2022

MEDICAL STUDENT RESEARCH DAY

BURRELL COLLEGE OF OSTEOPATHIC MEDICINE

The Burrell College Office of Research and Sponsored Programs

Imagination is more important than knowledge.

Albert Einstein (1879 - 1955)

Contents

President's Welcome Address

It is my privilege to welcome you to the Burrell College of Osteopathic Medicine 2022 Medical Student Research Day (MSRD)!

Thank you to our participants and our visitors for showing up to the College's premiere student research event. This is the forth year that the College has hosted this event and I could not be prouder of the students and faculty who have put forth so much effort to make this day possible. Medical Student Research Day is an immensely important day for our students. Not only does MRSD provide our students the opportunity to gain experience that will give them a competitive edge when applying for future residency programs, it also promotes the research and scholarly efforts of the College on a national scale.

I am pleased to see such an impressive turnout of student abstract submissions by our medical students. The research studies presented have significance because of their potential for translation to Osteopathic Medicine. I would like to take a moment to recognize the efforts of our faculty and staff members at the Burrell College Research Laboratories. Without our research mentors and the dedication of the staff of the Research Office, none of this would be possible. The Burrell College research community is a rising force, already making significant contributions to advancing knowledge in basic, clinical, and applied biomedical research.

It is my hope that you will engage with our student researchers and their mentors to learn both about their current projects and the ongoing investigative endeavors of the Burrell College Research Laboratories.

John L. Hummer, MHA
President & Co-Founder
Burrell College of Osteopathic Medicine
E-mail: jhummer@burrell.edu

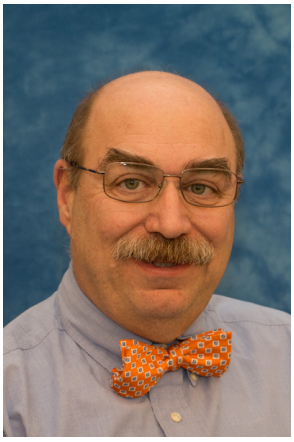
Dean's Welcome Address

It gives me great pleasure to recognize the many students who have traded their summer break for an opportunity to further their own education in the field of research. This year's Summer Research Program encompasses experiments in cytology, immunology, human physiology, population health and clinical medicine, a testimony to the varied interests of our students and their faculty mentors.

I am happy to see such interest in becoming the next generation of physician-scientists who will help advance our medical knowledge for the benefit of our profession and our patients. Please join me in appreciating their enthusiasm to share the skills and knowledge they have gained from this experience.

William Pieratt, DO, FACP
Dean and Chief Academic Officer
Burrell College of Osteopathic Medicine
E-mail: bpieratt@burrell.edu

Assistant Dean for Research Welcome Address



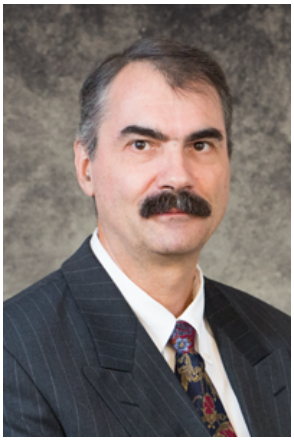
Welcome to our annual Medical Student Research Day and congratulations to all of students and faculty mentors on an outstanding array of research endeavors. This event marks the culmination of an intense summer research experience and for many of our participants the beginning steps towards a career that involves patient care, creation of new knowledge and incorporation of existing knowledge into the advancement of osteopathic medicine. This year's summer research experience incorporated an option for students to enroll in a credit-based course, which took a deeper dive into the topic of publication and presentation of data. We encourage our student researchers use the information to develop the work presented today for submission to professional conferences and scholarly journals.

I wish to thank the faculty who guided the many projects that you will see today. Your commitment, expertise, and mentorship makes student research opportunities at the College possible. Finally, I wish to acknowledge our Director of Student Research, Dr. Harald Stauss for leading our student research initiatives and the members of the Office of Research team, Ms. Martha Enriquez, Ms. Kalli Martinez, and Dr. Michael Woods, whose behind the scenes work keeps the research operations of the College running smoothly.

Join me in visiting the posters and participating in a day that celebrates the contributions of our students and faculty to the advancement of medical and basic science knowledge!

Joseph N. Benoit, Ph.D.
Assistant Dean for Research
Professor of Physiology & Pathology
E-mail: research@burrell.edu

Director of Student Research Welcome Address



Welcome to this year's Medical Student Research Day!
Medical Student Research Day is the culmination of the six-week Summer Research Experience (SRE) at Burrell College. This year marks the highest student participation since the inception of the SRE program. A total number of 46 Burrell Students participated in 18 different SRE projects led by 11 Burrell Faculty. Throughout the SRE, students also participated in a workshop on scientific writing. It is my hope that the workshop inspired students to work with their mentors to prepare manuscripts to be submitted for publication. This year, the Research Office will sponsor a manuscript competition to celebrate the best manuscript resulting from the SRE program. I am looking forward to receiving many submissions for the manuscript competition.

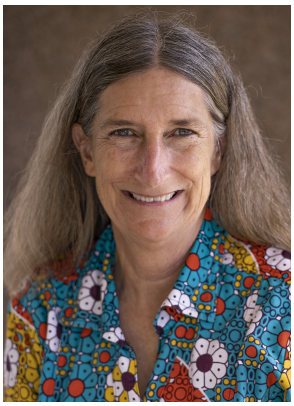
As the Director of Student Research, I would like to extend a big "Thank You" to the Burrell Faculty who enthusiastically submitted SRE projects. Once we realized that the number of student applications for the SRE program will largely exceed previous years' numbers, many faculty stepped in and offered additional research projects to accommodate all students. This faculty commitment to student research reflects the spirit at Burrell College where staff members, faculty, administrators, and senior leadership work together to provide the best medical school experience for our students.

In closing, I would like to wish all students, mentors, and participants a successful Medical Student Research Day 2022!

Harald M. Stauss, MD, PhD
Professor of Pharmacology
Director of Student Research
E-mail: hstauss@burrell.edu

Keynote Speaker

Terrie Taylor, D.O.



Terrie Taylor's battle against malaria, which she refers to as the "Voldemort of parasites", has been waged since 1986. An internationally recognized scientist and physician, Terrie spends six months of the year in the African nation of Malawi, conducting malaria research and treating patients, the vast majority of whom are children. The Blantyre Malaria Project, established by Terrie and Malcolm Molyneux, has carried out outstanding research and patient care in the area of pediatric malaria, specifically cerebral malaria, a syndrome in which the brain is involved. With the help of Dr. James E. Potchen (MSU Department of Radiology) and General Electric Healthcare, the first magnetic resonance imaging unit (MRI) in Malawi was brought to the hospital. The MRI has been invaluable for treating patients and conducting research. She and her team have saved countless lives. But there is more to learn.

Terrie Taylor, D.O.
University Distinguished Professor, Tropical Medicine
Department of Osteopathic Medical Specialties
Michigan State University
East Lansing, MI 48824
E-mail: ttmalawi@msu.edu

Program

Opening Ceremony **Lecture Hall 1 (Room 160)**

9:00-9:05 President's Welcome Address

9:05-9:10 Assistant Dean of Research Welcome Address

9:10-9:15 Director of Student Research Welcome Address

Poster Competition - Session 1: **Atrium Across From Lecture Hall**

Authors are required to be available at their posters during the indicated time to present to the judges. Coffee will be served during poster viewing.

9:30-11:10 [P01] Optimization of CRISPR-Cas13a SHERLOCK Assay for Rapid Detection of Arbovirus RNA
Brenes J, Osteria J, Pattarozzi D, Bramblett D
Department of Biomedical Sciences, Burrell College of Osteopathic Medicine, Las Cruces, NM

9:30-11:10 [P02] Development of Sensitive and Specific Loop-mediated Isothermal Amplification Assays in Buffer, Urine, and Homogenized Mosquito
Afrifa LO, Bhatia D, Buckley M, Bramblett D
Department of Biomedical Sciences, Burrell College of Osteopathic Medicine, Las Cruces, NM

9:30-11:10 [P03] Assessing the Anti-inflammatory Properties of Cannabinoids by Flow Cytometry
Best JP, Bandyopadhyay A, and Woods ME
Department of Physiology and Pathology, Burrell College of Osteopathic Medicine, Las Cruces, NM

9:30-11:10 [P04] Optimization of Splenic Pump to Induce Translocation of Immune Cells from the Spleen to the Systemic Circulation
Cerami KA, Wong JM, Huynh R, Romero FJ, Kania A, Stauss HM
Departments of Clinical Medicine and Biomedical Sciences, Burrell College of Osteopathic Medicine, Las Cruces, NM

9:30-11:10 [P05] Effects of Stress and Estrogen on Neuronal Activation in the Amygdala
Mackell L, Toohey W, Ripley K, Gosselink KL
Department of Physiology and Pathology, Burrell College of Osteopathic Medicine, Las Cruces, NM

Poster Competition - Session 2: **Atrium Across From Lecture Hall**

Authors are required to be available at their posters during the indicated time to present to the judges. Coffee will be served during poster viewing.

