LIBERTY UNIVERSITY

COLLEGE *of* OSTEOPATHIC MEDICINE

RESEARCH DAY

January 13, 2023

Program & Abstracts

Welcome!

It is my pleasure to welcome you to the fourth annual Liberty University College of Osteopathic Medicine (LUCOM) Research Day event! We are excited to celebrate the past year's accomplishments in research and share in scientific fellowship. Given that we just ended the Christmas season, it is truly a pleasure to start the year with wonderful colleagues seeking to understand God's creation and how to use that knowledge to alleviate pain and suffering in our world.

This celebration of research would not be possible without the contributions of numerous faculty, staff, and students. Particularly, I would like to thank the senior leadership of LUCOM who provided guidance and advice in the planning of this event, the IT staff who provided invaluable logistical support and contingency planning, and the faculty judges who will evaluate the presentations for awards. Last but certainly not least, I would like to thank Barb Lutz and Robbe Sisson for their assistance throughout the Fall in planning this year's event and for keeping me on track while organizing this meeting. Thank you all for your support, it is greatly appreciated!

In closing, may you all enjoy interacting with the many talented students who have worked so hard to prepare these presentations and share their research. I hope that new collaborations are fostered because of the connections you make with other participants. In closing, let's have a wonderful meeting as we learn something new!

Sincerely,

Michael S. Price, Ph.D.

Professor of Microbiology

Chair, LUCOM Research Day Committee

Program Overview

12:00 PM	**Boxed lunch**	**Pick up in 1st floor lobby**
12:30 PM	**Opening Prayer**	**Joseph R. Johnson, DO, FACOOG (Dist)** **Dean, College of Osteopathic Medicine**
12:35 PM	**Welcome & Opening Remarks**	**Michael S. Price, PhD** **Research Day Committee Chair**
	Oral Presentations*	
12:40 PM	**P01:** The Impact of Depression Upon Adults Living with Primary Immunodeficiency	Speaker: Harold Shin Mentor: Nicholas Rider, DO
1:00 PM	**P02:** A Nutritional Community Intervention for Low-Income, Low-Access Areas in Lynchburg, Virginia	Speaker: Anthony Ursua (OMS-II) Mentor: Olubukola Ojuola, MD
1:15 PM	**P03:** A rare case of EBV-related systemic T-cell lymphoma of childhood and considerations for medicolegal death investigation	Speaker: Jiayi Li (OMS-II) Mentor: Erin Brooks, MD
1:30 PM	**P04:** Adipose-Derived, HER2/neu Tumor-Targeted, Human Mast Cells have Antitumor Effects in vivo after Intravenous Injection	Speaker: Caelin Smith (OMS-II) Mentor: Christopher Kepley, PhD
1:45 PM	**P05:** Cadaveric Patent Foramen Ovale Correlated with Fossa Ovalis Area and Cardiovascular Comorbidities	Speakers: Katelynn Clark (OMS-II), Shannon Fehr (OMS-II), Eun-Mae Grace Lee (OMS-II), Natenael Nesibu (OMS-II) Mentor: Robert James Swanson, PhD
2:00 PM	**P06:** Integrating Cultural Sensitivity into a Skills-Based Approach of Delivering Bad News	Speakers: Nancy Chen (OMS-II) Swarnima Das (OMS-II) Mentor: Linda Mintle, PhD
2:15 PM	**BREAK**	
2:30 PM	**P07:** Interrelationship Between Diet and Exercise, Endoplasmic Reticulum (ER) Stress, and Metabolic Dysfunction: What Causes What?	Speaker: McClain Vail (OMS-II) Mentor: Joseph Gigliotti, PhD
2:45 PM	**P08**: Neonatal Opioid Withdrawal Syndrome (NOWS): Prenatal Morphine Exposure Dose-Dependently Changes Complex Ultrasonic Vocalization (USV) Characteristics in Novel Preclinical Model of NOWS	Speaker: Daniel Nguyen (OMS-II) Mentor: Shekher Mohan, PhD
3:00 PM	**P09:** Serum IGG Causes Colonic Submucosal Venule and Arteriole Construction	Speaker: Jonathan C. Kugler Mentor: Anthony J.M. Bauer, PhD
3:15 PM	**P10:** Societal Implications of Institutional Burnout Mitigation in Medical Students	Speaker: Jessica Scheve (OMS-II), Barbara Lutz, MA, CEAC Mentors: Scott Severance, PhD Stacey Lilley, PhD
3:30 PM	**P11:** The Management of Anxiety and Depression in Pediatrics	Speakers: Emma Brown, (OMS-II) Megan Pizzo (OMS-II) Jordan Delashmitt (OMS-II) Mentor: Raena M. Pettit, DO
3:45 – 5:00PM	**Poster Presentations**	**CMHS Main Street & Lower Lobby**

***Oral presentations WebEx link:**

Join from the meeting link

https://libertyu.webex.com/libertyu/j.php?MTID=m306ef8afe88c1afb8708e89ed2a9b90e

Join by meeting number

Meeting number (access code): 2633 188 3581

Meeting password: 94V3vJqmnwA

Oral Abstracts

P01: The Impact of Depression Upon Adults Living with Primary Immunodeficiency

Category: Clinical Research

Harold Shin (OMS-III)[1], E. Shea Brunner, MD[2], Tiffany Henderson, PhD[3], Joud Hajjar, MD[4], Christopher Scalchunes, MPA[5], and Nicholas Rider, DO[1]

[1]Liberty University College of Osteopathic Medicine, Lynchburg, VA
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Abstract

Purpose: Patients with primary immune deficiency/inborn errors of immunity (PI/IEI) are susceptible to increased risk of recurrent infections, immune dysregulation, and comorbid disorders. Disease burden confers lower quality of life (QoL) across several domains; however, the contributing determinants are not completely clear. Having any chronic disease, and PI/IEI for this matter, is a major risk-factor for the development of depression and decreased QoL. This study seeks to quantify how depression, anxiety, and associated medication use may contribute to impairments in social functioning among PI patients.

Methods: The present study used a questionnaire to survey 4,500 persons by the Immune Deficiency Foundation (IDF). The questionnaire incorporated the validated Social Function 12 version 2 (SF-12v2), Brief Fatigue Inventory (BFI), and the Patient Health Questionnaire 2 (PHQ-2) instruments. The SF-12v2 provides a measure of QoL and functional health by measuring physical and mental component scores (PCS and MCS respectively). For this study we utilized only the MCS. The BFI is a rapid means of assessing burden of general fatigue. Lastly, the PHQ-2 is used a general screening of depression.

Results: Out of the 4500 persons, 21% or 947 responded with usable data for analysis. Our findings revealed that patients with PI/IEI reported significantly higher ($\geq 3, \text{value range } 0-6$) PHQ-2 scores (59% vs. 9%; $\chi 2$ p-value < 0.001) compared to the general population. Patients with reported PI/IEI also had significantly more limitations, as shown by the BFI and SF12 instruments, due to emotional stress, fatigue, and concomitantly greater medication use to manage these complications.

Conclusions: These data suggests that patients with PI/IEI would benefit from routine screening for depression as well as a better clarification about drivers of this important aspect of impaired health.

P02 – A Nutritional Community Intervention for Low-Income, Low-Access Areas in Lynchburg, VA

Category: Educational Research

Anthony Ursua (OMS-II)[1], Kelley Goforth (OMS-II) and Olubukola Ojuola MD, MPH[1]

[1]Liberty University College of Osteopathic Medicine, Lynchburg, Virginia

Over the last two years, an estimated 54 million people in America faced the challenges of food insecurity, a factor directly associated with the deadliest, yet preventable, diseases in the US including hypertension, COPD, stroke, and diabetes.[i] In 2019, the USDA reported that an estimate of up to 27% of the U.S. population lived in low-income, low-access census tracts.[ii] These geographical regions, termed "food deserts", are often highly populated by convenience stores and fast-food vendors, allowing the convenience of unhealthy food options to perpetuate these chronic illnesses. The low socioeconomic status of these neighborhoods combined with many families having limited transportation to access healthy food options increases the probability of an unhealthy diet for millions.

In Lynchburg, VA, there were approximately 10,530 individuals facing food insecurity in 2019, with the nearest grocery store being over two miles away, a 30-minute ride on the City of Lynchburg's public transportation system for many.[iii] Over the past several years, the City of Lynchburg has been active in addressing the needs found within the food deserts of its community. Programs such as We Got the Beet, Live Healthy Lynchburg, and local recreational centers each address different aspects of the needs presented. Historically, We Got the Beet has partnered with the Lynchburg Community Market by hosting educational events on topics such as shopping on a budget, meal planning, and meal preparation.[iv] Additionally, Live Healthy Lynchburg presents the local resources available which promote a holistic, healthy lifestyle including personal diet, and local exercise programs in an accessible format.[v] While we must review and re-establish the current policies and programs which exist to combat food inequality, we must not forget to ask ourselves what can be done now to prevent further damage.

The goal of our project is to assist existing programs in promoting the well-being of those in the Lynchburg community by providing healthy recipes and meal preparation instruction based on low-budget items readily available within the food deserts of Lynchburg.

In the project's first phase, we will focus on creating recipes based on the federal dietary recommendations for those with diabetes mellitus. As the project progresses, we hope to expand our scope and provide recipes tailored to the dietary recommendations for other chronic illnesses such as hypertension and cardiovascular disease. For many of these individuals, pursuing a healthy diet on a low budget with limited access to healthy foods and little knowledge of food preparation is incredibly daunting. We aim to meet individuals suffering from these chronic diseases in their current realities and encourage them to seek a healthy lifestyle by taking ownership of their health in their own kitchen.

To date, we have taken an inventory of six convenience stores in the College Hill area of Lynchburg. We discovered that a healthy diet based on these resources alone would be impossible without the supplementation of protein and frozen veggies from larger local stores. This discovery led us to add instructions on how to safely freeze and store food items, such as meat products, that could be purchased on a single trip to a larger store about once a month. Our goal is to have 20 recipes by summer of 2023 with preparation instructions to begin empowering the members of our community towards healthy living, especially in a food desert.

[1] Balch, Bridget. *54 million people in America face food insecurity during the pandemic. It could have dire consequences for their health*. AAMC, Health Care, October 2020. https://www.aamc.org/news-insights/54-million-people-america-face-food-insecurity-during-pandemic-it-could-have-dire-consequences-their

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[1] https://www.livehealthylynchburg.com/

P03 – A rare case of EBV-related systemic T-cell lymphoma of childhood and considerations for medicolegal death investigation

Category: Case Report

Jiayi Li (OMS-II)[1] and Erin Brooks, MD[2]

[1]Liberty University College of Osteopathic Medicine, Lynchburg, Virginia
[2]Department of Pathology and Laboratory Medicine, University of Wisconsin-Madison, Madison, WI

A ten-year old male was found unresponsive at home. Despite resuscitation efforts, he was pronounced dead, and an autopsy was performed at University of Wisconsin Hospital and Clinics to determine the cause of death. Past medical history reveals past traumatic brain injury at four months of age which resulted in quadriplegia and recurrent seizures. Social history includes physical abuse by mother's boyfriend. Remarkable gross findings at autopsy include diffuse lymphadenopathy, ischemic cerebral infarcts, and cerebellar atrophy. Histology reveals positive CD3, CD20and EBER staining lymphocytic infiltrates in sections of lung, liver and spleen. Bone marrow is stained with CD163to highlight abundant histiocytes showing hemophagocytic histiocytosis (HLH), almost always associated with systemic EBV-positive T-cell lymphoma. The manner of death was determined as natural.

This case raises the importance of thorough autopsy and histology in medicolegal death investigation given the unusual circumstances and potential challenges in determining the cause and manner of death. Delayed homicide is defined as death due to remote injury inflicted by "hands of another". In this case, the same gross findings could fit the picture of aspiration pneumonia as the immediate cause of death which would in turn changed the manner of death to delayed homicide with entirely different legal consequences for the perpetrator. This is a reminder for all clinicians who practice evidence-based medicine to remain truthful to the physical findings despite the pressure from families of the deceased, law enforcement or society.

