miR)-214 can negatively regulate XBP1 expression and is down-regulated during ER stress. Here, we report studies to investigate the mechanism responsible for the down-regulation of miR-214 during the UPR. We performed in vitro experiments using mouse embryo fibroblasts (MEFs) wild-type or nullizygous for either Perk or Ire1α treated with tunicamycin, an inhibitor of N-linked glycosylation that causes ER stress and induces the UPR. Quantitative real-time RT-PCR revealed that ER stress-mediated diminishment of miR-214 is partially dependent on PERK, but not IRE1α. These data suggest a novel mechanism of regulatory crosstalk involving the PERK pathway, miR-214, and the IRE1-XBP1 pathway of the UPR. To complement these studies, we began in vitro experiments with mouse NIH-3T3 fibroblasts and pharmacologic inhibitors of PERK and IRE1α. Initial analysis using quantitative real-time RT-PCR to assess gene expression in tunicamycin-treated cells indicated that a PERK-specific inhibitor (AMG PERK 44) impeded induction of Ddit3, a PERK-dependent UPR target gene, and that an IRE1α-specific inhibitor (MKC8866) blocked induction of Xbp1s. These data indicate that these inhibitors can be used to further probe the respective roles of the PERK and IRE1α pathways in the regulation of miR-214 levels.
Tuberculosis Drug Discovery: An Overview of Phenotypic Screening and Formal Hit Assessment

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**Background:** The primary focus of our lab is drug discovery. Presently, the treatment regimen for tuberculosis (TB) lasts between six to nine months. We are looking to improve the current regimen by reducing treatment time and identifying novel compounds effective against drug-sensitive as well as drug-resistant strains of TB. The high-throughput screening (HTS) team aims to identify hits among thousands of compounds sent to us from various pharmaceutical companies and collaborators around the world. Hits are defined as the compounds selected based on statistically significant thresholds of inhibitory activity.

**Methods:** Screening of small molecule compound libraries is performed in high-throughput 384-well format using GFP *Mycobacterium tuberculosis* (Mt) in various carbon sources. We have screened diverse libraries with many collaborators, under the TBDA program, including: Merck, University of Dundee, Bayer, Pfizer, AstraZeneca, EISAI, Dupont, Johnson & Johnson, and Global Health Diversity. Compounds showing inhibition of Mt growth greater than 50% or z-scores less than -3 are chosen as hits and pursued further in the biological profiling cascade. These hits are triaged based on characteristics that we find undesirable, such as high levels of cytotoxicity.

Formal hit assessment includes four specific assays and twelve conditions. First, there is the cytotoxicity assay which uses human liver cells to test how harmful a compound is to humans. Then there is the lux reporter assay, which conveys information about cell wall damage and genotoxicity; two known mechanisms of action for anti-TB drugs. Next, the respiratory reporter assay reveals if the compound target is a part of the modestly understood cellular respiration pathway in Mt. And finally, minimum inhibitory concentration (MIC) assays are set up to reconfirm inhibitory activity and identify specific MICs in a variety of conditions that mimic the host environment.

**Results:** More than 5 million wells have been screened. Hit rates have varied depending on chemical composition and anti-microbial specificity of the libraries. After hit triage and preclinical assays conducted by the various TBDA consortium members, compounds from University of Dundee, Merck, and EISAI have progressed from hit to lead optimization. At least one compound is close to entering clinical development.

**Conclusion:** An effective and efficient design has been implemented to perform high-throughput screening of small molecule compounds against an infectious agent. The current, standardized screens have demonstrated their ability to elicit hits in *in vivo* relevant conditions. These hits have the potential to become leads, which are further examined in animal studies. However, prior to being classified as leads the compounds must go through a pipeline of phenotypic assays and assessments. The end result is the emergence of candidate drugs for clinical trial.
Exogenous Synthetic Oligonucleotide-Based Immunomodulatory Agonists Ameliorate Postoperative Ileus

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Introduction: Postoperative ileus (POI) is a near obligatory feature of postsurgical trauma costing in excess of $1 billion per year in the United States. Published works have demonstrated that POI is caused by the iatrogenic induction of an intense gastrointestinal muscularis molecular and cellular inflammatory response. Our goal was to investigate the therapeutic potential of exogenous synthetic DNA-based agonists to prevent the development of POI.