10:10-11:50 [P06] Effects of Hyperoxia, Normoxia and Hypoxia on Blood Pressure During Incremental and Sustained Exercise
Miller C, Prosser C, Acalin J, Kall H, and Del Corral P
Department of Physiology and Pathology, Burrell College of Osteopathic Medicine, Las Cruces, NM

10:10-11:50 [P07] Low-Fidelity Canthotomy Model Use in Resident Education, a Multifacility Study
Struhar A, Bouchibti S, and Mohar C
Emergency Department, AdventHealth, Orlando, FL

10:10-11:50 [P08] Attitudes About Cadaver Anatomy in an Institution That Started Without Cadaver Anatomy
Hansen M and Jackson Jon
Department of Anatomy & Cell Biology, Burrell College of Osteopathic Medicine, Las Cruces, NM

10:10-11:50 [P09] Validation of a New Blood Pressure-dependent Baroreflex Sensitivity Index Over the Use of Traditional Heart Rate Dependent Metrics in Assessing Baroreflex Function
Fuller AR, Gupta AK, Noguchi JA, and Stauss HM
Department of Biomediacal Sciences, Burrell College of Osteopathic Medicine, Las Cruces, NM

10:10-11:50 [P10] Efficacy and Predictors of Diagnostic Yield of Implanted Loop Recorders in Patients with Cryptogenic Stroke: A Systematic Review and Meta-analysis
Nolte EK, Rezaei M, Germaine C, and Bhatnagar UB
Department of Cardiology, Memorial Medical Center, Las Cruces, NM

General Poster Viewing: **Atrium Across From Lecture Hall**

Authors are expected to be at their posters during the indicated time.
Coffee will be served during poster viewing.

9:30-10:15 [P11] Investigation of the Cosmetologists' Role in Skin Cancer Detection
Crane A, Aluri B, Diep D, Mogilevsky R, Perdomo J, Pyatetsky I, Regal M, and Bramblett D
Department of Biomedical Sciences, Burrell College of Osteopathic Medicine, Las Cruces, NM

9:30-10:15 [P12] Post-Infantile Giant Cell Hepatitis Associated with Rheumatoid Arthritis
Martinez-Moad M, Wunderlich G, Diep D, Vasudevan A, Janitz T, Aluri B, Crane A, Rangaraju AM, Oviedo A
Department of Physiology and Pathology, Burrell College of Osteopathic Medicine, Las Cruces, NM

General Poster Viewing Continued: **Atrium Across From Lecture Hall**

Authors are expected to be at their posters during the indicated time.
Coffee will be served during poster viewing.

9:30-10:15 [P13] Post-Exertional Vestibulocular Markers in Pediatric Patients with Concussion and Exercise Intolerance (EI)
Chen A, Marx T, Paulsen K, Balaji A, Dusenberry B, Dill J, Ong C, Streeter L, Samsam L, Bailey J, Minor J, Mortazavi M
SPARCC Sports Medicine, Tucson, AZ

10:15-11:00 [P14] Clinical Efficacy of Transcutaneous Auricular Vagus Nerve Stimulation (taVNS) in Plaque Psoriasis
Tsang TB, Nguyen A, McLaren A, Bohman H, Zaidi M, and Stauss HM
Department of Biomediacal Sciences, Burrell College of Osteopathic Medicine, Las Cruces, NM

10:15-11:00 [P15] CT Scans of Lumbar Vertebrae Do Not Demonstrate Fryette Mechanics
Haughton DR, Rimer R, Stauss HM, and Kania A
Department of Radiology, Mountain View Regional Medical Center, Las Cruces, NM and Departments of Clinical Medicine and Biomediacal Sciences, Burrell College of Osteopathic Medicine, Las Cruces, NM

10:15-11:00 [P16] Reduction in Circulatory B Cells and Natural Killer Cells by Occipito-Atlantal Decompression, Transcutaneous Auricular Vagus Nerve Stimulation, and Splenic Pump Interventions
Viegas A, Chen M, Kania A, and Stauss HM
Departments of Clinical Medicine and Biomediacal Sciences, Burrell College of Osteopathic Medicine, Las Cruces, NM

10:15-11:00 [P17] Optimization of the Osteopathic Suboccipital Decompression Technique for Induction of Parasympathetic Activation
Maczka AR, Escano MG, Kania A, and Stauss HM
Departments of Clinical Medicine and Biomediacal Sciences, Burrell College of Osteopathic Medicine, Las Cruces, NM

11:00-11:45 [P18] Placental Exosomal Response to Endocrine Disrupting Chemicals
Barghouty S, Basista M, Prabhakar P, Valentino S, De La Rosa V
Department of Biomedical Sciences, Burrell College of Osteopathic Medicine, Las Cruces, NM

11:00-11:45 [P19] Potential Implications of Restraint Stress and Estrogen Modulation on Food Addiction
Ripley K, Puentes H, Toohey W, Mackell L, Gosselink KL
Department of Physiology and Pathology, Burrell College of Osteopathic Medicine, Las Cruces, NM

11:00-11:45 [P20] Effects of Early Life Stress on the Development of Anxiety in Adulthood
Mercado A, Montenegro S, Gosselink, KL
Department of Physiology & Pathology, Burrell College of Osteopathic Medicine, Las Cruces, NM

General Poster Viewing Continued: **Atrium Across From Lecture Hall**

Authors are expected to be at their posters during the indicated time.
Coffee will be served during poster viewing.

11:00-11:45 [P21] Parasympathetic Activation During Exercise Recovery and in Response to Transcutaneous Auricular Vagal Nerve Stimulation is Directed Towards Different Target Organs

Beyer B, Dwyer K, Mullins J, Patel A, Sheppard C, Del Corral P, and Stauss HM

Departments of Physiology and Pathology and Biomediacal Sciences, Burrell College of Osteopathic Medicine, Las Cruces, NM

Noon-1:00 Lunch Break (pizza provided)

Keynote Lecture

1:00-2:00 Terrie Taylor, D.O.

University Distinguished Professor, Tropical Medicine
Dept. of Osteopathic Medical Specialties, Michigan State University, East Lansing, MI.
The Endlessly Fascinating Story of Malaria Pathogenesis

Award Finalist Presentations: **Lecture Hall 1 (Room 160)**

The judges of the poster competition will select finalists to present during this session.
Moderator: Harald M. Stauss, MD, PhD, Professor of Pharmacology

2:00-2:15 Finalist Presentation 1

2:15-2:30 Finalist Presentation 2

2:30-2:45 Finalist Presentation 3

2:45-3:00 Finalist Presentation 4

3:00-3:15 Finalist Presentation 5

3:15-3:45 Coffee Break with Group Photo

3:45-4:15 Award Presentations

4:15-4:30 Final Remarks

Abstracts - Poster Competition

Optimization of CRISPR-Cas13a SHERLOCK Assay for rapid detection of Arbovirus RNA

Jessica Brenes, Jose Osteria, Daniel Pattarozzi
Mentor: Dr. Debra Bramblett

Context: In 2020 the U.S. reported 884 cases of domestic arboviral disease, 63% of which were neuroinvasive West Nile (WNV) disease and 31% of those were fatal [1]. Rapid detection of WNV is essential for prompt treatment due to a short incubation period of 2-14 days [2]. Although PCR is currently an effective method of diagnosis, it is costly and not widely accessible. The CRISPR-Cas SHERLOCK Assay is an emerging and potentially more accessible diagnostic tool for rapid detection of viral RNA [3, 4].

Objective: The aim was to optimize the SHERLOCK technique utilizing Cas13a for the detection of West Nile Virus (WNV) and Zika Virus (ZIKV). Such optimization would allow for the development of point of care methodologies which would increase the access of Zika and West Nile virus testing [5].

Methods: The CRISPR-Cas SHERLOCK Assay is based on CRISPR-mediated nucleic acid detection in which crRNA guide is used to direct RNase activity in response to target. Reverse Transcription-Recombinase Polymerase Amplification (RT-RPA) reactions were performed according to manufacturer's instructions. Target specific primers were designed and purchased from Integrated DNA Technologies (IDT), (Corralville, Iowa). RT-RPA Master Mix was prepared (0.5 µM Forward and Reverse RPA primer, TwistAmp rehydration buffer, EpiScript reverse transcriptase (Lucigen)). The RT-RPA Master mix was added to one of the lyophilized pellets from the Twist AMP basic kit (Twistdx, TABAS03KIT). A negative control was prepared using 1 μL ultra-purified H_2O. RT-RPA reactions were incubated at 42°C for 25 min on the MJ thermocycler. At 4 min mark reactions were briefly vortexed. Incubation continued for 21 minutes. RPA products were analyzed by agarose gel electrophoresis to ensure target sequences were amplified. Gel images were collected with UVP Imager. Amplified targets were used for Cas13 based detection using Cas13 enzyme expressed from the plasmid pCO13-Twinstrep-Sumo-huLwaCas13a (Addgene, Watertown, MA).