P04 - Adipose-Derived, HER2/*neu* Tumor-Targeted, Human Mast Cells have Antitumor Effects in *vivo* after Intravenous Injection

Category: Basic Science Research

Caelin Smith (OMS-II) [1], Mason Conine (OMS-II)[1], Rebecca Praetzel (OMS-II[1]), Benjamin James (OMS-II) [1], Jennelle Norem (OMS-II) [1], Samantha Vicenio (OMS-II) [1], Daniel Courter (OMS-II) [1], and Christopher L. Kepley, PhD[1]

[1]Liberty University College of Osteopathic Medicine, Lynchburg, Virginia

Abstract:

The use of one's own cells to treat tumors is typified by chimeric antigen receptor T cells (CAR T) therapy. The list of autologous immune cells with anti-tumor properties being investigated continues to grow. We have previously proposed a new strategy using tumor-targeted mast cells (MC) obtained from autologous sources and demonstrated proof-of concept previously *in vitro* and *in vivo*. We sought to exploit the anti-tumor mediators in MC granules to selectively target them to tumor cells using tumor specific immunoglobin E (IgE) and controllably trigger release of anti-tumor mediators upon tumor cell engagement. We used a human HER2/*neu*-specific IgE to arm human MCs through the high affinity IgE receptor (FceRI). The ability of intravenously (i.v.) injected HER2/*neu*-targeted MCs to effect HER2/*neu*-positive human tumors was assessed using a immunocompromised xenograft mouse model. It is shown for the first time that tumor targeted MC injected i.v. home to and shrink tumors. These studies provide further proof of concept that MC have anti-tumor properties and could possibly provide another strategy for developing adoptive cell transfer therapeutics for patients.

P05: Cadaveric Patent Foramen Ovale Correlated with Fossa Ovalis Area and Cardiovascular Comorbidities

Category: Basic Science Research

Katelynn Clark (OMS-II)[1], Shannon Fehr (OMS-II)[1], Eun-Mae Grace Lee (OMS-II)[1], Natenael Nesibu (OMS-II)[1], and R. James Swanson, PhD[1]

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PFO Abstract
Introduction: Foramen ovale, formed by the overlap of septum primum of the left atrium and septum secundum of the right atrium, is an interatrial septal structure essential for fetal circulation. Most fetal blood flow enters the right atrium and flows through the foramen ovale to the left atrium, bypassing the lungs [2]. Typically, within the first two years of life, septum secundum and septum primum fuse together to form an oval-like structure of the right atrium known as fossa ovalis (FO). Failure of septal closure results in a patent foramen ovale (PFO) which occurs in approximately 25% of the population [2]. Previous studies have associated the presence of PFO with multiple pathologies, including cryptogenic stroke, migraine headaches, platypnea-orthodeoxia syndrome and embolism [1]. Given the clinical significance of pathology related to PFOs, associations between PFO size and FO size as well as sex differences with cardiovascular comorbidities. Materials and Methods: Forty-nine human cadaver hearts were obtained from Liberty Universities' anatomy labs from 2017-2021. Measurements of length and width of the FO were taken, and length of the PFO was measured from both the right and left atrial septum using a Helios analog micrometer. Length was measured as the longest distance across the FO and width was measured as the distance perpendicular to the length. Cadaver age, sex, cause of death, and comorbidities were collected. Cardiovascular disease was defined based on comorbidities of stroke, hypertension, congestive heart failure, coronary artery disease, CABG, AFib, and CADASIL. Statistical analysis was completed using JASP and Python software. Objective: We aim to address the correlation between presence of a PFO with area of FO and cardiovascular comorbidities. Results: The area of the FO was significantly larger in hearts with PFOs than those without PFOs ($p=0.0109$). No statistically significant difference arose between presence of a PFO and sex ($\chi2$ (df=1, N=55) = 0.009, p = 0.925) or cardiovascular comorbidities ($\chi2$ (df=1, N=55) = 0.106, p= 0.745). However, significantly more male cadavers with a PFO had cardiovascular comorbidities compared to female cadavers with a PFO ($\chi2$(df=1, N=18) = 4.000, p = 0.046). Conclusion: The results show that increased fossa ovalis area positively relates to increased incidence of a patent foramen ovale in cadavers at Liberty. In addition, a higher percentage of males with PFOs had cardiovascular comorbidities than females with PFOs. These findings may provide clinical direction in patients presenting with PFO or related clinically relevant symptoms.

P06: Integrating Cultural Sensitivity into a Skills-Based Approach of Delivering Bad News

Category: Educational Research

Nancy Chen (OMS-II)[1], Swarnima Das (OMS-II)[1], and Linda S. Mintle, PhD[1]

[1]Liberty University College of Osteopathic Medicine, Lynchburg, Virginia

Abstract

Introduction:

According to the AAMC, "Academic medicine has a responsibility to prepare physicians who are culturally responsive, provide care that is equitable, and confront factors that drive racism, hate, and bias in health care." To accomplish this goal requires strategic integration of DEI into the formal curricula and the clinical learning environment. Medical schools have struggled to achieve this goal given the "crowded curriculum." However, because of the diversity of patients seen in clinical practice, DEI incorporation is needed. Thus, a teaching intervention was developed to better integrate DEI into the learning and assessment of teaching the clinical skill of delivering bad news to patients. A goal of this preliminary study was to develop an instructional model for incorporating DEI into existing curricula.

Method: A curriculum review was conducted in order to identify where in the existing curriculum cultural sensitivity could be integrated in order to better prepare students for serving diverse patient populations. A skills-building lecture was selected for revision. Cultural sensitivity learning materials were added along with videos demonstrating the skill with diverse populations. Student feedback was sought via TOP HAT at the end of the session as a preliminary step to developing an assessment of student learning and cultural sensitivity.

Results: The ethnography approach of Kleinman with the SPIKES protocol for delivering bad news was incorporated into a learning activity. A 50-minute OMS 2 lecture on delivering bad news was converted to a case application in order to provide new materials and learning on cultural sensitivity. Prior to the delivery of the content, students were given learning materials via a DIAL which included a PPT and an article on cultural sensitivity that combined the ethnography approach of Kleinman with the SPIKES protocol as a self-learning module to prepare for the case application. The in-class learning portion was comprised of videos in a case application format with interactive questions and active participation. Student feedback to be included.

Conclusion: Revising learning activities to integrate DEI into existing curricula is one method to boost cultural sensitivity and provide a model to assess already existing curricula and determine appropriate learning activities conducive to DEI integration. This was done by intentionally addressing cultural sensitivity in the teaching of the clinical skill of delivering bad news to patients. The change in teaching activity will be assessed in a future study to determine if the integration of materials to already existing activities increases cultural sensitivity.

References

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P07: Interrelationship Between Diet and Exercise, Endoplasmic Reticulum (ER) Stress, and Metabolic Dysfunction: What Causes What?

Category: Basic Science Research

McClain Vail (OMS-II)[1] and Joseph Gigliotti, PhD[1]

[1]Liberty University College of Osteopathic Medicine, Lynchburg, Virginia

Background
The relationship between diet, exercise, and metabolic dysfunction is widely established; however, the mechanisms responsible remain unclear. ER stress has recently been shown to play a significant role in homeostatic and disease processes and has been implicated in mediating the beneficial effects of diet and exercise. Given the interrelationship between diet, exercise, ER stress, and metabolic dysfunction, this project sought to examine how diet and exercise may interact to influence metabolic markers and whether ER stress may be involved.

Methods
30 male, weanling C57 Bl/6 mice were purchased from a commercial vendor and acclimated to a solid diet for one week. Mice were then randomly assigned to receive *ad libitum* access to one of three diets (n=10): standard chow, American Institute of Nutrition 93 (AIN93) formulation, and our in-house "Americanized" diet (AD). Within each diet group, mice were then assigned to an exercise (n=7) or control experience. The exercise condition consisted of three nights per week on an exercise wheel. Mice were exposed to the wheel for approximately 16 hours per night and level of utilization (distance traveled and exercise duration) was recorded using a bicycle computer/sensor. Mice were fasted for 6 hours, euthanized, and blood and liver tissues were collected. Serum was collected for quantification of circulating glucose and lipid levels, and liver was processed for histological evaluation (hematoxylin and eosin (H&E)), liver lipid content, and mRNA expression of genes related to ER stress by RT-PCR. ER stress was gauged by examining expression levels of prominent ER stress markers: Ddit3, Hspa5, and Erdj4. Data analysis was conducted using General Linear Model Procedures in SPSS**.**

Results
Despite having no influence on body weight, diet (P=0.04) significantly influenced liver weight, with mice fed AD having greater (P = 0.01) liver weight compared to mice fed chow. Exercise tended to increase liver weight compared to the control (P=0.095), while reducing liver lipid content in mice fed chow and AD. AD fed mice had the highest circulating glucose values, significantly greater than mice fed chow (P=0.013) and also tended to be greater than mice fed AIN (P=0.056). Exercising mice had significantly increased circulating triglycerides (P=0.03) and tended to have reduced circulating glucose levels (P=0.07). Diet significantly influenced insulin sensitivity (P = 0.003), with mice fed AD having less insulin sensitivity as compared to mice fed chow (P<0.001) and also tended to be lower than mice fed AIN (P=0.06). While non-significant (P=0.15), exercising mice tended to have less insulin sensitivity than controls. Exercise caused an increase in Erdj4 mRNA expression (P=0.02), especially so in mice fed AD, which had a greater ER-stress response with exercise than other diet groups (P=0.04).

Conclusion
Our results highlight a significant effect of diet and exercise on markers of metabolic health, both independently and dependently. Furthermore, our data suggest that ER stress does differ between the groups and may be responsible for some of the observed phenotypes. Further studies must be conducted to reevaluate these relationships and more broadly, to ask the question of whether a poor diet detracts from some of the beneficial aspects of exercise.

P08: Neonatal Opioid Withdrawal Syndrome (NOWS): Prenatal Morphine Exposure Dose-Dependently Changes Complex Ultrasonic Vocalization (USV) Characteristics in Novel Preclinical Model of NOWS

Category: Clinical Research

Daniel Nguyen (OMS-II)[1], Alexander Nguyen (OMS-III)[1], Shekher Mohan, PhD[1], and Sarah Stevens, PhD[1]

[1]Liberty University College of Osteopathic Medicine, Lynchburg, Virginia

Introduction
As the opioid epidemic continues to be prevalent in populations, opioid use in pregnant women is also on the rise. This has led to an increase in babies born with neonatal opioid withdrawal syndrome (NOWS). Short term effects of prenatal exposure can be assessed using a novel withdrawal behavior rodent model. Since babies with NOWS cry at a higher pitch, we analyzed ultrasonic vocalizations (USVs), a novel method to assess withdrawal from morphine in spiny mice pups. USVs fall within the frequency of 20-70kHz, above the human hearing range. Like human infants, the cries of mice can denote different moods (i.e., anxiety, depression, aggression) as well as behaviors (i.e., mating calls and play). The characterization of these calls by wave morphology in the context of NOWS in spiny mice may lead to the translation through the improved understanding of atypical behaviors in human babies with NOWS.

Methods
Treatments: Our study included 23 mice total. Fifteen mice were morphine-exposed NOWS mice; 10 mg/kg N=6 (3 males and 3 females), and 30 mg/kg N=9 (5 males and 4 females). Eight mice were saline-exposed (control). USVs from each pup were recorded at the same time each day from inside a sound chamber for approximately 2 min/day using an Echo Meter from postnatal day 0 to 7.

Recorded USVs were processed in the open-source software DeepSqueak, a MATLAB USV detection program that uses machine-learning, and region based convolutional neural networks (R-CNN) to filter out sounds not produced by mouse calls and decode “mouse chatter.” Manual entry and scoring for the spiny mouse calls was done and integrated into the machine-learning algorithm to fine-tune the system for detecting calls specific to a spiny mouse. The neural detection network was then run against the manual scores to assess for accuracy. All calls were then quantified and characterized by wave morphology, duration, and frequency.

Results
Mice treated with morphine 10 and 30 mg/kg experienced calls with higher frequency on P0-P4 in the early days post-birth compared to the control saline mice. The duration of calls was shorter in mice exposed to both doses of morphine on P0-P4 compared to the control mice. In the morphine 10 mg/kg group, males exhibited fewer calls than females on P0. From P1 onward, males exhibited more calls. In the morphine 30 mg/kg group, females exhibited more calls on P1-P2 and P4. Males exhibited more calls than females on P0, P3, and P5-P6. While there are variations in terms of which early vs late post-birth number of calls, both male and female morphine treatment groups (whether they were 10 mg/kg or 30 mg/kg) demonstrated an increased number of calls than the control saline mice in the immediate early post-birth period P0-P2. In comparing call complexities mice exposed to morphine had a much greater number of downward calls compared to the saline-exposed mice which exhibited a much greater number of composite and harmonic calls.

Conclusions
Mice exposed to both doses of morphine generally experienced higher call frequency, shorter call duration, and fewer number of calls compared to the controls group. As downward calls tend to signify low moods, i.e., anxiety, mice with NOWS tend to exhibit negative moods that may be related to opioid withdrawal.

P09: Serum IGG Causes Colonic Submucosal Venule and Arteriole Constriction

Category: Basic Science Research

Jonathan C. Kugler (OMS-II)[1], Ethan D. Rich (OMS-II)[1], Christina B. Bagnati (OMS-III)[1], Justin Go (OMS-II)[1], Connor A. Schroeder (OMS-III)[1], Jason Chung (OMS-II)[1], and Anthony J.M. Bauer, PhD[1]

[1]Liberty University College of Osteopathic Medicine, Lynchburg, Virginia

Objective: The objective of the present study was to investigate the acute effect of serum on the colonic microvasculature.