Methods: A standardized POI mouse model utilizing non-traumatic intestinal manipulation (IM) was used, which mimics the clinical procedure of “running of the bowel”. The effects of various synthetic DNA-based agonists were administered by intraperitoneal injection. Twenty-four hours after resuscitation from surgery, gastrointestinal transit was assessed by orally feeding a non-digestible, non-absorbable fluorescent marker (FITC-dextran 70kD) and plotting an intestinal distribution histogram with a calculated geometric center (GC). Muscularis neutrophil transmigration was quantified using a Hank-Yates myeloperoxidase stain and qPCR was performed to measure a molecular inflammatory response. (N=3-6, p<0.05)

Results: Alone, the prototypical synthetic oligonucleotide agonist ODN-1668, synthetic-A and synthetic-B did not significantly alter basal gastrointestinal transit compared to naïve saline control (GC saline= GC=11.1±0.37, ODN-1668-1668=10.3±0.12, synthetic-A= GC=10.5±1.19, and synthetic-B=10.3±0.86, each at 5mg/kg). Sham surgery treated mice had a typical gastrointestinal transit with the majority of the FITC-dextran localized to the ileum after 80 minutes exhibiting a calculated geometric center (GC) of GC=10.2±0.42. However, intestinal surgical manipulation resulted in a significant gastrointestinal postoperative delay with the fluorescence localized to the very proximal jejunum (GC=4.9±0.52, p>0.05). Pretreatment of the mice with ODN-1668 (5mg/kg) at 20 and 4 hours before surgical intestinal manipulation caused a further delayed gastrointestinal transit (GC=3.3±0.3). In contrast, both synthetic-A and -B significantly prevented the development of postoperative ileus (GC=8.9±0.69 and 8.9±1.24). The improvement in gastrointestinal motor function appeared to be due to the Tlr9 synthetic-A pretreatment cellular anti-inflammatory effect, because neutrophil extravasation into the muscularis whole-mounts was significantly prevented by synthetic-A (optical quantification of myeloperoxidase staining activity: sham control=0.003±0.00015, syn-A=0.002±0.00025, IM=17.7±3.80 and syn-A+IM=0.88±0.042% of optical field, p<0.05). In contrast to the diminished cellular inflammatory response, syn-A actually caused a molecular increase in IL-6 and MCP-1/CCL2 mRNA.

Conclusion: The oligonucleotide Tlr9 synthetic-A prevented the postoperative delay in gastrointestinal transit, maintained jejunal circular muscle contractility and reduced MPO+ phagocyte recruitment into the surgically manipulated gut. We hypothesize that oligonucleotide immunomodulation induces a protective, anti-inflammatory cellular response, which prevents the development of murine postoperative ileus that could be exploited clinically.
Diet Significantly Influences Indices of Renal Health in Mice Consuming Alcohol Chronically

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Diet and alcohol are known to have significant effects on health and disease development, especially in regards to liver disease. Interestingly, most preclinical animal studies have focused on these two risk factors (diet and alcohol) separately and have not considered how poor nutrition influences physiological outcomes associated with chronic alcohol intake. Furthermore, the diets used in animal studies are oversimplified representations of Western cultures that likely influence other organs (including the kidneys). Given the prevalence of hepatorenal syndrome in those with excessive alcohol intake, we set out to determine the effects of 3 different diets on liver and renal health in mice consuming alcohol in a chronic manner. Weanling C57Bl/6 male mice were purchased from the Jackson Laboratory and given 1-week to acclimate to a pelleted chow diet. Mice were then randomly assigned to receive 1 of 3 diets \textit{ad libitum}: a standard laboratory chow diet, a commercially available Western diet (WD), or a novel Americanized diet (AD) our laboratory created to model the median intake of several nutrients by Americans. Mice were fed their diets for 8 weeks and then further divided into 1) group consuming water, or 2) a group with 12.5\% (v/v) ethanol (EtOH) solution as their sole source of fluid. Mice remained on their assigned diets and beverage treatment for an additional 6-weeks, after which 24-hour food intake and water balance, mouse systolic blood pressure, and renal blood flow (RBF) were determined. Diet significantly influenced several physiological outcomes, with mice fed WD consuming less beverage (P=0.03) and less EtOH (P=0.03) as compared to mice fed chow and WD fed mice had the highest (P<0.001) body weight. Regardless of diet, all mice provided ethanol consumed more beverage (P=0.02) than mice provided water. Interestingly, mice fed the AD and consuming EtOH beverage had a greater dietary consumption than AD mice consuming water (P=0.03). Systolic blood pressure tended (P=0.1) to be increased in all mice consuming ethanol regardless of diet. Diet (P=0.003) and beverage (0.004) significantly influenced estimate of RBF. In chow fed mice, EtOH consumption resulted in a tendency (P=0.06) for increased renal blood flow. However, mice fed WD or AD did not have a similar increase in RBF as mice fed chow, even after a volume challenge. Histological evidence showed ethanol consumption resulting in hepatic steatosis in mice fed chow, while WD and AD mice also displaying fatty liver regardless of beverage intake. Taken together, our data highlight the significant effect of diet and alcohol on renal function and liver architecture in mice. High calorie diets in combination with chronic alcohol intake appear to influence hemodynamic regulation within the kidney and may contribute to the development of hypertension. Our data also suggest that diet and alcohol consumption interact and influences the preference for intake of both. Future studies will focus on understanding the molecular mechanisms responsible for these outcomes.
Diet Influences Immunological Outcomes of Animal Models of Polymicrobial Sepsis