Cas13a was expressed and purified in the laboratory of Eric Yukl at NMSU. Cas13a was prepared by dilution to a final concentration of 65.3μg/mL using storage buffer (50mMTris pH 7.4, 0.6 M $NaCl_2$, 5% glycerol and 2mM Dithiothreitol (DTT)). Target specific crRNAs were designed and synthesized using IDT PrimerQuest tool. Detection was performed following a 10-minute preincubation period between crRNA, (10ng/mL), and Cas13a. Then, the Cas13a-crRNA complexes were combined with the reaction master mix that contained the target RNA. RNAseAlertTM (ThermoFisher, 4479768) was used as a reporter to detect the Cas13a activity using the BioRad CFX96 Touch Realtime thermocycler. Incubated at 37°C with 1 minute data acquisition intervals for one hour and excitation/emission set to 490/520 nm.

Osteopathic Significance: The reciprocal nature of structure and function applies to the specificity of Cas enzymes to nucleotide sequences. As a result, we can manipulate Cas enzymes into markers that identify the presence of specific nucleotides.

Results: For experiments 1 and 2, enzymatic activity of WNV trained Cas13a was below 2400 RFU which matched our non-template controls. Fluorescence of enzymatic activity of ZIKV trained Cas13 was detected in both experiments above 2400 RFU. In experiment 1 ZIKV fluorescence

ranged from $\sim$3000-3250 RFU in all three triplicates. In experiment 2, only one of the triplicates had fluorescence above the non-template control at $\sim$3200 RFU. A third experiment with only WNV shown some fluorescence above its non-template control ($>$2400 RFU).
Conclusion: After repeated attempts to reproduce the correct fluorescence associated with Cas13a enzyme activity signifying the target RNA sequence, we successfully identified Zika RNA. Multiple ZIKV samples were analyzed with a fluorescence level above 3200 RFUs, showing that the Cas13a guides were successful. The West Nile virus fluorescence did not measure above 2600 RFUs, which shows some activity but was not as robust as the ZIKV guides. This suggests technical issues with the protocol still need to be addressed, and reproducibility with this method for Zika and West Nile needs to be demonstrated further. Additional goals can be addressed once consistent reproducibility of the protocol is attainted. The development of a positive mosquito RNA control specific to the highly conserved cytochrome oxidase subunit I (COX1) gene, as well as a Lateral Flow Assay (LFA), can help increase the validity of the test and increase point of care accessibility, respectively. Reagent optimization also needs to be addressed to meet the goals of a point of care methodology.

References:

1. Soto RA, Hughes ML, Staples JE, Lindsey NP. West Nile Virus and Other Domestic Nationally Notifiable Arboviral Diseases - United States, 2020. MMWR Morb Mortal Wkly Rep. 2022;71(18):628-632. Published 2022 May 6. doi:10.15585/mmwr.mm7118a3.

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4. Kellner MJ, Koob JG, Gootenberg JS, Abudayyeh OO, Zhang F. SHERLOCK: nucleic acid detection with CRISPR nucleases [published correction appears in Nat Protoc. 2020 Mar;15(3):1311]. Nat Protoc. 2019;14(10):2986-3012. doi:10.1038/s41596-019-0210-2.

5. Arizti-Sanz J, Bradley A, Zhang YB, et al. Simplified Cas13-based assays for the fast identification of SARS-CoV-2 and its variants [published online ahead of print, 2022 May 30]. Nat Biomed Eng. 2022;10.1038/s41551-022-00889-z. doi:10.1038/s41551-022-00889-z.

Development of sensitive and specific loop-mediated isothermal amplification assays in buffer, urine, and homogenized mosquito

Love O. Afrifa, Divya Bhatia, Maverick Buckley
Mentor: Dr. Debra Bramblett

Context: Delays in diagnostic testing of infectious diseases is costly to healthcare operations and can lead to worsened medical outcomes. Loop mediated isothermal amplification (LAMP) is a nucleic acid amplification technique (NAAT) that is more rapid and specific than current NAATs [1]. LAMP eliminates the need for expensive thermocycling equipment, facilitating point-of-care (POC) testing in less time. Additionally, LAMP products can be detected using multiple end point methods and in real time [2,3].

Objective: To further explore and better understand the applicability of LAMP using multiple pathogenic nucleic acid targets, namely Mycoplasma genitalium, Chikungunya virus (CHIKV), West Nile virus (WNV), and Zika virus (ZIKV), in various sample matrices.

Methods: LAMP primers were designed using *pdhD* gene sequences of the *M. genitalium* G37 strain (L43967.2) and the CHIKV *E* gene from a Brazilian genome sequence (MT526904.1) using GenBank [4,5]. BLAST and Clustal Omega were used to determine the universality of the primer sets across different sub-species [6,7]. WNV and ZIKV primers for RT-LAMP were designed previously against the *E* [8] and *NS2b* genes, respectively. The cytochrome c oxidase subunit 1 (*cox1*) gene served as the internal control for RNA isolation from mosquitoes. *cox1*-specific RT-PCR primers were designed using the PrimerQuest tool (Integrated DNA Technologies [IDT], Coralville, IA) [9]. RT-LAMP primers for ZIKV were designed using the PrimerExplorer tool while LAMP primers for *M. genitalium* and CHIKV were designed using the NEB LAMP primer design tool (New England Biolabs, Ipswich, MA) [7,9]. IDT synthesized all primers. WNV and ZIKV RNA samples were obtained from American Type Tissue Culture (Manassas, VA).

LAMP reactions were set up using the NEB WarmStart Colorimetric (or Fluorescent) LAMP 2X Master Mix (DNA & RNA) protocol. Results were visualized by color, fluorescence, or lateral flow assay (LFA). LFA was conducted using Milenia HybriDetect 1 detection strips according to the manufacturer recommendations (Milenia Biotec, Gießen, Germany). Fluorescent detection was monitored using a CFX96 Touch Real-Time Thermocycler (Bio-Rad Laboratories, Hercules, CA) with FAM absorption and emission spectra of 493nm/517nm.

Mosquitoes were trapped in the Las Cruces, NM or Houston, TX regions and stored frozen. Homogenates were prepared based on Rutkowski et al. using squash buffer (10 mM Tris pH 8.2, 1 mM EDTA, 50 mM NaCl) [10] and proteinase K (Qiagen, Hilden, Germany) [11]. RNA isolation was performed with Trizol (Invitrogen, Waltham, MA). RT-PCR was performed using the SuperScript IV with ezDNase (Invitrogen) according to manufacturer recommendations. RT-PCR end point detection was performed via gel electrophoresis.

Osteopathic Significance: LAMP will allow clinicians to gain quicker, accurate diagnoses, enabling earlier disease identification and augmenting the osteopathic physician's ability to treat viscero-visceral and viscero-somatic reflexes that may manifest from infection.

Results: The *pdhD* Color LAMP detected a concentration of 1x10^{-5} ng/μL of *M. genitalium* genome in ultra-pure water after 30 minutes, while the fluorescent LAMP detected 1.6 genomic copies, which equates to a concentration of 1x10^{-7}ng/μL, in a shorter amount of time (18 minutes). For CHIKV, RNA was detectable using fluorescence at a concentration of 6.2x10^{2} to 6.2x10^{3} viral copies.

Both primer mixes for ZIKV and WNV induced specific amplification in the presence of their targets and did not result in non-specific amplification. Synthetic WNV RNA was detected in the context of Culex mosquito homogenate. As part of our experiments with female mosquitoes, we discovered that one homogenate consisting of a single female mosquito produced a positive RT-LAMP result for WNV RNA the first two of three times that it was assayed, despite not being spiked with the WNV RNA target. To ensure that our homogenate preparation preserves RNA and that we can detect targets, we are designing an RT-PCR assay to amplify an endogenous mosquito transcript of the *cox1* gene.

Conclusion: In this study, we carried out multiple LAMP reactions for the detection of *M. genitalium*, CHIKV, and WNV nucleic acid targets. Our work demonstrates the specificity, sensitivity, and ease of operation of LAMP as a screening tool. LAMP maintains the high sensitivity of normal PCR assays while allowing for a more rapid result. LAMP removes the need for thermal cycling, making it an easier test to perform clinically. Colorimetric LAMP assays provide an alternative screening method to PCR for infectious pathogens and would be well suited for laboratory settings in which there is a lack of resources for other testing methods.