Methods: A novel whole-mount of the colonic submucosal vascular plexus was constructed. C57Bl/6 mice were euthanized, and the colon was removed through a surgical abdominal laparotomy. The colon was submerged in iced oxygenated Krebs solution, cut open along the mesenteric border, and pinned mucosal side up in a Sylgard dissecting dish. The mucosa was then selectively removed by sharp dissection and discarded. Next, the submucosal colonic microvascular plexus embedded in a connective tissue matrix was teased from the underlying muscularis externa as an intact stromal whole-mount sheet. The whole-mount vascular plexus was then transferred to a designed 3-D printed organ chamber and imaged using a confocal microscope, which was perfused and maintained with oxygenated Krebs solution at 37°C. The vascular plexus was exposed to various substances to assess the contractile responses of the colonic submucosal venules.

Results: Perfusion of increasing concentrations of the α-adrenergic agonist norepinephrine (0.1 – 10 μM) caused a dose-dependent increase in venule contractions, as measured by a % change in vessel lumen area (0.1μM = 95.7±3.28, 0.3 μM = 86.9±2.02, 1.0 μM = 74.1±5.73, 3.0 μM = 64.8±3.36, and 10.0 μ = 43.4±5.19). Epinephrine produced a similar concentration-contractile dose response curve. Interestingly, exposure of the venules to human serum also causes a dose-dependent contractile decrease in vessel lumen area (0.5% = 86.0±4.73%, 1.0% = 52.4±7.83%, 2.5% = 47.8±6.78%, and 5% = 35.5±4.76%. Preliminary data indicates that the serum induced contractile response is caused by serum immunoglobulin G.

Discussion: Increased vascular permeability in IBD results in vascular leak of serum proteins into the interstitial space of the gut wall. Our findings demonstrate that serum proteins in the gut wall cause a contracture of the colonic microvasculature. We hypothesize that the serum induced decrease in vascular perfusion would result in mucosal hypoxia and mucosal barrier failure, which would exacerbate the IBD inflammatory response to the intestinal microbiome. Preventing the vasoconstriction triggered by vascular leak may provide an additional therapeutic intervention for inflammatory bowel disease patients.

P10: Societal Implications of Institutional Burnout Mitigation in Medical Students

Category: Clinical Research

Jessica Scheve (OMS-II)[1], Barbara Lutz, MA, CEAC[1], Stacey Lilley, PhD[2], and Scott M. Severance, PhD[1]

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[2]Liberty University, Lynchburg, Virginia

Background

It is well known that medical students and physicians face many stresses, both emotional and physical, on a daily basis. This chronically demanding lifestyle often leads to increased levels of burnout with rates in medical school students, residents and physicians often reported to be as high as 50%[1]. The AMA estimates physician burnout costs the health care system approximately $5 billion a year[2]. Venture2Lead with Holistic Equine Assisted Resilience Training (HEART) was designed to provide practical, effective tools for acquiring necessary skills to increase heart rate variability, improve well-being, build resilience, and reduce burnout in students and professionals in the healthcare field.

Methods

The study utilized a mixed methods design of both qualitative and quantitative data consisting of a participant group that completed the Venture2Lead with HEART program and a control group that did not receive the training. Participants partnered with six different horses and two miniature horses over the course of five weeks. All horse-partnered activities were conducted from the ground and supervised by an equine specialist to ensure the welfare of participants and horses at all times. Heart rate variability (HRV) of the participants was collected pre and post training as a quantitative measure of skill acquisition. The Mayo Clinic Well-Being index for medical students and the RS-14 questionnaire were also completed by both the experimental and control groups at the beginning of the training, at the end of the five weeks of training, three months after the training, and six months following training as a quantitative measure of burnout. Qualitative measures were assessed through participant-logged journal entries as well as interviews within two weeks of training completion.

Result

Statistically significant improvement was observed in coherence scores for the participant group in the 3-minute HRV readings. The RS-14 measured a decrease in resilience in the control group, while the participant group showed an increase in resilience. Both groups showed improvements in well-being, with the participant group demonstrating a larger improvement. These differences were not statistically significant, however; there would need to be a total of 27 participants to show statistical significance, but only nine participants were enrolled in this phase of the study.

Conclusion

These findings indicate that HEART helps students acquire a proven resilience-building skill as seen by a baseline shift in coherence after completing 5 weeks of training. Additional studies are needed to quantitatively assess improvements in resilience via the RS-14. Longitudinal assessments may also be carried out to evaluate the role of HEART in not only increasing resilience in medical students but also decreasing burnout in physicians.

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P11: The Management of Anxiety and Depression in Pediatrics

Category: Systematic Review/Meta-analysis

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Anxiety and depression are among the most common psychiatric conditions affecting children and adolescents, and physicians in primary care settings often represent the first point of contact for these patients. Therefore, it is critical to provide these clinicians with an overview of current, evidence-based approaches for treating these conditions in pediatric and adolescent patients. Cognitive behavioral therapy (CBT) is an appropriate and effective first-line intervention for anxiety disorders in children and adolescents. For depressive disorders, treatment guidelines recommend either CBT or interpersonal therapy (IPT) as frontline treatment approaches. Pharmacologically, selective serotonin reuptake inhibitors (SSRIs) represent the most efficacious treatment for anxiety and depressive disorders in young persons. Combination therapies consisting of a psychotherapy plus an SSRI have produced greater therapeutic effects than either treatment alone. In particular, CBT plus sertraline is most effective in those with anxiety, whereas combining CBT or IPT with fluoxetine has been identified as the most effective treatment for depression in this population. Clinically, these combination therapies are especially useful in patients showing an insufficient response to treatment with only an SSRI or psychotherapy. A physician should also recommend lifestyle alterations to aid in the management of anxiety and depression, including diet, exercise, adequate sleep, limiting screen time, and spending time in nature. When used to complement standard treatment approaches, these interventions may provide the patient with additional symptom reduction while decreasing the return of symptoms in the long term.

Poster Abstracts

P12: What effects your superpower, Kryptonite or Kratom?

Category: Basic Science Research

Reni Gandhi (OMS-II)[1] and Shekher Mohan, PhD[1]

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Introduction:

Kratom, a forty-indole alkaloid known as an atypical opioid due to its synergistic or antagonistic pharmacological interaction has been on the rise. The use of novel opioids such as kratom has been exponentially growing within different populations, especially pregnant women, and it has become widely available. Kratom is marketed as an alternate opioid, and its use for the management of depression and anxiety has increased. Additionally, its ability to treat opioid withdrawal has increased kratom use. Collectively these factors continue to attract pregnant women and women of childbearing age to use kratom. The effects of kratom on neonates have been recorded in a handful of cases. With the continuing rise in the availability and abuse of kratom, the debate over the unregulated use of kratom, and FDA regulations remains open. A meta-analysis of published work on kratom was performed to determine the benefits and/or drawbacks of kratom.

Methods:

A non-systemic approach of keyword search on online literature databases such as PubMed and Dimensions was performed. Keywords such as “kratom”, “kratom and pregnancy”, “kratom pharmacology”, “kratom and animal models”, and “kratom and NOWS and animal models” were used.

Results:

The search word kratom on the dimensions database revealed 7170 publications. Compared to “methadone” word search on dimensions revealed 198,155 publications. Dimensions also revealed that in 2019 there was a spike in the number of publications about kratom. Before 2019 the average number of publications was between 100-200. In 2019, 4327 publications about kratom were published. “Kratom and pregnancy” revealed 10 results on PubMed. Three systematic reviews have been published regarding the outcomes of mothers and newborns to prenatal exposure to kratom. No clinical trials have been published and, in our search, only two animal models were revealed.

Conclusion:

The 2020 US National Survey of Drug Use and Health (NSDUH) revealed that 2.1 million US residents have used kratom in the past year. Additionally, the US poison control between 2011 to 2017 listed kratom as one of the four substances that had the highest rates of dangerous medical outcomes and hospitalizations. Currently, all the studies that exist are human studies, mainly observational surveys from Malaysia and Thailand. Further human and animal studies are required to understand this potential pain control alternative. Due to the wide availability of kratom and the lack of clinical research, the use of kratom by the public is dangerous because we are unaware of the consequences. These two aforementioned factors hopefully will signal researchers and clinicians to conduct further research about kratom and its effects on adults, neonates, and children. With the information gathered in this review, the goal is to apply the spiny mice model to further understand prenatal kratom use and its effects on neonates, infants, and brain development.

P13: The role of *DASH complex with microtubules* (*DCM1*) in pH adaptation and virulence of *C. neoformans*

Category: Basic Science Research

Ryker Heller (OMS-II)[1], Rebekah Satalino (OMS-II)[1], and Michael S. Price, PhD[1]

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Abstract

Fungal infections are on the rise in the modern era of medicine. *Cryptococcus neoformans* is a particularly lethal infection to individuals with compromised immune systems. One of the mechanisms that allows *C. neoformans* to be pathogenic is the ability to survive across a wide range of pH. Recently, the *C. neoformans* gene CNAG_02291 was identified as necessary survival at alkaline pH. CNAG_02291 is a putative homolog of the DASH/Dam1 (*DAM1*) gene in *Saccharomyces cerevisiae*. *DAM1* is crucial to the cell cycle and works by stabilizing the kinetochore complex to microtubules allowing chromosomes to separate properly. The *DAM1* homolog in *C. neoformans* is designated as *DCM1* (*DASH complex with microtubules*).

The *DCM1* deletion strain (*dcm1*Δ) exhibited impaired growth at pH 8 and reduced growth rate compared to wild type *C. neoformans*. Capsule, melanin, and growth at 37°C were not impaired in the *dcm1*Δ strain. Furthermore, the *dcm1*Δ and reconstituted strains were evaluated using an invertebrate model of virulence. *dcm1*Δ exhibited a profound virulence defect compared to wild type, suggesting an important role in *C. neoformans* pathogenicity. Survival of the *dcm1*Δ mutant strain in macrophages and virulence in a mouse model remain to be assessed.

P14: The Novel Interplay of Diet and Exercise in Relation to Systolic Blood Pressure, Renal Blood Flow and RAS Regulation Using a C57Bl/6 Mouse Model

Category: Basic Science Research

Austin Le Vey (OMS-II)[1], McClain Vail (OMS-II)[1], Thuy-Linh Nguyen (OMS-II)[1], Aaron Rodriguez (OMS-II)[1], Nathaniel Spears (UG)[2], Jeffrey F. Houghton[1], and Joseph C. Gigliotti, PhD[1]

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Introduction: Healthy diet and exercise are known to prevent cardiovascular disease; however, the exact mechanisms are still unclear. To determine how diet and exercise influence cardiovascular health, we quantified blood pressure, renal blood flow, and the mRNA expression of the intrarenal renin-angiotensin-aldosterone system (RAS) in mice fed different diets, with and without exercise treatments.

Methods: 30 weanling, male C57Bl/6 mice were purchased and acclimated for 1-week. Mice were then randomly assigned to receive 1 of 3 diets *ad libitum*: chow (n=10), American Institute of Nutrition 1993-formulation (AIN93) (n=10), and our novel Americanized diet (AD)(n=10). In each diet group, mice were randomly assigned to an exercise group (n=7) or a control group (n=3). Each mouse in the exercise group had the opportunity to exercise 3 times a week, for 16 hours, on an exercise wheel that recorded the total time spent on the wheel, distance traveled and average velocity of the wheel. The mice in the control groups were moved to a new cage without an exercise wheel 3 times a week for 16 hours to mimic the conditions of the exercise group. After 4 weeks of exercise treatments, blood pressure was recorded using the tail-cuff method, and renal blood flow estimated via doppler ultrasound. Animals were then euthanized, and the kidneys were collected and processed for quantification of mRNA expression of RAS components by RT-PCR. The gene expressions tested for were: renin (*Ren1*), *ACE1*, *ACE2*, angiotensinogen, angiotensin 2 receptor 1a (*Agtr1a*), angiotensin 2 receptor 1b (*Agtr1b*), *ANPEP*, *ENPEP*, *MAS1* and *MME*. Data were analyzed using General Linear Model Procedures in SPSS, with significance determined at $P<0.05$ and a tendency identified with $P<0.1$.

Results: Exercise significantly influenced the systolic blood pressure in the AIN-fed mice, with a significant (p=0.009) decrease in systolic blood pressure in the AIN exercise group (n=2) compared to the AIN control group (n=3). Interestingly, exercise also decreased RBF in AIN-fed mice as compared to AIN-fed mice with no exercise. No difference was observed in any of the other treatment groups in regard to SBP or RBF. Quantification of intrarenal RAS by RT-PCR identified a significant (p=0.025) increase in renal angiotensinogen expression in exercise mice compared to control mice independent of diet. There was also a significant (p=0.044) difference found between diet in combination with exercise compared to control groups in the expression of *MME*, a neprilysin derivative. Results showed a trend (p=0.054) in the upregulation of *ACE1* in exercise mice compared to control mice independent of diet. A trend was also seen (p=0.069) between *Agtr1a* expression in diet and exercise mice compared to diet control mice, where exercise caused an increase in *Agtr1a* expression in mice fed chow but decreased with exercise treatment in mice fed AIN and AD.