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Sepsis remains a serious health concern in the United States and is associated with 1 in 3 deaths in the hospital setting. Previous work has shown diet and obesity to be important in sepsis, with obesogenic diets resulting in worse outcomes in animal models. We have previously observed that high-calorie diets resulted in slower recovery from peritonitis induced by cecal-ligation and puncture or a fecal peritonitis challenge and found elevated circulating cytokines in these treatments. However, it is unclear how diet and/or obesity modulates the inflammatory response to microbial challenge. In the current study, we set out to determine how diet influences the immunological response of individual tissues to lipopolysaccharide stimulation ex vivo.

Weanling, male C57Bl/6 mice were purchased from the Jackson Laboratory and given 1-week to acclimate to a standard rodent chow. Mice were then randomly assigned to receive (ad libitum) standard chow, a commercially available Western diet (WD), or a novel Americanized diet (AD) formulated to match the median American intake of several nutrients. After 4-weeks, mice were euthanized and spleen and epididymal fat pads were collected, weighed, and incubated at 37°C in DMEM media containing either 0 or 10 µg/mL LPS. After 3.5 hour incubation, tissues were stored and representative samples from WD and AD treatment were processed for RNA isolation and cDNA synthesis following the manufacturer’s recommendation (Qiagen). Similar tissues from chow fed mouse with 0 µg/mL LPS served as the control. Samples were then screened for the expression of 84 different genes associated with innate and adaptive immune response (Qiagen). In the spleen, 21 of 84 assayed genes (25%) were upregulated and had at least a two-fold difference in response when comparing the tissues from WD or AD fed mice. Interestingly, 38 out of 84 genes were upregulated and had at least a two-fold difference when comparing tissues from mice fed WD and AD in epididymal adipose tissue. Mice fed WD tended to have the highest magnitude of expression, especially in the spleen. However, 14 of the 38 identified genes in adipose tissue had a higher magnitude of expression in mice fed AD as compared to WD. Despite being a secondary lymphoid organ, the magnitude of expression of many of the genes were similar between the spleen and adipose tissues. Taken together, our data further support the involvement of diet and obesity in the pathophysiology of sepsis. Further studies are needed to validate this array data and understand the cellular mechanisms dictating the impact of diet on inflammatory diseases.
Infant Safe Sleep and the VDSS Baby Box Program: Implementation at a Community Based Hospital

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In 2016, the AAP published recommendations for infant safe sleep environment. Since that time, the Virginia Department of Social Services has developed a program to provide an inexpensive but appropriate sleep space for newborns up to 6 months of age. This information had not been introduced to our local hospital that resides in the area of the state with the highest incidence of Sudden Infant Death Syndrome. The study used a questionnaire to elicit caretaker responses to knowledge of safe sleep and the baby box program at discharge, both before and after implementing a quality improvement procedure involving written and verbal information. Results indicated that given time and reinforcement with multiple messaging, parents were able to recall safe sleep information and increase participation in the baby box program.
Role of Microbiota in Autism

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The etiology and pathophysiology of autism spectrum disorders (ASD) remains unclear. Autism is speculated to be polygenic with contribution from environmental factors that interact with genetics to increase the risk of the disease ¹. In addition, evidence has shown that individuals with autism spectrum disorders (ASD) exhibit gastrointestinal (GI) symptoms, including abdominal pain, diarrhea, bloating, and metabolic disorders are frequently described in infants with (ASD)². There seems to be strong correlation between the GI symptoms of ASD patients and severity of their disease³,⁴.