The LAMP assay is a rapid, sensitive, and specific method of detection, but further evaluation using clinical samples is necessary to determine its efficacy as a diagnostic test. Fluorescent labeling of primers only allows for detection in the same target sequence and cannot be used with other end point detection methods [2]. LAMP also produces a high amount of amplicon and could pose a significant risk of contamination in post amplification analysis [12].

West Nile, Zika, Dengue, and Chikungunya are transmitted by mosquitoes, making viral screening of this vector a valuable tool for tracking and preventing the spread of disease. *M. genitalium* is also of vital concern, causing a variety of reproductive issues including infertility [1]. Screening for these infectious agents at the patient bedside and in the field, particularly in resource-poor conditions, would offer significant opportunities for clinical and public health interventions that could limit the acute health consequences and regional spread of several harmful diseases. We have demonstrated RT-LAMP's specificity for two distinct but related flaviviruses, WNV and ZIKV, and its capacity to amplify targets in homogenized mosquitoes. Our findings also include preliminary evidence that the assay may be able to detect pre-existing viral RNA in the mosquito.

References:

1. Edwards T, Burke P, Smalley HB, et al. Loop-mediated isothermal amplification (LAMP) for the rapid detection of Mycoplasma genitalium. Diagn Microbiol Infect Dis. 2015;83(1):13-17. doi: 10.1016/j.diagmicrobio.2015.05.010.

2. Tanner NA, Zhang Y, Evans TC, Jr. Simultaneous multiple target detection in real-time loop-mediated isothermal amplification. Biotechniques. 2012;53(2):81-89. doi: 10.2144/0000113902.

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8. Parida M, Posadas G, Inoue S, Hasebe F, Morita K. Real-time reverse transcription loop-mediated isothermal amplification for rapid detection of West Nile virus. J Clin Microbiol. 2004;42(1):257-263. doi: 10.1128/jcm.42.1.257-263.2004.

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10. Yeap HL, Axford JK, Popovici J, et al. Assessing quality of life-shortening Wolbachia-infected Aedes aegypti mosquitoes in the field based on capture rates and morphometric assessments. Parasit Vectors. 2014;7:58. doi: 10.1186/1756-3305-7-58.

11. Rutkowski N, Dong Y, Dimopoulos G. Field-deployable molecular diagnostic platform for arbovirus detection in Aedes aegypti. Parasit Vectors. 2020;13(1):489. doi: 10.1186/s13071-020-04357-y.

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Assessing the Anti-inflammatory Properties of Cannabinoids by Flow Cytometry

Justin P. Best and Anshika Bandyopadhyay
Mentor: Dr. Michael E. Woods

Context: Millions of Americans are afflicted by autoimmune diseases, many of which cause debilitating symptoms and require long-term management [1]. An evolving body of evidence indicates that Neutrophil Extracellular Traps (NETs), which are composed of decondensed chromatin and proteases produced by polymorphonuclear (PMN) granulocytes, contribute to the pathogenesis of many autoimmune diseases [2]. Consequently, reducing the formation of NETs may provide an alternative treatment strategy.
Objective: This study aims to determine whether cannabidiol (CBD), which has gained popularity for its use as an anti-inflammatory treatment [3], directly interferes with the NETosis pathway. Additionally, we hope to establish if this interference is accomplished by impeding NADPH oxidase-driven reactive oxygen species (ROS) production, histone citrullination, or both [4].

Methods: Using the MACSxpress® Whole Blood Neutrophil Isolation Kit, neutrophils were isolated from the peripheral blood of healthy volunteers and then treated with the MACSxpress® Erythrocyte Depletion Kit to further increase the purity of the collected cells. Concentration was determined using the EVETM Automated Cell Counter and then a small sample of the cells were stained with CD14, CD15, and CD16 antibodies. Using the Guava EasyCyte Pro 5HT flow cytometer, and defining neutrophils as CD14-, CD15+, and CD16+, the purity of the samples was assessed. Using this method, we routinely isolated $>$95% pure and unstimulated neutrophils from human blood. The remaining cells were resuspended in neutrophil culture medium (NCM) to a concentration of 2x10^6 cells/mL, then treated with either DMSO (control) or CBD (treatment group) to a concentration of 10 μM. The tubes were incubated for 1 hour at 37°C, 5% CO_2, protected from light.

Experiment 1: SYTOX Green Stain: Following this incubation, the cells were stained with SYTOX Green, a high affinity nucleic acid stain. Next, samples were divided and stimulated with either PMA or DMSO at a concentration of 50nM. This mixture was homogenized and seeded in 200 μL technical quadruplicates in wells of a sterile black 96-well suspension plate, then incubated for 2 hours. Finally, flow cytometry was used to measure the fraction of cells positive for SYTOX Green in each well.

Experiment 2: CellROX Green Stain: Following this incubation, the samples were divided and stimulated with either PMA or DMSO at a concentration of 50nM. This mixture was homogenized and seeded in 200 μL technical quadruplicates in wells of a sterile black 96-well suspension plate, then incubated for 1 hour. Next, the CellROX® reagent (high affinity for ROS) was added at a final concentration of 1000 nM to the appropriately induced cells, and incubated an additional 1 hour. Finally, flow cytometry was used to measure the fraction of cells positive for CellROX Green in each well.

Osteopathic Significance: This research aims to support the osteopathic tenet of rational treatment based upon the principles of body unity, self regulation, and the interrelation of structure and function by establishing an alternative, effective treatment for inflammation.

Results: We established a consistent method to obtain $>$95% pure samples of neutrophils, which was verified by staining cells for CD14, CD15, and CD16, then assessing the purity using the flow cytometer. Once it was verified which cell population was composed of neutrophils (CD14-, CD15+, CD16+), we were able to apply a region to the graph and assess whether there was a shift to the right corresponding to an increasing number of cells stained with SYTOX Green. Since SYTOX Green is a membrane impermeable nucleic acid stain, we know that it can reliably be used to calculate the number of cells undergoing NETosis [5]. We applied "Region 2" to the graph and used that to count the number and percent of cells undergoing this shift. The neutrophils were pretreated with either DMSO or CBD, then divided and incubated with either DMSO or PMA. The percent of cells found in Region 2 that were pretreated with DMSO (control) and stimulated with DMSO was 1.022% whereas the cells stimulated with PMA was 20.34%. The percent of cells found in Region 2 that were pretreated with CBD (treatment group) and stimulated with DMSO was 4.73% whereas the cells stimulated with PMA was 20.82%.

A similar strategy was used to assess ROS production because CellROX is also a green stain that is able to be quantified using a flow cytometer. The percent of cells in R2 that were pretreated with DMSO and stimulated with DMSO was 6.02% whereas those stimulated with PMA was 8.54%. The percent of cells pretreated with CBD and stimulated with DMSO was 5.50% whereas those stimulated with PMA was 9.23%.

Conclusion: The SYTOX Green and CellROX Green stains were used to determine if cannabidiol (CBD) directly interferes with the NETosis pathway. As expected, there was a significant increase in the amount of NETosis and ROS production after being treated with PMA. However, the samples that were pre-treated with CBD failed to show consistently lower amounts of NETosis and ROS production, which does not support the hypothesis that CBD interferes in the pathway. There were issues with obtaining a high enough cell count, leading researchers to believe the cells may settle and adhere to the 96-well plate during the incubation period. Preliminary studies performed by our team that account for these issues indicate that CBD affects NETosis, but the precise pathways of interference are still unknown. There was not a significant change in the amount of ROS found in samples pretreated with CBD, suggesting that if interference does occur, it is somewhere later in the NETosis cascade. Overall, these results suggest that the experiment should be modified and repeated to establish more consistent results. By doing so, in the long term, we hope to establish more specific and effective treatments against autoimmune conditions.