Conclusion: Results of this pilot study suggest that diet significantly influences the vascular response to exercise, where mice fed AIN had significantly lower SBP and RBF. Diet and exercise also significantly influenced the renal expression of RAS components; however, it is unclear if this data is responsible for the observed changes in SBP and RBF. Future studies using a longer exercise treatment period, and manipulation of the intrarenal RAS, will help determine the mechanisms by which diet and exercise influence cardiovascular health.

P15: *PRM1* influences pH adaptation but not virulence in the human pathogenic yeast *Cryptococcus neoformans*.

Category: Basic Science Research

Rebekah Satalino (OMS-II)[1], Ryker Heller (OMS-II)[1], and Michael S. Price, PhD[1]

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ABSTRACT

Cryptococcus neoformans is an opportunistic pathogen that has been shown to adapt to changes in pH in its environment. Previous work at Duke University identified numerous genes displaying altered growth at alkaline pH in *C. neoformans*, including gene CNAG_05866, a homolog of *PRM1* in *S. cerevisiae* that is involved in plasma membrane fusion. It was our goal to verify whether this gene is involved pH adaptation in *C. neoformans* by deleting the CNAG_05866 gene in wild-type (WT) *C. neoformans* strain CM2049. To reconstitute the mutant to WT phenotype, the CNAG_05866 gene was cloned into plasmid pSDMA25 and transformed into the CNAG_05866 knockout strain via electroporation. Transformants were selected by their ability to grow on pH 8.0 medium. Spot assays on YPD pH 8.2 showed similar growth between the wild-type strain and transformants, so additional studies will be done utilizing different media to better delineate the phenotypes. A *Galleria* model showed no significant difference in virulence between the WT and CNAG_05866 deletion strains. These data support the hypothesis that CNAG_05866 is involved in the pH regulation of *C. neoformans*, but the role of *PRM1* in virulence of *C. neoformans* remains inconclusive. Further studies will include evaluating PRM1 virulence using a murine model.

P16: Prenatal Opioid Exposure Decreases Pain Threshold in A Nobel Preclinical Model of Neonatal Opioid Withdrawal Syndrome (NOWS)

Category: Basic Science Research

Martin Bava (OMS-II)[1] and Shekher Mohan PhD[1]

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Introduction:
The ongoing opioid epidemic is coupled with heightened opioid use among pregnant women, thus leading to an increased number of babies born with neonatal opioid withdrawal syndrome (NOWS). This novel study aims to assess how prenatal buprenorphine exposure with the rodent *species Acromys Cahirinus*, commonly known as spiny mice, effects the nervous system's response to thermal pain sensation. Spiny mice, when compared to other mice species, have a longer gestation period of 38 - 40 days. This extended time *in utero* fosters increased organ development prior to birth, and results in the birth of pups with a more mature nervous system, similar to that of humans, when compared to other mice species. This allows assessment of pain sensation in spiny mice immediately after birth.

Methods:
Pregnant Dams were treated once daily with saline or buprenorphine (0.3 mg/kg S.C.) from gestation day 19 to postnatal day 0. This is a single blinded study with a control and treatment group. Prior to assessing thermal withdrawal latency a series of acclimation sessions were performed (15 minutes per day for 3 days) to habituate individual pups to the Hargreaves testing apparatus. Once acclimated, the paws of the mice were subject to 52^{o} - 62^{o} C and their withdrawal time (secs) from the heat was recorded. After each test mice were given at least 5 minutes prior to being tested again and were tested no more than 3-5 times per day. Spiny mice pups were tested at 1 week, 1 month, and 2 months of age.

Results:
Preliminary data showed that on average the opiod-exposed group (n=2) had an increased withdrawal latency response when compared to control (n=4). This observation was consistent throughout the study. However, as the pups aged the withdrawal latency response between both groups became shorter. In the first seven days of life, the opioid exposed group had approximately a two times slower withdrawal response than the control group (4.376 sec vs. 2.656 sec respectively). As the mice aged, withdrawal time was reduced and the time difference between groups narrowed. At one month of age the control group averaged 2.33 sec and the treated group averaged 2.87 sec. At two months the control group averaged 1.39 sec and the treated group averaged 1.912 sec.

Conclusion:
Preliminary data shows that prenatal opioid exposure may affect withdrawal latency to thermal pain. Shortly after birth there may be an increased withdrawal latency in opioid withdrawing pups when compared to control. However, as both groups mature their withdrawal times reduce and become relatively similar. These results provide a promising model of assessing NOWS and may suggest that prenatal opioid exposure may slow the nervous system's maturation process in spiny mice.

P17: Preliminary study on the effect of progesterone and pregnenolone on expression of the renin-angiotensin system in proximal tubule cells

Category: Basic Science Research

Yusra Malik (OMS-II)[1], Jeffrey Houghton[1], and Joseph C. Gigliotti, PhD[1]

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The proximal tubule is an important functional component of the nephron and the kidney as it significantly influences the regulation and homeostasis of volume, electrolytes, and the acid base status, both locally and systemically. The renin-angiotensin system (RAS) and oxidative stress play a critical role in these normal processes, and pathophysiological processes as well. Sex steroids and their precursors are also known to have an influence on renal physiology, but the mechanism and the extent of their role are unknown. This preliminary study aimed to assess the impact of progesterone and pregnenolone on renal proximal tubule cell health and function in the context of oxidative stress. Murine TKPTS cells were grown and treated with 0.5mM H2O2 and varying concentrations of either progesterone or pregnenolone. The concentrations of progesterone and pregnenolone were determined based on available circulating values in humans and mice. Upon treatment, the cells were counted and processed for quantification of RAAS genes through RT-PCR. Analysis of the data demonstrated that both progesterone and pregnenolone influence the expression of renin, angiotensin converting enzyme, and angiotensin, some of which in an apparent dose-dependent manner. This preliminary study indicates that sex steroids and their precursors significantly influence cell viability and the activity of RAS in renal proximal tubule cells, however more research is needed to validate these findings and elucidate their physiological significance.

P18: Inhibition of Murine Tumor Growth by Decitabine is Correlated with Elevated RNA Levels of the Mouse Mammary Tumor Virus

Category: Basic Science Research

Jieyu Zhang (OMS-II)[1], Savannah Higgins (OMS-II)[1], Riley Smith (OMS-II)[1], Tania Reginald (OMS-II)[1], Andrew Qi (UG)[2], Anna King (UG)[2], and Yingguang Liu, PhD[1]

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The purpose of this study is to continue research on the antineoplastic effect of decitabine, a DNA methyltransferase inhibitor. Previously in an *in vitro* study, decitabine inhibited the proliferation of the 4T1 breast cancer cell line and the expression of the mouse mammary tumor virus (MMTV) in a dose-dependent manner. Interestingly, an *in vivo* study showed elevated expression of MMTV env in treated mice inoculated with 4T1 cells and yet successful suppression of tumor development. The current abstract reports the findings of an experiment studying the effect of decitabine on MMTV expression in C57BL/6 mice inoculated with MC38 cells derived from colon adenocarcinoma**.** The results confirmed previous data demonstrating that decitabine slows tumor growth and elevates MMTV env expression. Decitabine increased the relative mRNA quantity of MMTV *env* in the tumors, which confirmed the result from *in vivo* 4T1 cell study. Both the control and treated groups demonstrate an overall negative correlation coefficient between MMTV env and tumor mass. Decitabine also significantly mitigated tumor-induced splenomegaly. Our data suggest decitabine inhibits tumor growth *in vivo* and *in vitro* via possibly different mechanisms. The findings also support the current understanding of the upregulation of ERVs by decitabine to increase the targeting of the tumors through interferons (Chiappinelli et al., 2015). Decitabine-induced mice ERV may trigger viral defense mechanisms which target ERV as tumor-associated antigens (Cooper et al., 2015). Further studies of the interaction between decitabine and ERV on tumor suppression using immunodeficient mice will help us to better understand the role of ERV-induced immune response.

P19: Determining the Effect of Diet and Voluntary Exercise on the Gut Microflora in Young Male Mice

Category: Basic Science Research

Aaron Rodriguez (OMS-II)[1], Austin Le Vey (OMS-II)[1], Thuy-Linh Nguyen (OMS-II)[1], McClain Vail (OMS-II)[1], Nathaniel Spears (UG)[2], Jeffrey Houghton[1], and Joseph C. Gigliotti, PhD[1]

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Background: Two leading environmental factors that influence the overall health of humans are diet and physical exercise. However, it is still unclear how these factors influence health and disease susceptibility. The majority of past animal studies have been performed using an optimized, vegetarian diet which does not accurately model the typical American diet. The microbial flora has gained attention as a significant modulator of health and disease and both diet and exercise have been shown to modulate the microbial flora. However, it is unclear if diet will modulate the effects of exercise on gut microflora. Therefore, the goal of this study was to determine how diet and exercise modulate the microbial content of the mouse cecum.

Methods: Male, weanling, C57Bl/6 mice were purchased from a commercial vendor and acclimated to a solid diet. Mice were then randomly assigned to receive one of three diets ab libitum: standard laboratory chow, the American Institute of Nutrition 93 formulation (AIN), and our novel Americanized diet (AD). Within each diet, mice were then assigned to voluntary exercise regimen (wheel access, 16-hours per day, 3-days per week) or a control experience (individually housed in cage without wheel for same duration and frequency). After 2-weeks of exercise, mice were euthanized and cecal content was collected and stored in RNAlater solution. Total DNA was isolated using a commercially available assay, and 16S rDNA for *Firmicutes* (*FIRM*), *Bifidobacterium* (*GBIFD*), *Lactobacillus* (*LB*), and *Bacteroides* (*BACT*) was quantified using real-time RT-PCR. Data were analyzed using General Linear Model Procedures in SPSS with significance determined at $P<0.05$.

Results: Pilot results found that diet, exercise, and their interaction significantly influenced *FIRM* expression. Mice fed the standard chow diet had the lowest expression of *FIRM*, which was significantly lower than mice fed AIN ($P<0.001$) and AD ($P=0.001$). Exercise caused a significant decrease ($P<0.001$) in *FIRM* presence, especially in mice fed AIN and AD. Diet and exercise also significantly influenced *GBIFD* expression. Mice fed AIN had significantly more *GBIFD* than mice fed chow and AD ($P<0.001$ for both). Exercise also caused a significant reduction ($P=0.016$) in *GBIFD* expression. Finally, in a comparison study, it was found that diet significantly influenced the effect of probiotics on *LB* expression. Here, exercise reduced *LB* expression in mice fed chow and AD, but not AIN ($P=0.03$).

Conclusions: A lifestyle consisting of regular exercise and diet similar to standard chow generated support for a mechanism for reducing presence of cecal *Firmicutes*. *Firmicutes*, especially in high concentrations compared to *Bacteroidetes* has been found in other studies to be associated with the development of obesity. Strains of *Bifidobacterium* have historically been linked to inflammatory bowel disease through their modulation of specific immune cells and their outputs, although this mechanism is unclear. Other strains of *Bifidobacterium* have been found to have anti-inflammatory effects through T-cell modulation. Diets modeled after AIN with exercise thus show promising benefits, however, further analyzation and studies are needed to better understand the mechanism as well as the applicability to humans, with the aim of improving clinical outcomes and providing novel therapies.