Various studies have demonstrated an increase in Clostridiaceae, Bacteroidetes, Proteobacteria and Akkermansia muciniphila (phylum Verrucomicrobia) and a decrease in Faecalibacterium and Bifidobacterium (phylum Actinobacteria) in (ASD) patients when compared to healthy controls (HC)²,³,⁵,⁶.

The purpose of this presentation is to contrast the microbiota differences in patients with ASD compared with healthy controls [HC] and to understand how this dysbiosis plays a role in contributing to the disease process.

The aim of this presentation is to shed light on microbiota biomarkers which may serve as early screening tools for Autism.

Pilot Study Utilizing MRI “Single Shot” 3D TGSE PASL (Arterial Spin Labeling) Differentiating Clearance Rates of Labeled Protons in Patients with Early Alzheimer Disease from Normal Subjects

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Objective: Determining feasibility of 3D-TGSE-PASL MRI with long inversion times to estimate CNS perfusion clearance, comparing normals to Alzheimer disease patients.

Methods: This pilot study used 3D–TGSE-PASL MRI with long TI’s to estimate signal clearance of labeled blood/ultra-filtrate (CSF) from brain. Signal averages of 7 inversion times (TI) from 6 regions of the brain in 18 normal subjects’ ages 18-70 before and after exercise were studied. Arterial pulse corrected signal average per TI versus TI was plotted. The slope (linear regression) indicated clearance rate. Three subjects with mild Alzheimer disease (AD) were studied pre-exercise only.

Results: In normals, signal decay rate variance among brain regions, age groups and post-exercise failed to demonstrate statistical significance except in middle age group pre to post exercise dominant temporal lobe. We found highly statistically significant reduced signal clearance rate in the AD group.

Discussion: Signal decay in normal age groups correlates with decay of $T_1^{blood}$, thus CSF paravascular flow egress and is inseparable from venous outflow. The AD group correlates with decay rate $T_1^{CSF}$, indicating a proportion of labeled blood ultra-filtered within the brain (paravascular fluid), is retained. This provides indirect evidence of reduced paravascular clearance in AD. Further development may produce an efficient biomarker identifying neurodegenerative diseases, and future treatment efficacy.
Rapid Clinical Screening Test for Dementia

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Objective: Given the need for time efficiency in clinical medicine, a validated simple rapid screen for dementia would be advantageous for bedside use identifying patients requiring future more in-depth evaluation.

Methods: Our study used timed categorical recall of US cities by 100 clinic subjects between the ages of 45-85 recording the number named in 1 minute after 1 minute to mentally prepare. The number of cities named was tallied along with whether any apparent method of recall was used or not. To avoid performance anxiety, response duration was not revealed to the subjects. This exercise was followed by administration of the Folstein mini-mental status exam (MMSE). All subjects were queried for maximal level of education, US travel, medications and health status.

Results: We found the mean number of cities named among 93 subjects was 16.88 with a SD of 8.989, and 7 subjects named a mean of 5 with standard deviation of 2.582. Three independent variables were identified, using a naming method p =.003, level of education achieved p=.004 and results of the Folstein mini-mental status exam p=.017 the sensitivity of the screen was 71% and the specificity was 92%.

Discussion: The simplicity and brief testing time (2 minutes) make this screen ideal for clinical use. The method interrogates the frontal cortex (method planning), temporal lobe (storage and recall), and the thalamic accelerator process (accelerating verbal and recall function, along with the speech pathways). Clinical adoption of this test for general screen is validated given the correlation with the Folstein MMSE.
Patient Rationale Behind the Refusal of Hospital Transportation