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Optimization of Splenic Pump to Induce Translocation of Immune Cells From the Spleen to the Systemic Circulation

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Mentors: Dr. Adrienne Kania and Dr. Harald M. Stauss

Context: Nearly 3 million Americans are affected by Inflammatory Diseases. Osteopathic Manipulative Treatment (OMT) may serve as adjunct therapy for these diseases. OMT lymphatic techniques augment lymphatic flow and may modulate immune cell numbers by facilitating exchange of immune cells between reticular organs and the blood. Previous observations suggested that

circulatory monocytes isolated following Splenic Pump (SP) application have lower IL-6 mRNA expression than those isolated before SP.
Objective: This study evaluated how different compression rates and pressures applied while performing SP impacts its effect on circulatory immune cell numbers. We hypothesized that a higher rate and pressure will result in a larger effect on immune cell translocation between reticular organs and the systemic circulation compared to a lower rate and pressure. We also hypothesized that IL-6 protein expression in monocytes following SP application is lower than before SP application.
Methods: This study was approved by the Institutional Review Board of Burrell College (IRB# 0089_2021). The study participants (n=6) were adults ($>$18 years) of both genders (2 male, 4 female). Exclusion criteria included pregnancy, previous abdominal surgery, abdominal infections, inability to lie on their back for 20 minutes, recent blood donation, and current drug or alcohol abuse. The SP technique was performed on 3 consecutive days, with 10 compressions/min of rhythmic pumping of the left upper abdominal region at a pressure of 2-4 mmHg (n=3) or 30 compressions/min at a pressure of 6-8 mmHg (n=3). Pressure was verified by monitoring manometer readings on a blood pressure cuff placed behind the subject's left lower back. Frequency of compression was timed using a metronome. Venous blood samples were collected pre-intervention on the first day and post-intervention on the third day. For flow cytometry, leukocytes were isolated (ACK lysis buffer) on the first and third days, and T-helper cells (CD3+, CD4+), cytotoxic T-cells (CD3+, CD8+), B-cells (CD19+), monocytes (CD14+, CD11b+), and natural killer cells (NK, CD3-, CD56+) were detected using fluorescence-labeled antibodies. For IL-6 protein expression in monocytes, the isolated leukocytes were incubated with lipopolysaccharide (LPS, TLR4 ligand) and monensin (captures synthesized proteins within the Golgi apparatus, preventing exocytosis) for 24 hours. Cells were then stained with fluorescence-labeled antibodies for detection of monocytes (anti-CD14, anti-CD11b) and intracellular IL-6. Flow cytometry data were analyzed using a stepwise multiple linear regression analysis using the R Statistical Platform. The dependent variables were the relative number of immune cells (percent of total cells). The independent variables were rate of compression and applied pressure of the splenic pump technique performed. Statistical significance was assumed at $P<0.05$ and trends were considered at $P<0.10$.
Osteopathic Significance: There is no consensus on the pressure and rate of compression for the SP. Optimizing these parameters based on the effect on immune cell number and function may improve the clinical outcomes when using the SP for chronic inflammatory diseases.
Results: The SP did not affect the relative abundance of T-helper cells, cytotoxic T-cells, B-cells, or monocytes. However, application of the SP tended ($P<0.10$) to reduce the relative number of natural killer cells (-34$\pm$20%, P=0.08) with no significant difference between the two modes of SP (low frequency of compression and low pressure vs. high frequency of compression and high pressure). No effect of the SP technique was observed on intracellular IL-6 protein expression in monocytes after 24 h of incubation with monensin and LPS. Variability in monocyte numbers and IL-6 levels due to extended incubation and cell death led us to re-evaluate the optimal monensin and LPS incubation time. To test if the absence of an effect of the SP on intracellular IL-6 protein expression in monocytes was due to the long monensin and LPS incubation time of 24 h, we assessed the time course of intracellular IL-6 protein expression in monocytes after 4 h, 6 h, 8 h, 12 h, 16 h, and 20 h of monensin and LPS incubation. IL-6 expression started to increase after 6 h of incubation and reached a maximum between 8h and 12 h of incubation. At incubation times above 12 h, we noticed a significant decrease in cell numbers, including granulocytes and monocytes.

Conclusion: A decrease in circulatory NK cells was observed following the SP application across all subjects regardless of different compression rates and pressures. It is possible that the SP technique facilitates the translocation of immune cells between reticular organs and the systemic circulation according to cell densities. i.e., cells that are more abundant in reticular organs than in the blood will translocate into the blood, while cells that are more abundant in the blood than in reticular organs, such as natural killer cells, will translocate into reticular organs.
Our time course experiment demonstrated maximal IL-6 protein expression in monocytes after 8-12 h of LPS/monensin incubation and significant decreases in monocyte numbers after more than 12 h of LPS/monensin incubation. This suggests the absence of an effect of the SP on intracellular IL-6 protein expression in monocytes observed in our experiment may be due to the long LPS/monensin incubation time of 24 h. At this time point, most monocytes may have already been destroyed through activation of cytotoxic immune responses to LPS/monensin incubation. A limitation of our study is the relatively low number of subjects (n=6 total and n=3 for each of the two SP variations). This low number may have resulted in a low statistical power.

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Effects of Stress and Estrogen on Neuronal Activation in the Amygdala

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Mentor: Dr. Kristin L. Gosselink

Context: The body generates stress responses to threatening stimuli, producing a momentary rush of adrenaline as part of fight-or-flight but also activates the hypothalamic-pituitary-adrenal (HPA) axis for longer term responses and recovery. Chronic stress can lead to mental illness or other diseases, some of which affect females more than males. The amygdala plays a major role in this

process, since it regulates the stress response and elicits emotional responses such as fear, anger, or aggression.

Objective: To determine how acute and repeated stress affect neuronal activation, measured by Fos protein expression in different subregions of the amygdala. Also, to examine how amygdala responses to stress vary based on the presence or absence of estrogen. We hypothesized that acute stress would increase Fos in the central (CeA), basolateral (BLA) and medial (MeA) nuclei, that this response would decrease with repeated stress exposure, and that removal of estrogen would block these responses.

Methods: All animal procedures were conducted at the University of Texas at El Paso, in accordance with the Public Health Service Guide for the Care and Use of Laboratory animals and under a protocol approved by the Institutional Animal Care and Use Committee. Sprague Dawley female rats were kept in individual cages in a vivarium with a controlled climate and full access to food and water. At an adolescent age, before estrus cycling began, the rats underwent OVX or sham surgery, with different groups receiving subcutaneous implants of pellets of vehicle or 17β-estradiol (E2) at a low or high dose. The rats were exposed as adults to acute or repeated restraint stress for 30 min/d for 1 or 14 consecutive days, respectively. Control rats were never restrained but were handled and exposed to open restraining devices every day for 14 d. Post-stress, the rats were perfused transcardially with ice-cold 4% paraformaldehyde and brain tissues collected and sectioned on dry ice on a tabletop sliding microtome. Coronal sections 30 μm in thickness were stained immunohistochemically for Fos protein, using a peroxidase method on free-floating sections that were then mounted on gelatin-coated slides and coverslipped. Sections were photographed on a light microscope coupled to a digital imaging system, and analyzed using ImageJ software. Counts of positively-stained cells were taken from 5 sections throughout the rostrocaudal extent of the CeA, BLA, MeA, and also the lateral amygdalar nucleus (La). Counts were summed for each animal and comparisons made between group averages using a t-test and significance determined at $p \leq 0.05$.

Osteopathic Significance: Understanding how the amygdala and its subnuclei respond to varying levels of stress could inform both pharmaceutical and osteopathic approaches for treating stress-induced mental and other illnesses.

Results: The number of neurons expressing Fos protein was counted in a total of 15 animals in the following groups: Sham-vehicle-control, n=1; Sham-vehicle-acute, n=1; Sham-vehicle-repeated, n=1; OVX-vehicle-control, n=2; OVX-vehicle-acute, n=2; OVX-vehicle-repeated, n=2; OVX-low E2-control, n=2; OVX- low E2-acute, n=2; OVX- low E2-repeated, n=2. Rats given the high dose of estrogen after OVX were not stained. As expected, acute restraint in sham animals appears to have increased Fos expression over the levels seen in sham-control rats in the CeA, the BLA, and the MeA (see table below). OVX may increase basal Fos expression in the CeA, and seems to decrease the acute stress response in CeA and MeA. The effects of low dose E2 replacement are variable across the regions we studied. Analysis of the La was not completed.

	CeA	BLA	MeA
Sham-vehicle-control	61	67	183
Sham-vehicle-acute	112	111	353
Sham-vehicle-repeated	28	50	140
OVX-vehicle-control	90.5 $\pm$ 20.5	76.5 $\pm$ 11.5	176.0 $\pm$ 42.0
OVX-vehicle-acute	101.0 $\pm$ 18.0	106.0 $\pm$ 5.0	222.5 $\pm$ 39.5
OVX-vehicle-repeated	54.0 $\pm$ 8.0	80.0 $\pm$ 9.0	154.5 $\pm$ 7.5
OVX-low E2-control	89.5 $\pm$ 4.5	34.5 $\pm$ 1.5	155.5 $\pm$ 35.5
OVX-low-E2-acute	63.5 $\pm$ 13.5	58.5 $\pm$ 13.5	213.0 $\pm$ 22.0
OVX-low E2-repeated	46.0 $\pm$ 8.0	102.0 $\pm$ 35.0	292.0 $\pm$ 31.0

Conclusion: Acute stress effects on intact (non-OVX; Sham) female rats in all three amygdala regions analyzed were consistent with our hypothesis. The number of Fos-expressing neurons was increased by acute restraint and adapted with repeated restraint exposure. Removal of the ovaries decreased the peak response to acute stress but did not change the overall pattern of stress responses. It is difficult to interpret the result of replacing E2 at a low dose. Due to the low number of animals per treatment group in our study so far, our confidence in our results is somewhat limited. This project will continue, and more data will be generated, including the high-E2 rats, to improve our data and our understanding. Overall, the increased Fos seen in the CeA and BLA is in agreement with previously published studies [1]. The basolateral amygdala is responsible for responding to second-order drug conditioning cues and plays a key role in addiction reward pathways [2], which are also known to be impacted by stress. Chronic stress is associated with dendritic hypertrophy and glutamate-related synaptic remodeling of basolateral amygdala projection neurons [3]; this may be relevant to the higher Fos counts in our repeatedly restrained OVX rats that had a low E2 replacement dose. The CeA expresses corticotropin-releasing factor and is known to modulate HPA axis responses to stress [4]. Our findings help us more fully understand how stress affects the amygdala and may contribute to the regulation of the HPA axis and the development of anxiety. In addition, we are beginning to understand the role that estrogen plays in these processes, since women are more likely to have anxiety diagnoses than men [5].