P20: Determining the effect of diet and probiotic administration on metabolic function in mice

Category: Basic Science Research

Jonathan Westlake (OMS-II)[1], Brian Kagel (OMS-II)[1], Nathan Lichti (OMS-II)[1], Shreya Singh (OMS-II)[1], Jeffrey Houghton[1], and Joseph C. Gigliotti, PhD[1]

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Diet has an established influence on health and disease susceptibility, however the mechanisms responsible are still unknown. The microbial flora in the gastrointestinal tract is known to be mediated by diet and is thus one potential mechanism by which diet influences health. Furthermore, probiotic supplementation also affects various biological markers, however it is unclear if a diet and probiotic interaction exists. Therefore, the goal of our study was to assess how diet and probiotic administration influenced metabolic health in mice. Weanling, male C57Bl/6 mice were purchased from a commercial vendor and acclimated to a solid-diet for one-week. Mice were then randomly assigned to receive one of three diets: chow, American Institute of Nutrition 93 formulation (AIN93), or our novel Americanized diet (AD). Mice were then administered a commercially-available probiotic for 2-weeks and the euthanized (after 6-hour fast). Blood was collected for serum analysis of circulating metabolic markers. Liver and adipose tissue were collected and processed to quantify the mRNA expression of key metabolic genes by real-time RT-PCR. All data were analyzed using General Linear Models in SPSS with significance determined at $P<0.05$. It was found that diet ($P=0.001$), but not probiotic supplementation ($P=0.27$), significantly affected insulin sensitivity. The AD group showed the lowest responsiveness to insulin as compared to the chow ($P<0.001$) and AIN-93 ($P=0.001$) diet groups. Diet alone tended ($P=0.09$) to affect circulating HDL, but not triacylglycerides or LDL levels. Chow fed mice had lower HDL than did the AIN-93 and AD fed mice. RT-PCR showed that gene transcription of SREBF2, a transcription factor regulating cholesterol synthesis, was upregulated in the liver of probiotic fed mice, especially those in the AIN-93 diet group ($P=0.024$). Increased transcription of a carbohydrate metabolism regulating gene, MLXIPL, was found to occur in liver of the chow and AIN-93, but not AD diet groups ($P=0.06$). Probiotic supplementation also modulated liver transcription of NF-κB1: increasing in the chow, but decreasing in AD diet group ($P=0.016$). In the adipose tissue, diet ($P<0.001$), probiotic ($P<0.001$), and their interaction ($P=0.01$) influenced transcription of the androgen receptor (Ar) gene. The chow group had the most Ar transcription, while the AD had the lowest transcription in both the diet alone and probiotic treated group. Taken together, our data highlight the significance of diet in mediating the beneficial metabolic effects of probiotic administration. Diet appears to play a greater role in dictating the expression of key metabolic enzymes and transcription factors and future studies are needed to validate these findings and determine their physiological significance.

P21: Determining the effect of diet and probiotic administration on gastrointestinal microbial content in male mice.

Category: Basic Science Research

Shreya Singh (OMS-II)[1], Brian Kagel (OMS-II)[1], Jonathan Westlake (OMS-II)[1], Nathan Lichti (OMS-II)[1], Jeffrey Houghton[1], and Joseph C. Gigliotti, PhD[1]

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The gut microbiome is a growing topic of discussion due to its varying effects on the gut brain barrier, infections, obesity, allergies, and mental health. With a newfound public desire to ameliorate gut health, there has been an increase in probiotic consumption and dietary changes, but more research is needed to better understand the mechanisms responsible for the observed phenotypes. In the current study, we set out to determine how different diets and administration of a commercially available probiotic influence the gastrointestinal microbiome in mice. Weanling, male C57Bl/6 mice were purchased and given a week to acclimate to a solid diet and group housing. Mice were then randomly assigned to receive one of three diets *ad libitum*: chow, the American Institute of Nutrition 93-formulation (AIN93), and our novel Americanized diet (AD). Mice from each diet were then administered a control solution (distilled water) or 30 uL of a commercially available probiotic mixture, orally five days a week for two weeks. After 8-weeks, mice were euthanized and the cecal content was collected and stored in RNAlater solution. Total DNA was isolated from the cecal samples using a commercially available assay, and the presence of microbial species was quantified by RT-PCR analysis of 16S rDNA specific for *Firmicutes, Bifidobacterium, Lactobacillus*, and *Bacteroides*. Data were analyzed using General Linear Models in SPSS with significant difference identified as $P<0.05$. Diet significantly impacted *Bifidobacterium* ($P=0.015$), *Lactobacillus* ($P=0.001$), and *Bacteroides* ($P<0.001$) levels, with mice fed the AIN diet having the greatest *Bifidobacterium* levels but lower *Lactobacillus* and *Bacteroides*. Mice fed the AD had the highest *Lactobacillus* and *Bacteroides*. Probiotics significantly decreased *Bifidobacterium* and *Firmicutes*, with a decrease in *Firmicutes* being associated with improved physiological phenotypes in other studies. This preliminary study highlights that dietary changes, even within “normal” ranges, significantly influence the gut microbiome. Furthermore, our data supports the claim that commercially available probiotics do modulate GI microbial flora and that this effect is modified by the diet being consumed. More research is needed to understand the mechanisms underlying this change to maximize its potential benefits and improve gut and overall health.

P22: Determining the effect of diet and probiotic administration on behavior and forebrain inflammation in male mice.

Category: Basic Science Research

Nathan Lichti (OMS-II)[1], Brian Kagel (OMS-II)[1], Shreya Singh (OMS-II)[1], Jonathan Westlake (OMS-II)[1], Jeffrey Houghton[1], and Joseph C. Gigliotti, PhD[1]

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There is a high degree of comorbidity between psychological and gastrointestinal disease, which implies a functional relationship exists between the two systems. Early research investigated a top-down model in which psychiatric disorders impacted gastrointestinal disease, while more recent research has investigated how the gut can effect the brain in a bottom-up manner. Collectively, research suggests that the gut-brain axis is bidirectional, and involves complex relationships between immune, neural, endocrine, and metabolic pathways. Another factor to consider is the gastrointestinal microbiome, which interacts with each of these pathways. In this pilot study, we seek to further investigate the microbiome's role in a bottom-up model by studying the short-term effects of different diets and probiotic supplementation on behavior and the expression of inflammatory biomarkers in the forebrain. Weanling, male C57Bl/6 mice were purchased from a commercial vendor and acclimated to a solid diet for one-week. Mice were then randomly assigned to receive one of three diets for eight weeks: chow, the American Institute of Nutrition 1993 formulation (AIN), or our novel Americanized diet (AD). After six-weeks of diet, mice were then administered a commercially available probiotic, or water control, for the final two weeks of the study. Aggressive tendencies were measured during the probiotic administration period and dietary intake was recorded during the final week of the study. Animals were then euthanized and the forebrain was dissected and processed for the quantification of mRNA for BDNF, NFKB, GFAP, PTGS2, and NR3C1 by real-time RT-PCR. Data were analyzed using General Linear Model Procedures in SPSS with significance determined at $P<0.05$. There was a significant decline ($P<0.001$) in aggressive behavior over the testing period, and mice administered the probiotic tended ($P=0.15$) to display less signs of aggression regardless of the diet. For dietary intake, there was a numerical increase in daily caloric intake in mice consuming the AD (~13 kcal/day), while mice consuming chow and AIN were similar (9 and 10 kcal/day, respectively). Additionally, there were numerical differences between probiotic and control groups for two of the diets. Quantification of gene expression in the forebrain revealed that diet significantly ($P=0.002$) affected Glial Fibrillary acidic protein (GFAP) expression, with mice consuming chow having significantly lower values than mice fed AIN or AD. Probiotic treatment significantly ($P=0.02$) affected NF-κB expression, with mice administered the probiotic having greater forebrain NF-kb as compared to control mice. This was most obvious in mice fed the chow and AIN diets, but not the AD. Collectively, our pilot study's findings suggest that short term probiotic supplementation and dietary variation have potential effects on neuroinflammation, brain function, and satiety. Furthermore, our data suggests that the efficacy of probiotic administration is dependent upon the quality of diet consumed.

P23: Determining the effect of diet and probiotic administration on adipose tissue in male mice.

Category: Basic Science Research

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Diet plays a major role in regulating many factors related to overall health. As obesity numbers in America continue to climb, it is becoming increasingly important to understand the mechanisms of how nutrition contributes to our total level of well-being. Similarly, the makeup of our intestine's microbiome is recently being studied as a key modulator for how certain nutrients are metabolized and the function it plays in both digestive and systemic processes. Taken together, the purpose of our study is to demonstrate the influence of probiotic supplements in the context of different diets on the expression of the adipokines leptin and adiponectin. Weanling male C57Bl/6 mice were purchased and acclimated to a solid diet and group housing for 1 week. Mice were then assigned to receive one of three diets *ad libitum*: a control "chow" group (standard vegetable base), the synthetic American Institute of Nutrition 93 formulation (AIN), and our novel "Americanized Diet" which is similar to the proportion of nutrients consumed in Western societies and were fed for 8 weeks. Mice within each diet group received either a commercial probiotic (30 uL) or saline control five days a week for 2 weeks. Afterwards, the mice were then euthanized, adipose tissue was collected, and tissues processed for the quantification of leptin and adiponectin by real-time RT-PCR. Diet significantly influenced adipose tissue weight ($P=0.02$), with mice fed the AD having the greatest degree of adiposity. Interestingly, there was a significant diet*probiotic interaction ($P=0.003$), where probiotic administration caused an increase in adipose tissue weight in mice fed the AIN, but a decrease in mice fed the AD. Diet also significantly influenced adipose adiponectin expression ($P<0.001$), with mice fed the AD having the lowest expression as compared to mice fed chow and AIN. There was also a significant ($P=0.002$) diet*treatment interaction, where probiotic administration increased adipose adiponectin expression in mice fed AD to levels similar to the other diets at baseline. There was also a tendency for diet*treatment to influence adipose leptin expression ($P=0.054$), where probiotic administration increased leptin in mice fed chow, but decreased in mice fed the AD. Taken together, our data suggest that diet and probiotic administration significantly influence the structure-function relationship of adipose tissue. The results of this study underline the significance of maintaining a healthy gut microbiome in today's culture and the impact that the gut microbiome has on our overall health.

P24: Adipose-Derived, HER2/*neu* Tumor-Targeted, Human Mast Cells have Antitumor Effects *in vivo* after Intravenous Injection

Category: Basic Science Research

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Abstract:
The use of one's own cells to treat tumors is typified by chimeric antigen receptor T cells (CAR T) therapy. The list of autologous immune cells with anti-tumor properties being investigated continues to grow. We have previously proposed a new strategy using tumor-targeted mast cells (MC) obtained from autologous sources and demonstrated proof-of concept previously *in vitro* and *in vivo*. We sought to exploit the anti-tumor mediators in MC granules to selectively target them to tumor cells using tumor specific immunoglobin E (IgE) and controllably trigger release of anti-tumor mediators upon tumor cell engagement. We used a human HER2/*neu*-specific IgE to arm human MCs through the high affinity IgE receptor (FcεRI). The ability of intravenously (i.v.) injected HER2/*neu*-targeted MCs to effect HER2/*neu*-positive human tumors was assessed using a immunocompromised xenograft mouse model. It is shown for the first time that tumor targeted MC injected i.v. home to and shrink tumors. These studies provide further proof of concept that MC have anti-tumor properties and could possibly provide another strategy for developing adoptive cell transfer therapeutics for patients.

P25: Disease Induced Intestinal Vascular Leak of Serum IgG Inhibits Gastrointestinal Motility Via Nitrergic and Prostanoid Mechanisms

Category: Basic Science Research

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Background: Vascular leak syndrome is a key feature of many inflammatory disease states in the GI system including trauma, sepsis, colitis, and postoperative ileus. Recently, we have shown that IgG leaks out during microvascular leak which causes subsequent dysmotility of the colonic smooth muscle. The objective of this project was to elucidate the physiological mechanism underlying the IgG initiated dysmotility after microvascular leak to further understand the progression of microvascular leak and dysmotility in inflammatory GI disease states. *Methods:* C57Bl/6 mice were euthanized, the colon removed, and the mucosa was stripped from the underlying colonic muscularis externa. Colonic circular muscle strips were cut (2mm x 15 mm) from the proximal colon. The strips were then mounted in a mechanical organ bath chamber with Krebs solution flowing at a constant temperature of 37° C to preserve biological activity. Muscles were stretched to L_0 and spontaneous activity of each muscle strip was measured in response to increasing concentrations of serum (0.05%, 0.1%, 0.25%, 0.5%, 1.0%, and 2.5%) and pure human IgG (0.25%, 0.5%, 1.0%, and 2.5%). These control trials using serum and IgG were followed by trials that blocked different mechanisms of neuromuscular transmission in the presence of the serum or IgG. Preserved contractility of the strips in the presence of both IgG and a specific neuromuscular transmission or COX antagonist would indicate the proportion of IgG mediated dysmotility that is attributable to the blocked mechanism. The role of nitrergic neuromuscular transmission during serum and IgG exposure was explored using the neural nitric oxide synthase blocker L-nitroarginine (300 μM). The role of prostaglandin production was explored using indomethacin (10 μM and 30 μM), a COX inhibitor. Combinations of LNA and indomethacin with serum or IgG were then perfused over the muscle strips to explore how much of the dysmotility was attributable to nitrergic and prostanoid mechanisms respectively.

Results: Increasing concentration of human serum cause a dose-dependent decrease in colonic circular muscle contractility. Treatment of the muscle strips with 2.5% serum decreased contractility to 37.0±9.98% of control activity. Treatment with 2.5% IgG decreased contractility to 20.0±4.46% of control. Treatment with LNA and 2.5% serum decreased contractility to 36.5±5.14%. Treatment with indomethacin (10 μM) and 2.5% serum decreased contractions to 94.7±23.05%. Indomethacin (30 μM) with 2.5% serum decreased contractions to 95.2±30.28. Serum (2.5%) in the presence of both blockers (LNA and indomethacin (10 μM) resulted in a significant excitatory contractility response (241.7±28.75%) compared to control. Similarly, LNA and indomethacin (30 μM) with 2.5% serum also increased circular muscle contractility (155.1±31.64%).