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Up to 25% of out-of-hospital calls results in patient refusal of hospital transportation [1, 2]. Consequences of such decision can lead to poor prognosis, as 72.6% of these patients refusing hospital transportation later seek care in an emergency department while 39% end up in an intensive care unit with worsened conditions [3-5]. Multiple studies have attempted to uncover the reasons underlying a patient’s refusal of medical aid and to be transported to the hospital after an Emergency Medical Team (EMT) has been mobilized on site. Patient demographic, socioeconomic status, education status, marital status, previous healthcare experiences, and EMT team dynamic were identified as recurrent factors behind patients refusing to be transported to the hospital. However, the methods used to identify these factors were vastly based on retrospective database searches and telephone follow ups. These methods have lots of biases and do not necessarily reflect actual patients’ rationale behind their refusal to be transported to the hospital. This study takes a different approach by attempting to directly and prospectively characterize factors potentially leading to patients refusing transportation to the hospital in Lynchburg, Bedford, and the Counties of Amherst, Appomattox, Bedford, and Campbell areas. Collecting input directly from patients can help improve understanding of subtle and often unspoken barriers to healthcare. This project aims to improve healthcare in this region, benefiting patients, EMS, and potentially improving outcomes. Additionally, potential new programs or approaches may be suggested to fill in identified gaps in patient education/perceptions and/or alternative delivery of pre-hospital care.

References

Influence of Aerobic Training Dose during Pregnancy on Infant Heart Function

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The leading cause of death globally has consistently been cardiovascular disease, however exercise during pregnancy may offer a form of cardioprotection. Maternal exercise is safe and beneficial for pregnant women and their children. Previous research demonstrated lower fetal heart rate (HR) associated with duration of maternal exercise during pregnancy, though this was self-reported. The effects of supervised exercise during pregnancy has not been assessed in infants. This study will determine if heart function of one-month old infants is improved with supervised maternal aerobic exercise, and if there is a dose-response with these outcomes. We hypothesize that one-month infants of aerobic exercisers during pregnancy will have increased heart function compared to same age infants of controls, and that greater differences will be seen with increased exercise. Pregnant participants were randomized into either aerobic exercise or non-exercise control group. Aerobic exercisers completed moderate intensity exercise for 50 minutes, tri-weekly from 16 weeks gestation until delivery; control participants received no intervention. At one-month postpartum, a blinded echocardiographic recording was performed on all infants. A blinded pediatric cardiologist analyzed recordings and infant activity state (active, quiet). Training data was grouped into exercisers, active or control based on maternal exercise attendance and compliance. Data was analyzed using regression analyses, ANOVA, and post-hoc Tukey tests, with an alpha level set a priori at \( p<0.05 \). SPSS was used for the analyses. Findings show significantly different one-month infant HR between groups (\( p = .02 \)), with the lowest infant HR in those exposed to maternal aerobic exercise. Increased number of aerobic exercise sessions during pregnancy is associated with lower one-month infant HR (\( p = .047 \)). Trends of increased cardiac output, stroke volume, ejection fraction, and cardiac index are seen in infants from aerobic exercisers compared to the other groups. Findings suggest that aerobic exercise during pregnancy at recommended levels is beneficial for infant heart function, this finding is dose-related. This study is important in encouraging women to exercise during pregnancy at recommended levels, and that any level of exercise will be beneficial for infant heart outcomes.
The Tale of Tail Bone Pain: A Case Study of Sacral Chordoma

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A 78-year-old Caucasian male with a medical history of diabetes and hypertension presented to the office with a chief complaint of “tail bone pain”. This pain began approximately one year prior to the visit when he was retrieving a coy pond filter from the creek, slid down the bank and landed on his bottom. The following day while riding his lawn mower he noticed more pain in his “tail bone” region and thus sought help from urgent care where he received a pelvic X-ray which showed no tailbone fracture. He received a steroid injection into the right sacroiliac joint in order to relieve the pain. This provided some pain relief for a month. Throughout the course of the year, he had received six steroid injections into his sacroiliac joint between urgent care and his primary care provider (PCP). His PCP ordered a computed tomography (CT) scan one year after the onset of symptoms. When he presented to outpatient surgery, the patient complained that the pain was worse when sitting and at nighttime. He also admitted to a 7-pound weight loss over the past two months without change in diet or appetite. He denied symptoms of bladder incontinence, urinary retention, bowel incontinence, sharp stabbing pains in the lower extremities, night sweats or anorexia. On exam he was a well-developed male, alert and oriented. His neurologic exam was unremarkable with an intact motor and sensory exam and no symptoms of cauda equina syndrome. He was tender to palpation over the lower sacrum and coccyx and some boggy soft mass was palpated.
A Case of Meige Syndrome