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Effects of Hyperoxia, Normoxia and Hypoxia on Blood Pressure During Incremental and Sustained Exercise

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Mentor: Dr. Pedro Del Corral

Context: An exaggerated exercise blood pressure response (EEBP) can serve as an early warning sign of cardiovascular disease. Studies suggest the etiology of EEBP is related to systemic endothelial dysfunction, reduced proximal aortic compliance, and high exercise-induced angiotensin II levels in otherwise healthy individuals [1]. Though progress has been made on what causes the exaggerated response, some factors affecting EEBP are less well understood, including the effects of FiO2.

Objective: To explore the differences seen with hypoxia (FiO2=0.137), normoxia (FiO2=0.208), and hyperoxia (FiO2=0.504) on systolic blood pressure (SBP) during and after exercise and provide insight into the factors affecting EEBP and therapeutic use of hyperoxic and hypoxic devices by the general population.

Methods: Sixteen subjects from the Las Cruces area, ages 20-40 years old (mean age 27.3$\pm$2.8 years, 12 male, 4 female), with a BMI of $\leq$ 30 Kg/m^2, a resting SBP of $<$140 mmHg (mean SBP = 120.0$\pm$9.9 mmHg SBP), and resting diastolic blood pressure (DBP) of $<$90 mmHg (mean DBP = 75.0$\pm$mmHg) were recruited using word of mouth, flyers, and community forums. Each subject performed 3 graded exercise tests to maximal effort (up to 7 stages, 2 minutes each) and 3 submaximal effort tests (2 minutes at 45% maximal effort and 28 minutes at 65% of the maximal effort) on a cycle ergometer (Lode Corival, Netherlands). One test was performed per day, with an average of 11 days between tests. Testing sequence used a balanced approach. Subjects were required to inspire either normobaric normoxia (FIO2=0.208), normobaric hypoxia (FIO2=0.137), or normobaric hyperoxia (FIO2=0.504). Gas concentrations were determined using an oxygen concentrator (Pro-10, Oxidation Technologies, LLC.). The trials were single-blinded. Blood pressures (SBP/DBP) and heart rate (HR) (Tango Plus M2, Suntech Medical) were taken at the end of each stage (stage = 2 min) and 2, 5, and 10 min post-exercise for the maximal test, and at 5, 10, 20, 30 min during exercise and 5, 20, 40, 60 min post-exercise for the submaximal test. Subjects ranked their perceived effort (RPE = Rate of perceived exertion) at the end of each stage for the maximal test and at 5, 10, 20, and 30 min for the submaximal test. Blood lactate was measured for both

the maximal and submaximal tests at 3 min post-exercise and at 5 and 30 min during exercise for the submaximal test. Muscle oxygen saturation (Moxy, Fortiori Design LLC) was measured during exercise and 3 min post-exercise. Moxy device placement on the subject's right vastus lateralis was determined using ultrasound (GE VScan w/Dual Probe Handheld Portable Ultrasound). Respiratory parameters were calculated using a metabolic cart (TrueOne 2400, ParvoMedics).
Osteopathic Significance: Determining the effects of different oxygen concentrations on SBP during and after exercise may facilitate early diagnosis of hypertension. This will enable patients to make lifestyle changes, allowing their bodies to self-regulate and self-heal.
Results: In comparing hypoxia versus normoxia max trials, the systolic blood pressure (SBP) was lower in normoxia in the first two exercise protocol stages ($p<0.005$). However, there was no significant difference in the SBP during stages 3-6 ($p>0.16$) or max SBP ($p>0.15$). In contrast, the DBP tended to be lower in hypoxia versus normoxia in stages 1-3 ($p<0.07$), but differences in these two conditions diminished in stages 4-6 ($p<0.17$). There was no difference in 2, 5, or 10-minute post exercise SBP or DBP between hypoxia and normoxia ($p<0.15$). During exercise, subjects reported a higher peak RPE in hypoxia (19.6 ± 0.8) versus normoxia (18.8 ± 1.6) ($p=0.03$). Maximum wattage obtained during exercise was lower in hypoxia (190 ± 57 W) than in normoxia (211.5 ± 63.2 W) ($p<0.001$). Additionally, total exercise time was greater in normoxia (680.0 ± 98.3 sec) versus hypoxia (579.4 ± 79.2 sec) ($p<0.001$). Peak VCO2 was higher in normoxic (41.1 ± 10.0 ml/kg/min) versus hypoxic (35.0 ± 7.8 ml/kg/min) incremental exercise ($p<0.001$).
When evaluating hyperoxia versus normoxia, there was no significant difference in the SBP during stages 1-6, but the SBP was lower in normoxia (205.4 ± 29.1 mmHg) versus hyperoxia (208.4 ± 40.2 mmHg) ($p=0.03$) in stage 7. Similarly, the DBP was higher in hyperoxia (75.2 ± 8.8 mmHg) versus normoxia (71.6 ± 10.4 mmHg) ($p=0.03$) during the 7th stage but was not different in stages 1-6. There was no difference in 2, 5, or 10-minute post exercise SBP or DBP between hyperoxia and normoxia ($p<0.69$). Subjects reported a lower peak RPE in normoxia (18.8 ± 1.6) versus hyperoxia (19.6 ± 0.8) ($p=0.05$). Maximum wattage obtained during exercise was greater in hyperoxia (211.8 ± 67.7 W) versus normoxia (211.5 ± 63.2 W) ($p=0.03$). Additionally, total exercise time was greater in hyperoxic (723.9 ± 102.7 sec) versus normoxic (680.0 ± 98.3 sec) incremental exercise. Peak VCO2 was higher in hyperoxia (43.8 ± 10.7 ml/kg/min) versus normoxia (41.1 ± 10.0 ml/kg/min) ($p<0.01$).
Conclusion: Breathing moderately hypoxic air leads to a faster rise in SBP and drop in DBP during initial stages of incremental exercise. However, differences in blood pressure became insignificant between hypoxic and normoxic conditions after six minutes of incremental exercise, as they begin to approach similar peak SBP. The peak RPE was lower in normoxia when compared to hypoxia. It follows that subjects under normoxic conditions were able to bike for a longer duration and achieve a higher maximum work capacity, resulting in higher maximum VCO2.
SBP/DBP response in hyperoxic environments was similar to normoxic conditions until the seventh stage of incremental exercise, at which point both the SBP and DBP climbed to higher pressures in hyperoxic conditions. This is in congruence with previously demonstrated vasoconstrictive effects of hyperoxia at an FiO2 $>30\%$ [2]. The peak RPE was lower in normoxia when compared to hyperoxia. However, under hyperoxic conditions, subjects were able to bike for a longer duration and achieve a higher maximum work capacity, resulting in a higher maximum VCO2. There was no difference in post-exercise blood pressure at 2, 5, or 10 minutes in each of the three gas conditions following incremental exercise. Whether the various oxygen environments affect peak lactate levels and post exercise hypotension during sustained submaximal exercise is

still being explored.

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Low-Fidelity Canthotomy Model Use in Resident Education, a Multifacility Study

Anna Struhar, Salma Bouchibti
Mentor: Dr. Camilo Mohar

Context: The aim of this study is to demonstrate an economical method of training healthcare professionals on performing the lateral canthotomy and implementing it into training of emergency department residents. Because this is an emergent procedure which is not often encountered, many physicians are not likely to have the opportunity to come across this in vivo during their training. The current synthetic and organic models are expensive or single use so they are typically forgone during residency.
Objective: To determine if a synthetic, low-fidelity, low-cost, eye model can adequately prepare residents to perform a lateral canthotomy.
Methods: Our study participants consisted of men and women in their first, second, or third year of a US emergency medicine residency program. We recruited the residents via email sent directly to residency program directors across the country. Each of the programs received a package with a premade low-fidelity canthotomy trainer, along with the supplies needed for each resident to recreate their own. The supplies provided for each eye model were a ping pong ball, a 4 oz Ziploc container and lid, microfoam surgical tape, 3M Transpore surgical tape, a circular sized rubber band, scissors, and a scalpel. The instructions for the assembly of the trainer and the subsequent lateral canthotomy were demonstrated in a video by Dr. Mohar, which was provided to ensure uniformity in the teaching process. The eye model demonstrated in the video was a replica of the one proposed by Kong et al. as it was found to be the most reproducible and cost effective non-cadaveric model totaling less than four dollars per model. The video itself was optimized by having medical students, with no prior knowledge of the procedure, utilize it to ensure that the steps could be accurately replicated with little to no external help. Results of the study were collected via a survey which was provided to the participants as a QR code/link and was made accessible to them at the completion of the procedure.