Conclusions: The enteric leak factor IgG at clinically relevant concentrations causes a near complete inhibition of colonic circular muscle spontaneous contractile activity. The loss of this inhibitory mechanism in the presence of LNA and indomethacin combined, implies that the dysmotility observed in inflammatory states is attributable to the downstream production and release of nitric oxide and prostaglandins. These novel mechanisms of dysmotility observed in many clinical disease states may proved to be novel sites of pharmaceutical intervention.

P26: Conceptual Design, Construction, and Implementation of a Microscopic Live Imaging System to Record Alterations in Jejunal Lamina Propria Microvascular Contractile Activity and Endothelial Calcium Signaling

Category: Basic Science Research

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The physiological regulation of the intestinal microvasculature and its functional alteration during various pathophysiological states remain virtually unknown due to the inaccessibility of the terminal microvascular networks. Our objective was to design, construct and implement a live imagining system which could explore the physiology and pathophysiology of the intestinal microvasculature and endothelium.
An Olympus BX15WI mounted on a motorized Burleigh Gibraltar manipulator with a UPLAN FLN 10X objective and three optional wet dipping objectives were used (XLUMPLAN FLN 20X, LUMPLAN FLN 40XW or LUMPLAN FLN 60XW). For video imaging a Panasonic CD-GH5 was mounted to the Olympus BX15WI with a DSLRM43TCW_Pro adapter and connected via USB to a Dell Alienware Aurora Ryzen Edition R10 with a NVIDIA GeForce RTX 3080 32GB video board. Panasonic LUMIX-Tether software provided live streaming capabilities between the GH5 and the PC. To image fluorescently labeled structures a U-LH75XEAPO 75/W U-RX-T Xenon burner was used, as it provides a wide excitation spectrum from DAPI to near-infrared fluorescent dyes such as DAPI, GFP/fluorescein, Cy3 or Cy5.

A two stage *SYLGARD™ 184* silicone elastomer chamber was poured creating a 10 ml chamber with a transparent base for preparation pinning in a 60 mm culture dish. The chamber was perfused with a Rainin Dynamax RP-1 peristaltic pump flowing a Krebs buffer at 5 ml/min through a condenser coil thermoregulated with canola oil flowing from a Haake C1 circulating water bath. The recording chamber solution was aspirated by a second Dynamax RP-1 peristaltic pump running at 15 ml/min.

A novel whole-mount of the jejunal mucosal vascular plexus was constructed. C57Bl/6 mice were euthanized, and the jejunum was removed through a surgical abdominal laparotomy. A segment of jejunum was submerged in iced oxygenated Krebs solution, cut open along the mesenteric border, and pinned mucosal side up in a Sylgard dissecting dish. The lamina propria and attached mucosa was then selectively removed intact from the underlying muscularis externa. The lamina propria vascular plexus whole-mount was then transferred to organ chamber and microscopically imaged while the chamber was perfused with an oxygenated Krebs solution at 37°C. The vascular plexus was exposed to various substances to assess the contractile responses of the jejunal lamina propria microvasculature. Additionally, C57Bl/6 mice measured calcium signaling in a newly developed mouse line that constitutively expresses GCaMP8 in the endothelium under the VE-cadherin (Cdh5-GCaMP8) promoter.
Conclusion: The implemented live video imaging system will allow the lab to explore the physiology and pathophysiology of the intestinal endothelium and microvasculature.

P27: Establishing Histological Methods to Study the Effect of Diet and Therapy on Kidney and Liver Health in Mice

Category: Basic Science Research

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Diet is a leading risk factor for disease and death worldwide, yet the physiological consequences of poor diet is poorly understood and misrepresented in preclinical animal studies. While much research has focused on the effect of diet on obesity and metabolic dysfunction, our preliminary data suggest that diet directly impacts kidney and liver health prior to the development of overt metabolic dysfunction. Therefore, the goal of this study is to determine how diet and therapeutic interventions influence histological evidence of inflammation and fibrosis. Weanling male and female C57Bl/6 mice were given *ad libitum* access to standard rodent chow, a high-calorie Western diet (WD), or our novel Americanized diet (AD) for 5 months. Mice were then assigned to receive 1) no treatment, 2) dietary modification (changing to chow), or 3) a pharmacological therapy consisting of an angiotensin II receptor blocker (candesartan) and a statin (atorvastatin) for an additional 3-months. At the end of the study, kidney and liver tissues were dissected and processed for immunohistochemistry. Tissues were submersion fixed in a zinc-based fixative for 36-hours and 8 um, paraffin-embedded sections were prepared for CD45 or Picrosirius red staining. Representative images were captured from each slide and abundance of $CD45^+$ or Picrosirius red staining was calculated using Image J software. Preliminary results suggest that both diet and therapeutic intervention influence the presence of immune cells within the liver, with dietary modification having lower evidence of inflammation than no treatment or pharmacological treatment. This appears to be specific for the liver, as pharmacological treatment had lower evidence of inflammation in the kidneys. This trend was also true for fibrotic staining (Picrosirius red), as sections from pharmacological inhibition had less apparent positive staining than sections from no treatment or dietary modification. Additional samples are needed to verify our preliminary results and determine how diet and therapeutic intervention influence the structure-function relationship in liver and kidney tissues.

P28: Role of Interleukin-1 Receptor (IL-1R) in Morphine-induced Hyperalgesia Using a Mouse Model of Postop. Pain

Category: Basic Science Research

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Introduction: Opioids are commonly prescribed for pain, in 2012, 259 million prescriptions for opioid pain medications were written (CDC). Most people who are prescribed opioid pain relievers take them due to a genuine medical need, e.g. post-op. pain. Patients can experience tolerance and loss of effectiveness of opioids over time and this has contributed to the current tolerance epidemic here in the USA. Interleukin-1β (IL-1β) plays a major role in host defense and inflammation, and is associated with inflammatory pain, opioid analgesia, and pain sensitivity. *The aim of this study was to determine the role of IL-1R, the receptor activated by IL-1β in morphine-induced hyperalgesia using a mice model of incisional pain.* Hypothesis: deletion of the IL-1R receptor will alleviate pain and decrease the incidence of morphine-hyperalgesia.

Methods: Surgical incisions: An 5mm incision to the right hind paw was performed on adult, male WT and IL-R1$^{-/-}$ (22-35 g) as described previously (Brennan et al., Pain 1996). Behavioral studies: Mice were acclimated to the testing environment for 3 days before baseline testing. The person testing was blinded to treatments and genotype. Withdrawal latency to radiant *thermal* stimulus was assessed using a Plantar Test (Hargreaves' Method) Analgesia Meter (Harvard Apparatus). Withdrawal threshold to *mechanical* stimuli was assessed using Dynamic Plantar Aesthesiometer (Harvard Apparatus). Both the filament and IR-heat source was applied to the center of incision site (ipsilateral) on the right hind paw and on the plantar of the left hind paw (contralateral). The time required to cause withdrawal of the hind paw from the stimulus was measured to the nearest 0.1 s (cutoff time 20 s). The results of three trials 5 to 10 min apart provided the average paw withdrawal latency (PWL) (sec). *Drug administration*: 0.9% Saline or morphine (10 mg/kg) was subcutaneously injected into the scruff of the neck once-daily from post-op., day 0-3 (3d-study) and from post-op., day 0-6 (6d- study**).**

Results: *3-day study* – 1) Mechanical stimuli – on PoD1 and 2, paw withdrawal latency was less in morphine treated WT mice compared IL-1R$^{-/-}$ mice. 2) Heat stimuli – on PoD1, paw withdrawal latency was less in morphine treated WT mice compared IL-1R$^{-/-}$ mice. *6-day study* – 1) Mechanical stimuli – on PoD5 and 6, paw withdrawal latency was less in morphine treated WT mice compared IL-1R$^{-/-}$ mice. 2) Heat stimuli – on PoD5 and 6, paw withdrawal latency was less in morphine treated WT mice compared IL-1R$^{-/-}$ mice

Conclusion: This study suggests that IL-1β and its cognate receptor might be involved in morphine-induced hyperalgesia.

P29: ***Urgency in the Treatment of Sudden Sensorineural Hearing Loss***

Category: Case Report

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Abstract

Sudden Sensorineural hearing loss (SSHNL) is considered an otolaryngologic emergency that must be treated within 72 hours. Failure to treat within 72 hours often results in permanent hearing loss. Primary care providers must be able to recognize the clinical presentation of SSHNL for prompt ENT referral and management. Here we present a case of SSHNL confirmed by audiogram that was initially treated as eustachian tube dysfunction. Aggressive management measures started at follow-up failed to improve the hearing loss. This resulted in a permanent unilateral hearing loss. Our case attempts to educate primary care providers on the need for early recognition of the clinical presentation of this disease, along with the need for urgent ENT referral.

P30: Mirizzi Syndrome: A Case Report

Category: Case Report

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Abstract:

Mirizzi Syndrome (MS) describes a rare complication of cholelithiasis resulting from extrinsic compression of the common hepatic duct by impacted gallstones in the cystic duct or Hartman's pouch. MS typically peaks in late adulthood, and although gallstones are more prevalent in women, the incidence of MS is equivalent between genders. Due to the pathophysiology of MS being similar to other causes of cholecystitis and biliary obstruction, the symptomatology is rather nonspecific. While ultrasound is typically the first line imaging study of choice, MRCP is also a highly effective and safe tool for working up patients suspected of MS. The mainstay treatment of MS most often involves cholecystectomy or subtotal cholecystectomy. While laparoscopy has its advantages, there is a high rate of conversion to open laparotomy. In this case report, we describe a case of MS diagnosed in a 32-year-old male who presented with nonspecific abdominal pain and other signs of obstructive jaundice.

P31: Isolated fallopian tube torsion: A case report

Category: Case Report

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Background: Isolated fallopian tube torsion (IFTT) is a rare gynecologic emergency that requires a high index of suspicion and immediate surgical intervention. IFTT is rarely diagnosed preoperatively due to the lack of pathognomonic signs and symptoms.

Case: We present a case of a nulligravid 15-year-old female with no medical history who presented with acute lower abdominal pain, nausea, and diffuse abdominal tenderness. Ultrasound revealed a large cystic adnexal mass with patent vascular flow to the ipsilateral ovary. Vital signs were stable and laboratory evaluation was unremarkable. Due to an uncertain diagnosis and suspicion for incomplete ovarian torsion, laparoscopy was performed revealing an IFTT and an ipsilateral hemorrhagic ovarian cyst. Unilateral salpingectomy with cystectomy was performed. Grossly, the fallopian tube measured 12.0 X 3.2 x 1.5 cm with an attached cyst measuring 15.5 X 8.0 X 0.3 cm. Histopathology revealed hemorrhagic infarction of the fallopian tube and cyst.

Conclusion: A high index of suspicion is needed for prompt intervention of an isolated fallopian tube torsion. Diagnostic laparoscopy can be useful in the setting of an uncertain diagnosis. Doppler ultrasound findings of a unilocular, cystic, adnexal mass with patent vascular flow to the ipsilateral ovary, and absent tissue edema are suggestive of isolated fallopian tube torsion.

P32: Substantiation of Piriformis Counterstrain

Category: Clinical Research

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Introduction: Counterstrain is an osteopathic manipulative technique that involves passive shortening of a muscle that possesses a point of tenderness. The body is positioned by the physician to shorten the targeted muscle to the point that the muscle's tenderness is relieved, and then the position is held for 90 seconds.[6] Counterstrain literature regarding the piriformis muscle is varied. Each of the accepted standards of technique, however, require marked flexion between 110 and 135 degrees with abduction and rotation of the hip. The largest disparity between the two techniques is a difference in hip rotation.[4,5,6] Considering this contradiction and the mechanism of action for counterstrain, an appreciation for which technique achieves a greater shortening of the muscle would ideally identify the most effective method for treating the piriformis muscle's tender point. The piriformis muscle is implicated in many clinical conditions, including the commonly diagnosed piriformis syndrome in which the piriformis muscle irritates the sciatic nerve. This model aimed to produce data discerning the most effective technique.

Materials and Methods: The experiment included a plastic skeleton with an artificial muscle modeled after several supporting studies which enabled evaluation of muscle length at varied hip positions.[1,2,3] The joints were placed into the neutral position using a laser level with plumb line and three anatomical landmarks: the body of the L3 vertebrae, the greater trochanter of the femur, and the lateral epicondyle of the femur. Length changes in the muscle were measured using a caliper at varied hip positions within normal range of motion.[6] All angles were measured with a goniometer.