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Meige Syndrome, also known as blepharospasm-oromandibular dystonia, is a neurological movement disorder that involves the involuntary muscle contractions of the eyes, mouth, tongue, and jaw. It is often associated with other disorders such as Parkinson’s Disease. The author describes a case of an 87-year-old man with Meige Syndrome who was successfully treated with oral baclofen.
Chronic Kidney Disease: Detection and Evaluation

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Chronic kidney disease (CKD) is a prevalent disease that continues to affect more than one-tenth of the American population. Early detection is essential to slow the natural progression of CKD. This can be accomplished by urine and blood screening tests, which are analyzed for creatinine, urine albumin, and urine protein. Screening is often indicated for individuals with known comorbidities such as cardiovascular disease, mineral and bone disorders, and diabetes. Asymptomatic patients with early renal disease can make detection problematic, requiring clinicians to recognize risk factors that may warrant further testing. When symptoms do appear, the renal manifestations are often broad, including changes in kidney size, electrolyte abnormalities, and proteinuria. Changes in biomarkers may be evaluated in the early stages of CKD before significant kidney damage. The current, most accurate determination of renal function is the estimated glomerular filtration rate (GFR), which must be less than 60 mL/min to prompt further testing for CKD. Novel biomarkers may allow for earlier diagnosis of CKD as they can be detected at lower levels than standard biomarkers. Biomarkers such as homocysteine, cystatin C, and kidney injury molecule-1 are predicted to become more prevalent in a clinical setting. The current gold standard for diagnosis of CKD is a renal biopsy, but MRI is a less invasive alternative. Proper staging of CKD allows for appropriate evaluation and treatment of the patient. The early stages of CKD should be treated to limit complications and to prolong the life and health of patients.
Medical Error Recognition by Medical Students during Simulated Asystole: Teamwork and Assertiveness from Aviation

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Medical error continues to result in poor clinical outcomes. Teamwork training developed in the aeronautics profession has decreased adverse flight events and may also assist to reduce clinical errors. Following a literature review we will present results of our mixed-methods research among a sample of 21 first-year medical students without current CPR training. We provided American Heart Association training in CPR techniques to all participants, then divided students into a control group (n=10) and an intervention group (n=11). The intervention group participated in a 90-minute discussion on teamwork and error recognition modeled after crew resource management from aviation. In a videotaped scenario, both groups of students individually entered a simulated emergency room setting. A simulated nurse shortly thereafter announced asystole of a standard mannequin, asked the student if they knew CPR, and departed to get assistance. Following one cycle of student CPR compressions, a simulated Physician entered the room, took over compressions and directed the student to complete breaths using a bag mask ventilator. The simulated physician intentionally performed compressions slowly pending the students’ reaction. Observers counted the elapsed time (in seconds) for the subject to verbally correct the simulated Physician’s improper CPR technique. Forty-two percent of intervention group participants spoke up within a critical 10 second time period, compared to 30% of control group participants. Intervention group response time (in seconds) was lower as compared to the control group (9.56 +/-2.47, 15.86 +/- 11.19, p=0.11). During audiotaped debriefings respondents from both groups commented on the difficulty of speaking up while working with an unfamiliar senior supervising physician. We employed mind mapping and nVivo analysis to review qualitative data and yield rich thematic clusters increasing the body of literature for improving team behavior. Based on our findings, we believe team training in medical education has promise to reduce patient harm.
Toward a Better Understanding of Non-Academic Factors in Academic Success

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It is a tremendous challenge for students to adjust to the academic rigors of the pre-clerkship years of medical school. Although students matriculating to medical school have faced academic challenges previously, very few of them have faced them to the extent and in the environment as they do in medical school. Their ability to assimilate large amounts of information presented in a short time frame, which is assessed by their performance on high-stakes exams, is important. Other factors, however, such as mental health and time management, play a much more integral role in medical school success than in other academic settings. Multiple studies have reported that gender, age, emotional intelligence, confidence, punctuality, and exercise also affect academic performance in medical school.

The goal of this research project is to understand some of the many non-academic factors in Liberty University College of Osteopathic Medicine (LUCOM) students that contribute to both personal and professional success in their first year of medical school. We attempted to identify attitudes and behaviors that are perceived to play a role in academic success. We conducted two web-based surveys and personal interviews with student groups of varying sizes. All students who participated in the study began the academic year as first-year students in the LUCOM class of 2022.