Osteopathic Significance: It is important to ensure that physicians are comfortable altering the ligamentous structure to relieve intraocular pressure as a means of preventing functional deterioration of an affected eye. Vision is dependent on maintaining balanced pressures.
Results: At this time the only results available are from 1 local community hospital. A 5-point Likert scale was established in the survey to measure the subjects' comfort level in performing a lateral canthotomy, with 1 being very uncomfortable and 5 being very comfortable. A total of 18 residents from the local community hospital completed the lateral canthotomy procedure and the post procedure survey. The survey indicated each participant's PGY level, determined their familiarity with the procedure, and assessed their comfort on the Likert scale both before and after the training as a way to evaluate if the session was a valuable preparative tool. Only one resident had performed a lateral canthotomy on a live patient prior to this simulation. Their results showed a mean increase in comfortability in performing a lateral canthotomy from 1.9 to 4.5 and those values were used to run a two-sample t-test with a 95% confidence interval in order to assess the results, yielding a p value of <0.0001. The mean change in comfort level significantly increased after the lateral canthotomy simulation session. This indicates that the low-fidelity trainers used were a pragmatic addition to the alternative teaching method. Four additional residency programs were provided supplies and the video tutorial to complete the lateral canthotomy training whose results have yet to be collected and included.
Conclusion: Based on initial data from a single community hospital, the low fidelity trainers are a cost effective and efficient way to demonstrate the lateral canthotomy procedure, which is very often left untaught due to its low prevalence. The procedure itself vastly decreases morbidity in patient cases of orbital compartment syndrome when done promptly and precisely. Limitations exist as the results thus far only reflect a single hospital. We hope that the pending results from the four additional residency programs will further solidify our study in proving the effectiveness of this low-fidelity, low-cost, eye model for medical education.

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Attitudes About Cadaver Anatomy in an Institution That Started Without Cadaver Anatomy

Madeline Hansen
Mentor: Dr. Jon Jackson

Context: Since the 14^{th} century, cadaver dissections have been used in medical education as a vital tool for understanding the foundational relationship between structure and function [1]. But Burrell did not use cadaver anatomy for the first two years, then added cadaver anatomy. This provided an opportunity to examine the attitudes and feelings of Burrell's students, staff, and faculty about adding cadaver anatomy to the curriculum.
Objective: To analyze survey data to better understand attitudes about cadavers especially focusing on the impact that having two classes underdo anatomy without any cadaveric anatomy or donor bodies.
Methods: A qualitative study with both rating and open-ended questions was created and underwent full IRB approval. Due to the nature of the questions, the only potential risk this survey had was emotional hardship if any painful memories where relived.
The survey was sent to all enrolled students and employees at that time. The survey was designed to be anonymous and only collected names that only admin assistants had access to which were then put into a door prize drawing.
The analysis of the data began manually. The responses were stripped of personal information and then review diligently. Looking for any patterns of feelings or beliefs associated with position at Burrell, gender, past experiences like any past anatomy experience or witnessing a natural death. Averages of the rating scaled questions were calculated. Open responses were categorized based on what the response focused on like emotions, impact on learning, no response, or concerning response. At the time of writing this abstract, the ATLAS.ti program was just found and its use as an analysis tool was just starting.
Osteopathic Significance: There currently is not osteopathic significance.
Results: Since the survey was sent to all possible students, faculty, and staff in the fall of 2018, the exact number of people it was sent to is unknown but only 150 responses were received. The 150 survey responses include 66 students without undergoing cadaver anatomy curriculum, 68 students with, 10 staff members, and 6 faculty members. The survey responses include 95 female and 56 males. Only 126 surveys were fully completed (84% completion rate) but incomplete surveys were still included in the analysis process. The only patterns observed at this time were that staff members did not feel that the staff should have received the survey and that people who had passed anatomy experience were more likely to have also witnessed a natural death.
Conclusion: No significant patterns were manually observed from the free response answers or the rating the truth of a statement. At this time, no conclusions can be drawn from the data as the data groups have similar spread of responses and emotions. The data needs further analysis to allow any significant conclusions to be drawn from. Further research into the statistical differences between the class that did not have cadaver experience and those that have could shed a better light on the impact that cadaver anatomy has on the long-term success of medical students.

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Validation of a New Blood Pressure-dependent Baroreflex Sensitivity Index Over the Use of Traditional Heart Rate Dependent Metrics in Assessing Baroreflex Function

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Mentor: Dr. Harald M. Stauss

Context: Baroreceptor reflex function is impaired in a variety of conditions, including hypertension and diabetes [1,2,3]. Thus, accurate assessment of baroreflex function is of clinical significance. Baroreflex function is often measured by the heart rate response to blood pressure (BP) perturbation, not the BP itself. Thus, using BP to assess baroreceptor reflex may be a more accurate reflection of true baroreflex function than the baroreceptor-heart rate reflex sensitivity.
Objective: The aim of this study was to establish a novel measure of baroreceptor reflex sensitivity that is based on assessing the effect of the baroreflex on spontaneously occurring BP variability contrasted with the more traditional baroreceptor-heart rate reflex sensitivity. We tested the hypothesis that the novel measure better distinguishes between patients admitted to the Intensive Care Unit (ICU) who passed away during their stay vs. those patients who recovered and were discharged.
Methods: The subjects used in this study come from the MIMIC-III database, which is composed of 53,423 patients admitted to Beth Israel Deaconess Medical Center intensive care units in Boston, Massachusetts between 2001 and 2012. This database contains a variety of measurements from these patients and their ICU stay. This study examined the patients in the MIMIC III dataset who also had corresponding arterial BP waveforms available. Using filtering logic written in Python 1,870 patients were identified from the complete data set of 53,423 patients that had arterial BP waveforms available. From those 1,870 patients, we only considered the ones that stayed in the ICU for a minimum of 48 hours. Only the first 24 hours of the initial BP recording after ICU admission and the last 24 hours before ICU discharge or death were included in the study. These admission and discharge data were further divided into day- and night-time recordings. Within these timeframes, artifact-free segments were selected based on predetermined criteria (e.g., BP within normal physiologic range, no movement artifacts, etc.). This further reduced the number of included patients to 685 patients. From this set of patients, 59 patients were randomly selected and analyzed using the HemoLab software (http://www.haraldstauss.com/HaraldStaussScientific/hemolab/).
The BP waveforms were automatically scanned for BP ramps, that were defined by a continuous increase or decrease in systolic BP over a span of 4 or more heart beats. For each ramp, the RR-intervals corresponding to the systolic blood pressure values that determined the BP ramps were also determined. Ramps for which BP and RR-interval both increased continuously or both decreaesed continuously were considered baroreflex-mediated sequences. Ramps for which BP increased and RR-intervals decreased or vice versa were considered non-baroreflex-mediated sequences. The sensitivity of the baroreceptor-heart rate reflex was determined as the average slope of the linear regression line between RR-intervals (y-axis) and systolic BP values (x-axis) for all baroreflex-mediated sequences.
We also calculated a novel baroreflex index. For this, the BP perturbation, defined as the difference between the largest and lowest systolic BP value within a BP ramp was calculated for all BP ramps. The baroreflex index was then defined by the ratio of the BP perturbation of the non-baroreflex-mediated sequences to the BP perturbation of the baroreflex-mediated sequences. For patients

with strong baroreflex function, the baroreflex index is expected to be high and for patients with impaired baroreflex function, the baroreflex index is expected to be low.

Osteopathic Significance: The baroreceptor reflex is a mechanism aimed at regulating BP to avoid dangerous hypotensive or hypertensive episodes. This mechanism is in line with the osteopathic tenet that states that the body is capable of self-regulation.

Results: The average age of the patients was 65±15 years. The average length of ICU stay was 15.5±14.3 days. Of the 59 randomly selected patients, 11 patients (19%) died during their ICU stay.

Patients who survived and were subsequently discharged from the ICU had a baroreceptor-heart rate reflex sensitivity of 18.4±17.9 ms/mmHg, while those who passed away during their ICU stay had a baroreceptor-heart rate reflex sensitivity of 24.8±28.1 (not significant). Thus, the baroreceptor-heart rate reflex sensitivity cannot differentiate between these two groups of patients. In contrast, the baroreflex index was able to differentiate between these two groups of patients (survivors: 0.73±0.51 vs. non-survivors: 0.42±0.19 ($p<0.05$).