Results: When the model hip was placed into either position used for piriformis counterstrain-120 degrees flexion, 30 degrees abduction, and external rotation or 120 degrees flexion, 30 degrees abduction and internal rotation- the simulated muscle was lengthened by 3.276 and 0.179 cm respectfully. The position which achieved the greatest muscle shortening, -3.380 cm, was 0 degrees flexion, 20 degrees abduction and 45 degrees external rotation of the hip joint.

Discussion: This study shows that the degree of piriformis muscle stretch induced by flexion overwhelms any desired shortening from passive manipulation in the other two planes of motion with external rotation exaggerating this trend at angles greater than approximately 65 degrees. The treatment modality found in the *Clinical Applications of Counterstrain* textbook may achieve better results than those found in the other two sources.[4,5,6] However, neither technique seemed to shorten the muscle from the assessment position. This is contrary to the current understanding of counterstrain's mechanism of action.

Conclusion: Our model demonstrates a lengthening of the piriformis from the counterstrain assessment position, anatomical neutral, to the current standard treatment positions. It also demonstrates that shortening is achieved readily when the patient's hip is placed in a state of 20 degrees abduction and 45 degrees external rotation while maintaining neutral flexion.

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P33: Lynchburg Head Start Vision Screening Initiative

Category: Clinical Research

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Introduction: Amblyopia, often referred to as 'lazy eye', is a vision disorder with bilateral or unilateral decrease in vision caused by abnormal vision development in childhood and infancy. Amblyopia is a common problem in infants and children and if left untreated, could result in permanent visual loss.[1] Amblyopia can arise from many causes including refractive errors, cataracts, or strabismus.[2] The US Preventive Services Task Force (USPSTF) currently recommends vision screening in all children aged 3 to 5 years to detect amblyopia; however, studies have shown that screening rates among children vary by race, ethnicity, and family income.[3]

Vision screening is offered by pediatricians at well child visits as well as in the first years of schooling. Typical testing includes visual acuity assessments and evaluation for strabismus; however, it is often more difficult for younger children to cooperate and thus these tests may result in false-positive results. Photoscreeners can be used with young children and infants to improve cooperation and accuracy. They detect amblyopia risk factors such as refractive error, ocular malalignment, and lens opacity.[3] There is a critical period to complete developmental screening in children to ensure prompt follow up to correct their vision before mature vision occurs. This critical period extends up to age 8 years, making early vision screening imperative to giving a child the best chance for visual recovery if amblyopia is detected.[4]

Methods: Local data regarding the incidence of amblyopia in children in the Lynchburg area has not been published, even though screening is done annually at the Head Start program. We therefore developed a vision screening study to determine the burden of amblyopia in children attending Lynchburg's HeadStart program, using the PlusOptix Vision Screening® device. After obtaining approval from Liberty University's Institutional Research Board (IRB), we partnered with the local HeadStart leadership to conduct vision screening using the PlusOptix Vision Screening® device for all children in the program who met the eligibility criteria and for whom we had obtained informed consent. Vision screening was conducted at the three HeadStart locations in the Lynchburg area, specifically, in Lynchburg City, Bedford, and Madison Heights.

Results: Vision screening was successfully completed for 77 children out of 91 eligible children at the HeadStart programs. The average age for children screened was 3.84 years old and the population consisted of 44 females and 33 males. Of the participants, 61 identified as Black/African American, 7 identified themselves as White/Caucasian, and 9 did not wish to provide this information. Every child with an abnormal screening result was referred for further ophthalmologic evaluation with a local pediatric ophthalmologist. The referral rate among the screened population was 32.5% (25/77). Of those referred for services, the average age was 3.88 years, with 14 females and 11 males; 21 Black/African American, 2 White/Caucasian, and 2 who did not wish to provide their race or ethnicity. In addition to identifying abnormal vision, the PlusOptix® device provides the specific indication for referral. Of the 25 children with abnormal screening results, 12 were for astigmatism, 8 for anisometropia, 6 for hyperopia, 3 for myopia, and 2 for gaze asymmetry.

Conclusion: Our study revealed a higher abnormal vision screening rate compared with national data of about 20% abnormal results in children in HeadStart programs[6]. The small sample size of our study is a limitation that could account for the discrepancy.

P34: Comparing Hysterectomy Care Among Persons in a Medicaid Population to a National Benchmark

Category: Clinical Research

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Objective: We reviewed the diagnostic and procedure codes associated with hysterectomies obtained from a US Medicaid cohort to understand patterns of care within a traditionally medically underserved population. Our principal aim was to ascertain differences in surgical approach frequency across indications in comparison with a national benchmark.

Methods: Using 12-months of claims data from a single-payer, Medicaid population (n=427,110 individuals) we restricted our analysis to subjects who had undergone a hysterectomy (n=30). We then extracted procedure codes and enumerated counts across 3 surgical approaches (abdominal, vaginal, and laparoscopic for 5 typical indications (Bleeding, Prolapse, Endometriosis, Malignancy, Leiomyoma/cysts). We computed frequencies for hysterectomy by indication and approach within our population in comparison to a large, published US Cohort (Wright et al., 2013) using Fisher's exact test.

Results: We note significant differences in the routes used for hysterectomy surgery in the US Medicaid cohort compared to the national benchmark. The laparoscopic route was used significantly more in the US Medicaid cohort for bleeding ($p < 0.001$), leiomyoma ($p = 0.016$), malignancy ($p = 0.001$), and endometriosis ($p = 0.023$). The abdominal route was used significantly less in the US Medicaid cohort for bleeding ($p = <0.001$) and leiomyoma ($p = 0.001$). There was no significant difference in cohort frequency for use of the vaginal route to treat prolapse ($p = 0.56$).

Conclusions: Aligning with recently published reports of hysterectomy care, analysis of this US Medicaid cohort suggests a trend towards adopting the laparoscopic approach. Our analysis suggests that Medicaid patients are receiving standard hysterectomy care. Limitations of this study include lack of clear demographic and age differences between the cohorts studied which may shed light on differences noted above.

P35: Can Osteopathic Manipulation Improve Post-Partum Urinary Incontinence?: A Retrospective Study Evaluating the Efficacy of Osteopathic Manipulation in Combination with Pelvic Health Physical Therapy for Post-Partum Urinary Incontinence.

Category: Clinical Research

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Introduction: In treating urinary incontinence (mixed, stress, or urgency), the American Urogynecologic Society and American College of Obstetrics and Gynecology recognizes pelvic health physical therapy (PT) as an efficacious therapy option for patients to reduce symptom burden. There is a lack of evidence, however, of the effects of osteopathic manipulation medicine (OMM) on reduction of urinary incontinence burden. This study aims to validate the effects of OMM on reducing urinary incontinence burden when paired with standard pelvic health PT techniques by evaluating participants' Urinary Problem (UP) score to track their symptom burden.

Objective: The purpose of this retrospective quality improvement study was to evaluate the effectiveness of combining pelvic health PT techniques with OMM on reducing symptom burden for women experiencing urinary incontinence.

Methods: The study was approved by Liberty University's IRB and Centra Health System's IRB. All new pelvic health patients (seen by Dr. Brandy Wilson) from June 1, 2021 to June 1, 2022 were evaluated for enrollment in the study. If inclusion and exclusion criteria were met, the participant was enrolled in the study. The study evaluated female patients with any urinary symptoms that do not have an infectious cause, focusing mainly on types of incontinence (mixed, urgency, or stress). The inclusion criteria are the individual must be at least a G1, not currently pregnant, age 55 or younger, and must have at least 2 Urinary Problem scores documented during their course of treatment. The data was de-identified to protect participants' identities and extracted from the electronic medical record at the pelvic health physical therapy clinic. It was then analyzed for mean difference of urinary problem score, mean difference from goal score to actual score, and a paired t-test comparison in Microsoft Excel.

Results: Of the 234 new female pelvic health patients that presented in the 1-year period of the study, 135 had urinary symptom complaints and 50 of those 135 met criteria to be followed for the study. Each received treatment of pelvic health PT and OMM. The pelvic health PT included muscle training, manual therapy, and biofeedback. The OMM included muscle energy to treat pelvic somatic dysfunction including rotations and pubic shears, lumbar roll to treat sacroiliac joint dysfunction, and strain-counterstain and myofascial release to treat tender points (ASIS and pubic).

The average change from initial UP score to final UP score is an improvement of 7.36. The average difference between the goal and final UP scores is the final UP score is 2.3 lower than the goal. When analyzing the change from a participants initial UP score to their final UP score, 39 of the 50 participants had improvement in their scores meaning a reduction in urinary symptoms (78%). A paired T-test comparing the two sets of data shows a significant improvement in urinary problem scores from the initial value to the final value recorded (P=5.5869E-07; Alpha 0.05). When analyzing the difference between a participants goal UP score to their final UP score, 19 of the 50 participants met their goal (38%).

Conclusion: The incorporation of OMM with pelvic health PT in treating urinary incontinence results in a significant reduction in urinary symptoms. Further studies are required to compare OMM vs expectant management and OMM vs pelvic health PT.

P36: Assessing Harm Reduction Attitudes in Preclinical Medical Students

Category: Clinical Research

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Introduction:
Given the on-going opioid crisis, harm reduction approaches to treating people with substance use disorders are used to prevent overdose and infectious disease transmission. Yet controversy remains over the use of such programs. Thus, it is important to understand how medical student attitudes toward substance use treatment may impact their approach to treating patients. The puSrpose of this study is to assess preclinical medical students' attitudes towards harm reduction approaches using standardized surveys.

Method
This IRB-approved exploratory study used survey data to assess medical student attitudes toward harm reduction. The sample included 119 preclinical medical students who were recruited from OMS-1 and OMS-2. Surveys used for assessment were: 1) The Harm Reduction Attitude Survey-R (HRAS-R); and 2) The Harm Reduction for Opioid Use Disorder Supplemental Questionnaire. Surveys were distributed via Qualtrics at the beginning of the 2022-2023 academic school year, from August to October. Data collected were de-identified, coded, and converted from text to number scoring for analysis.

Results:
Almost a third of students agreed that people with substance use who want to reduce but not eliminate their alcohol or drug use are in denial. However, over 90% agreed that treatment goals including abstinence and harm reduction should be discussed with 79% agreeing that treatment options unrelated to abstinence would likely lead patients to seek professional help. 35% agreed with abstinence only treatments for illegal drug users while 50% disagreed. Participants were split on whether or not opioid users benefit from safe, legal spaces where harm reduction is practiced (39.49% agreed; 35.14% disagreed). In terms of housing, over half of students felt people should be required to be drug free to live in government-funded housing but students were split on whether housing programs for opioid users should be linked to use (36.97% agreed; 36.97% disagreed). Regarding mothering, 40% of students were neutral as to whether women who use illegal drugs during pregnancy should lose custody of their babies. Approximately, one-third agreed that women can be good mothers if their drug use did not interfere with day-to-day functioning with approximately half of participants in disagreement

Conclusion
Overall, medical students held positive attitudes towards harm reduction approaches for people with substance use. However, attitudes were split regarding government funded housing, the specific application of harm reduction methods, and mothering.

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P37: Utilizing Bloom's Taxonomy in Medical Education

Category: Educational Research

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Bloom's taxonomy is an educational tool that has been used across an array of subjects including biology, anatomy, histology, and even radiology training. It utilizes a hierarchical structure to classify orders of thinking into a more specific structure than lower order or higher order. These classifications, moving from lower order to higher order, are: Knowledge/Remembering, Comprehension/Understanding, Application, Analysis, Synthesis/Creating, and Evaluation. From the perspective of medical education, multiple choice questions are the traditional way to examine students, and the Bloom's ordering for these questions generally falls around the first through fourth classifications (Knowledge/Remembering, Comprehension/Understanding, Application, and Analysis). Encouraging students to understand where a practice question fits into this structure may allow them to better identify practice questions that are at or above exam level for independent study , as well as to incorporate more advanced levels of critical thinking routinely. Our project aimed to modify previously existing definitions described in the literature with the use of action verbs and question types for each of these Bloom's classifications to better fit the expectations of medical learning, specifically in the context of physiology. Additionally, we compiled and generated practice questions at classification levels two through four to be utilized in the Cardiovascular, Respiratory, and Hematology course (CVRH) for future OMS-I students.

Our team developed a new Bloom's tool that built on previous descriptions of Bloom's levels in order to help students to better understand the depth of thinking required in medical education. The team of three researchers independently rated previously written physiology practice questions based on Bloom's level using the criteria determined by the previous lab group. Next, the team discussed the ratings as a group and worked toward a consensus of what factors lead to a specific classification. After several rounds of ranking, each individual wrote a new description for each Bloom's classification level. Further analysis, was used to create a combined description table based on the strongest components of each individual's definitions. Once the classification levels had new, agreed upon descriptions and examples, the team generated new practice questions at different levels to be utilized by future CVRH classes.