We identified a number of factors that seem to affect the success of medical students at LUCOM. Not surprisingly, establishing learning methods and earning good grades were identified as important. However, survey results indicate that non-academic factors such as handling stress and emotions, establishing life priorities, getting adequate sleep, and exercising regularly were factors in academic success. Specifically, grades are deleteriously affected when these “non-academic” elements are not properly addressed.

Based on the results of our surveys, it seems that LUCOM students would benefit from being given practical, concrete, jargon-free steps that would help them learn how to succeed in all facets of life during their first year in medical school. The old adage, “Practice makes perfect” holds true. All experiences in medical school are new, and students need to be taught both in and out of the classroom. Future directions in this project include the following: to use input from a wide variety of students to provide practical advice for new students to use as a guide in establishing solid learning methods, healthy sleep patterns, exercise habits, and constructive methods of handling emotions; and to determine if incorporating these steps translates into improved academic performance.
Applying Functional Medicine in Clinical Practice for Medical Students

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Functional Medicine is a holistic model of healthcare being used in a collaboration between the Cleveland Clinic and physicians seeking to treat the underlying cause of disease. There is a growing interest among medical students in novel methods of improving their patient’s quality of life. The proposed course would address a desire of medical students for evidence-based discussion of lifestyle and dietary protocols. These discussions are best begun at the medical student level, especially for osteopathic medical students who are trained to see the whole patient as a self-healing unit of body, mind, and spirit. Researchers contacted the Institute for Functional Medicine (IFM) and proposed a course in functional medicine to be added between the first and second year, optimally as a research elective. The course would cover all the didactic material during the first week via a self-guided video lecture series, and the second week would meet on the LUCOM campus for discussion facilitated by Functional Medicine practitioners in the Lynchburg and surrounding areas. This course would integrate case-based training, aiding the transition between first and second-year didactics. This model would build an increasingly collaborative relationship between rising clinicians and the IFM, have the potential to grow the number of certified and enrolled osteopathic physicians and be a conduit for dissemination of innovative tools to improve clinical practice.
Religiosity as a Protective Factor in the Psychological Well-being of Medical Students in the Pre-clinical Years

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Objective: Psychological well-being is a vital aspect of an individual’s overall health because of how it impacts one’s thoughts, emotions, and behavior. Maintaining positive emotions and mental health practices are important for all individuals, but especially critical for those responsible for the care and support of others. In order for physicians to provide optimal care for their patients, it is believed that they must foster psychological well-being. Research findings indicate that the cumulative effect of medical school stress and mental health influence psychological well-being. Medical students in the United States report that after matriculating into medical school, well-being decreases overtime with moderate to severe depression and burnout reported at higher rates than other graduate students or population samples. Religiosity has been determined to have a significant relationship with mental health in other populations; however, few studies have demonstrated its impact on the mental health of medical students. This study looks to better understand the role religiosity/spirituality may play as a protective factor for psychological well-being in medical students.

Participants: Participants include OMS-1s at Liberty University College of Osteopathic Medicine who will graduate from medical school in 2023. Remediating students currently in this class were excluded from the study to eliminate any possible bias that their first year in medical school may have had on their survey responses.

Methods: A Qualtrics-based survey was administered to students prior to the start of their first year at Liberty University College of Osteopathic Medicine. This survey included the Ryff Psychological Well-Being Scale: 42 Item Instrument, the Duke University Religiosity Index (DUREL), and demographic questions including age, biological sex, marital status, education prior to medical school, race/ethnicity, current living status, religious affiliation, and current financial debt. The survey will be re-administered at the conclusion of the OMS-1 student’s first semester of medical school, the conclusion of their second semester of school, prior to the beginning of their first semester of their second year of school, at the conclusion of their first semester of their second year of school, and at the conclusion of their second semester of their second year of school to assess their responses throughout their pre-clinical years. Data will be analyzed to determine the impact of religiosity on psychological well-being.

Hypothesis: Our team hypothesizes that a higher religiosity, as measured by the DUREL index, is a protective factor in the psychological well-being of medical students in their pre-clinical years. Data collection from this study will not be completed until spring 2021.