The results of our project involved new definitions, action verbs, and question type examples for each previously described Bloom's level. In addition, a bank of practice questions at Bloom's levels specifically targeted to the expected examination level for first year medical students has been generated to enhance their learning opportunities and provide a better structure for the utilizing the taxonomy. The hope for the future is that this tool, which has been tailored for first year physiology courses, will help first year medical students to better understand what differentiates the levels of Bloom's taxonomy and lead to more consistent use during the school year, promoting better educational outcomes.

P38: Tracking Sleep and Measuring the Influence of Sleep Information on the Sleep Habits of First- and Second-Year Medical School Students

Category: Educational Research

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Abstract: Healthy sleep habits play crucial roles in maintaining mental health, high-level learning, and overall quality of life.[1-5] Medical students are especially prone to sleeping problems.[6-10] Studies show a high prevalence of sleep deprivation, extensive daytime sleeping, and poor sleep quality among medical students.[11-13] However, when informed, medical students implement healthy sleeping habits that lead to better overall quality of life and enhanced academic outcomes.[6-10] With these in mind, we are seeking to identify the sleeping habits of first- and second-year LUCOM students as well as their willingness to alter those practices with additional education. There are multiple factors that lead to getting good sleep and developing strong sleep hygiene. Participants will be asked to focus on two factors – maintaining a consistent sleep time for the duration of the study and stopping the use of electronic devices 30 minutes before bedtime – that are likely to not be characteristics of a medical student's sleep habits. Prior to participating in the study, interested students were instructed to complete a pre-study survey to assess their knowledge of healthy sleep hygiene and satisfaction with their current sleeping practices. Upon completing the pre-study survey and agreeing to participate in the sleep study, participants in the OMS-I and OMS-II classes will fill out a sleep survey daily to assess their sleeping habits, such as duration and quality, for six weeks. During this phase, students will also receive weekly emails containing summaries of scholarly articles detailing the benefits of healthy sleep habits. At the end of the study, participants will complete a post-study survey asking if participating in the study and receiving information about the importance of healthy sleep habits caused them to think about their sleep and implement healthy sleep habits. The study is just beginning because the data was purposefully scheduled to be collected during the CVRH and Neph-Uro courses, which coincide with seemingly extra stressful and tiring times of the fall semester. Based on conclusions reached in other studies, we predict that sleeping patterns and habits will improve due to the interventions and information provided throughout the course of the study.

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P39: Investigating the Utility of Interprofessional Discussions on Shaping Students' Views on Ethical Issues

Category: Educational Research

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Background
Bioethics is a relatively new academic field that is emerging out of the necessity to address issues and conflicts that cannot be addressed by another single professional academic field. Guiding principles have been defined in this field as a framework to evaluate ethical conflicts that arise surrounding biology and medicine. These principles are beneficence, non-maleficence, autonomy, and justice; and issues in bioethics can be viewed as conflicts between these guiding principles. Learning to evaluate and think critically about issues is bioethics is vital in the practice of multiple careers including medicine, nursing, law, and pastoral care. However, several barriers affect a professional's ability to effectively engage with these topics. These barriers include, but are not limited to, differences in cultural, religious, and academic backgrounds. A study was designed to evaluate these barriers, their impact on one's ability to engage with issues in bioethics, and the role of interprofessional discussions on a student's view of these ethical issues.

Methods
Three lunch meetings – each seeking to facilitate discussion on an important issue in bioethics – were scheduled throughout the academic year. The first meeting sought to address end-of-life conflicts in bioethics. The second meeting will address research ethics conflicts, and the third meeting will seek to address conflicts that arise in genetic editing and testing. The layout of each meeting includes a brief orientation to the purpose of the meetings, time to fill out a pre-discussion survey, an introduction to the tenants of bioethics, 20 minutes to break into small groups and discuss the pertinent cases, 10 minutes to recap in a large group the main points from each case, and time to fill out the post-discussion survey. Professional students from Liberty University's College of Osteopathic Medicine, School of Nursing, School of Theology, School of Law, and School of divinity all received emails from their respective deans, inviting them to participate. The pre- and post-discussion surveys comprise ten questions, which seek to answer the question of whether discussion can influence perceptions of bioethics topics in individuals of varying academic and religious backgrounds.

Results
There were approximately 60 students that attended the first discussion meeting, with 43 students participating in the pre-discussion survey and 33 students participating in the post-discussion survey. The preliminary data suggests that discussion can potentially impact engagement with and evaluation of multiple perspectives on issues in bioethics, but more data must be collected before definitive conclusions can be drawn.

Conclusion
Discussion appears to be a valuable tool in evaluating and engaging with difficult topics in bioethics on a deeper level. While it is impossible to remove all the barriers that prevent critical thinking and unbiased evaluation of topics in bioethics, interprofessional discussion may play an important role in addressing barriers. As the study continues, this impact may be further refined by modifying the structure of the meetings to make them more accessible to students from all of the professional schools at Liberty University and to allow more time for large group discussion.

P40: Review: Potential Local Mechanisms for Exercise Induced Hypoalgesia in Response to Blood Flow Restriction Training

Category: Systematic Review/Meta-analysis

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Abstract

Overall, there is a great need within sports medicine to ensure that athletes can return from injury in an efficient, yet thorough manner. It is crucial to not avoid necessary difficulties in this process, but also ensure rehabilitation in a time efficient fashion. One of the more promising techniques to achieve timely recovery is blood flow restriction (BFR) training. BFR training is a growing and novel development that could be a vital tool to lighten the burden of recovery from injury in athletes. In a field where time away from sports can have massive implications, the need for tools to assist in the acceleration of the rehabilitation process are vital. Much of the work that has already been done in the field has been able to exploit the benefits of exercise induced hypoalgesia (EIH) and further enhance the body's capabilities through blood flow restriction. Studies have compared EIH in both low and high intensity resistance training, as well as low and high intensity aerobic exercise with and without BFR. The results of these studies show comparable beta endorphin levels with high intensity exercise and low intensity exercise with BFR. Low intensity training with BFR had a greater local pain relief pointing to BFR significantly improving the pain diminishing responses of the body through EIH. By reviewing the current literature on this topic, we hope that further progress can be made to better understand the mechanism behind BFR and its ability to enhance EIH. Although no link has been made to EIH yet, studies have shown peripherally located Mrgprs contribute to local pain pathways via mast cell degranulation. In a related pathway, CCR2/CCL2 may also contribute to mast cell degranulation and the resulting inflammation-induced pain. Furthermore, anti-inflammatory IL-10 signaling and anaerobic metabolites via TRPV1 have been shown to reduce pain. Through understanding these mechanisms, it is possible to further exploit the use of BFR to not only serve athletes recovering from injury, but also apply this information to better serve all patients.

P41: The Effects of High Fat Diet, American Diet, and Exercise on Collagen Deposition and ER Stress in Mouse Skeletal Muscle

Category: Basic Science Research

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Background: Poor diet can detrimentally affect skeletal muscle repair and regeneration after injury. Prior studies have shown that consuming low-quality diets induces endoplasmic reticulum stress (ERS) and increases collagen gene expression in skeletal muscle, which can lead to negative regulation of skeletal muscle homeostasis during exercise. However, human diet is routinely misrepresented in preclinical animal studies. As such, it is unclear how diet and exercise influences the structure-function relationship in skeletal muscle. Therefore, our objective was to determine the extent to which moderate intensity exercise training can attenuate markers of fibrosis (*Col1α1* and *Col3α1*) and ER stress (*Hspa5*, *Ddit3*, and *Erdj4*) in animals fed different diets.

Methods: 30 3-week-old male C57BL/6 mice were purchased from Jackson Laboratory and given one week to acclimate to the lab vivarium and a pelleted diet. Mice were then randomly assigned to 1 of 6 interventions for 2 weeks: 1) CHOW-No Exercise, 2) CHOW-Exercise, 3) AIN-No Exercise, 4) AIN-Exercise, 5) AD-No Exercise, and 6) AD-Exercise. They were given ad libitum access to 1 of 3 diets manufactured by Teklad (n=10): (a) standard laboratory chow (CHOW) - the control diet, (b) American Institute of Nutrition 93-Growth diet - the HFD, or (c) American diet (AD). Mice in the "Exercise" groups were given access to an exercise wheel thrice weekly at night. The mice in the "No Exercise" groups were placed in a new environment thrice weekly without an exercise wheel. After 2 weeks, the mice were fasted for 4 hours. 21 mice were then euthanized for the collection of gastrocnemius and soleus muscles. Collagen (*Col1α1* and *Col3α1*) and ER stress (*Hspa5*, *Ddit3*, and *Erdj4*) gene expression were evaluated in skeletal muscle by qPCR. Data was analyzed using multivariate ANOVA in SPSS with significance identified with $p < 0.05$.

Results: In general, mRNA expression of markers of ERS and fibrosis were increased with exercise and this effect was dependent on the diet fed. AD-No Exercise and AIN-No Exercise significantly increased *Hspa5*, *Col1α1*, and *Col3α1* expression in skeletal muscle compared to CHOW-No Exercise (diet main effect, $p < 0.05$). This increase trended larger in AIN compared to AD. AD and AIN also showed a statistical trend in increasing *Erdj4* expression. Diet and exercise altered total *Hspa5*, *Erdj4*, *Col1α1*, and *Col3α1* expression significantly (diet x exercise interaction, $p < 0.05$). Exercise significantly decreased *Col3α1* in AD ($p < 0.05$). Exercise showed a statistical trend in decreasing *Ddit3* expression in AD. There is also a trend in exercise attenuating the increase in *Hspa5*, *Erdj4*, and *Col1α1* in AD as compared to the increases of these genes in the exercise groups of CHOW and AIN.

Conclusions: This study demonstrates that synthetics diets with higher simple sugar content, AIN and AD, had increased markers of fibrosis and ER stress. These changes can be attenuated with exercise training in AD mice. The results suggest that diet influences the magnitude of fibrosis and ER stress in skeletal muscle with exercise. This also suggests that AD is a promising diet formulation to model typical American diets for further studies on the interactions between diet and exercise in muscle repair. Future studies should provide a better understanding of how fibrosis and ER stress negatively affect skeletal muscle repair as well as the long-term effects of diet and exercise on these pathways.

P42: Considerations of Left Coronary Dominance as an Independent Risk Factor for Pathology and Iatrogenic Damage

Category: Clinical Research

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Background: The right or left coronary artery is classified as dominant if it gives rise to the posterior descending artery, also known as the posterior interventricular artery or inferior interventricular artery. Left dominance is viewed as a normal option of development, despite its documented potential to cause increased pathological risk. Multiple studies have suggested that left dominant anatomy can contribute to a greater risk of iatrogenic injury and incidence of pathology. The objective of this study, and the review of current literature, is to highlight the influence that coronary dominance has on pathologies and outcomes as well as emphasize the need for further research into this area of study.

Methods: Cadaveric hearts were sourced from LUCOM's 2021-2022 cohort of cadavers as well as hearts from previous years from the prosection collection. The estimated distribution of dominance type within the general population which will be used as the reference for standard is 70-80% right dominant, 10-20% codominant, and 5-10% left dominant. The coronary dominance of each cadaveric heart was assessed where possible. A retrospective cohort study was conducted using available clinical data to look for patterns of pathology that may be associated with the left dominant hearts. This study was limited by the small number of available hearts for assessment and the potential recall limitations and recall biases of the clinical data provided by family members of the donors.

Results: Of the 41 hearts assessed, 34 (83%) were found to be right dominant, 5 (12%) were found to be codominant, and 2 (5%) were found to be left dominant. One of the left dominant hearts belonged to an 82-year-old male donor whose cause of death was Alzheimer's dementia with additional pathology including type I diabetes. The second heart belonged to a 100-year-old female donor whose cause of death was congestive heart failure and additional pathology including hypertension, high cholesterol, cardiomyopathy, atrial fibrillation, and chronic kidney disease.

Conclusion: *Gray's Anatomy* describes left dominant or codominant anatomy as arising in response to insufficient coverage or absence of right dominant anatomy. This description, paired with the incidences described in current literature, could suggest that left dominant may not be an option of normal anatomical development. It may be more beneficial for left dominance to be seen as a congenital defect in which the body is compensating for the lack of the development of normal right dominant anatomy. Framing left dominance as anomalous rather than a normal variant in the population highlights the importance of knowing a patient's cardiovascular anatomy and the impact dominance could have on a patient's clinical care. Patients with left dominant anatomy may require different pharmaceutical regiments, considerations for procedures, or treatment plans. Moving forward, increasing cohort size, with long-term accumulation of data and by collaborating with other institutions, would help in understanding the potential associations between left dominance and clinical outcomes. Investigations into coronary vessel embryological development would answer the question of left dominance as a congenital anomaly.