There was no significant difference between day and night baroreceptor-heart rate reflex sensitivity or baroreceptor index at admission. However, at discharge, the daytime baroreceptor-heart rate reflex sensitivity was 11.8±5.6 ms/mmHg, while the nighttime sensitivity was 24.5±21.9 ms/mmHg ($p<0.05$). During the same period, the baroreflex index was 0.43±0.24 at daytime, and 0.70±0.33 ($p<0.05$) at night-time.

Conclusion: Our data demonstrate that the traditional baroreceptor-heart rate reflex sensitivity cannot differentiate between ICU patients who deteriorate clinically and pass away during their ICU stay from those who improve clinically and eventually are discharged from the ICU. In contrast, the novel baroreflex index was able to discriminate between these two ICU patient populations. However, both techniques to assess baroreflex function were able to detect differences in baroreflex function during the day- and night-time towards the end of the ICU stay. Because only the baroreflex index but not the baroreceptor-heart rate reflex sensitivity was able to discriminate between patients who improved clinically and those who deterorated clinically, we propose that the baroreflex index is a more useful measure of baroreflex function than the baroreceptor-heart rate reflex sensitivity. In addition, the baroreflex index better reflects the true physiologic role of the baroreflex which is to regulate arterial BP and not heart rate. Future follow-up studies may explore if clusters of patients with impaired baroreflex indices can be identified based on the admission diagnoses for the ICU stay.

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Efficacy and Predictors of Diagnostic Yield of Implanted Loop Recorders in Patients with Cryptogenic Stroke: A Systematic Review and Meta-analysis

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Mentor: Dr. Udit B. Bhatnagar

Context: About 1 out of 4 people over the age of 25 will suffer from a stroke during their lifetime, and 10-40% of these are cryptogenic strokes (CS) (1,2). Atrial fibrillation (AF) is thought to be a contributing factor of CS. ECG monitoring is recommended for rhythm detection after CS but is limited to a max of 30 days. There is evidence that prolonged rhythm monitoring with Implanted Loop Recorders has a higher yield of AF detection, which suggests a shorter monitoring period may underdiagnose AF (3).

Objective: Our meta-analysis aims to systematically review studies to determine the optimal length of time for monitoring patients for post-CS AF, determine the efficacy of ILRs, as well as to analyze the clinical predictors of AF in patients with ILRs.

Methods: A systematic review of randomized controlled trials (RCT) and observational studies was carried out following a protocol with specific inclusion criteria, including the use of ILRs following a cryptogenic stroke and patient follow-up after implantation of an ILR. The search strategy on PubMed, Cochrane, and Google Scholar databases included the use of several keywords and key phrases. A specified set of study characteristics were noted for each study found, and studies were excluded if baseline characteristics in patients with and without AF were not analyzed. The data from all of the studies was extracted and analyzed in a Google Sheets form using the PRISMA workflow.

Osteopathic Significance: Understanding the clinical predictors of higher diagnostic yield may help clinicians to better identify patients who would benefit the most from this form of rhythm monitoring.

Results: 13 studies were found to meet all of the inclusion criteria, totaling 1351 patients. Patients were monitored for a median of 435 days. Overall, an average of 26.75% of patients were diagnosed with AF and the average length of time to detect an episode of AF was 81 days. The average age of patients with post-CS AF detection was 70 years old, compared to the average age of patients without post-CS AF detection being 63.6 years old. Most of the included studies also demonstrated that higher CHA2DS2–VASc score is a predictor of post-CS AF. Some studies showed that enlarged left atrium (LA) volume index and increased ectopy/atrial premature contraction (APC) burden were significantly higher in patients who had AF detection.

Conclusion: Patients with cryptogenic stroke had a high incidence (26.75%) of atrial fibrillation on long-term rhythm monitoring and the average length of time to detection was 81 days. Higher age and CHA2DS2–VASc score were noted to be significant predictors in detecting AF.

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Abstracts - General Category

Investigation of the Cosmetologists' Role in Skin Cancer Detection

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Mentor: Dr. Debra Bramblett

Context: Skin cancer is the most common cancer diagnosis in the U.S., with melanoma contributing the highest mortality [1]. While highly prevalent, these cancers have favorable outcomes with early detection. Patients are often educated on the importance of skin cancer prevention but detection of cutaneous neoplasms holds equal significance. Many patients face challenges accessing dermatologic care, especially those living in underserved areas. Cosmetologists may play a role in addressing this disparity.
Objective: To determine the efficacy of the skin cancer education course, Eyes on Cancer (EoC), in preparing hairdressers to recognize and alert their clients of skin neoplasms. Hairdressers often see clients on a regular basis and providing training on skin cancer detection not only helps them to recognize lesions but empowers them to recommend further follow up. This is crucial for cancers such as melanoma that can grow undetected on the scalp and carry some of the highest rates of mortality [2].
Methods: Between November 2021 and April 2022, researchers contacted salons and cosmetology schools in Las Cruces, New Mexico to determine interest in study participation. In total, 15 hair professionals signed informed consent forms and completed the study. This study was designed around the Eyes on Cancer (EoC) course, which is a 20-minute virtual module that reviews the "ABCDE's" of skin cancer recognition. Participants were given a survey, both pre-course and post-course, that each included questionnaire and assessment portions. The questionnaire in the pre-course survey served as a method to assess a participant's career background, comfort in recognizing skin cancers and willingness to communicate these suspicions with clients. The assessment portion used photos and diagnoses of lesions from DermNet with permission obtained. This set of five images provided a selection of benign and malignant lesions followed by two Yes/No questions per image. These questions asked the participant if the lesion appeared to be normal or abnormal and whether they would recommend a client with said lesion to seek follow up care with a physician. Following completion of the pre-course survey, the participant would view the Eyes on Cancer (EoC) presentation and complete the post-course survey to assess whether this course improves a participant's capacity to correctly identify skin lesions as well as their level of comfort when sharing these concerns with clients. Of note, images provided in the pre-course survey were identical to the images provided in the post-course survey.
Osteopathic Significance: The second tenet states the body is capable of self-regulation, self-healing and health maintenance. This study highlights the value of preventative care, such as performing routine skin exams to monitor for changes that may affect patient health.
Results: After running a simple T test on all pre- and post-survey data, we discovered a significant improvement in participants' comfort level in recognizing skin cancers, based on a measured p-value of 0.0001. The cosmetologists' comfort level in bringing up abnormal lesions to their clients also showed significant improvement with a p-value of 0.0049. While the total average on the assessment portion of the survey improved by 10% after reviewing Eyes on Cancer (EoC) course,

comparison of correctly identified skin lesions was insignificant with a p-value of 0.32. However, participants did report developing a stronger sense of their unique relationships with clients and the importance of their role in initiating a conversation around the sensitive subject of skin cancer. *Conclusion:* The purpose of using the Eyes on Cancer (EoC) course was to educate cosmetologists on skin cancer recognition and referrals, but not to replace diagnostic criteria by healthcare professionals. After reviewing the image assessment portion of the surveys, our data demonstrated that accuracy of detection increased, but not significantly. However, cosmetologists reported significantly increased confidence in recognizing and discussing skin lesions with their clients. This course also substantially increased the number of cosmetologists who would apply the principles of "ABCDE's" to lesions observed on their clients, given their newfound educated approach to skin cancer recognition. Although the sample size of this pilot study was small, our favorable results showed potential for the use of educational courses in cosmetology.

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Post-Infantile Giant Cell Hepatitis Associated with Rheumatoid Arthritis

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Mentors: Ayyappa Mysore Rangaraju, MD and Angelica Oviedo, MD

Context: While giant cell hepatitis is typically observed in children, post-infantile giant cell hepatitis (PIGCH) is a rare and poorly understood adulthood disorder. There have only been 100 reported cases in the last 30 years [1]. PIGCH presents with large multinucleated hepatocytes in the hepatic parenchyma [2]. PIGCH can lead to some degree of periportal fibrosis ending in acute liver failure or rapid cirrhosis [2,3]. Post-infantile giant cell hepatitis has multifactorial etiologies including pharmacologic side effects, autoimmune disorders, hematologic disorders, and infectious diseases [3]. Management of PIGCH largely depends on the underlying mechanisms; thus, treatment is often etiology specific.
Objective: To showcase a unique clinical case of post-infantile giant cell hepatitis secondary to rheumatoid arthritis in a 41-year-old female.
Methods: Patient underwent multiple laboratory analysis, various imaging, and specimen biopsy with clinical correlation to arrive at a diagnosis of post-infantile giant cell hepatitis secondary to rheumatoid arthritis.

Osteopathic Significance: This clinical vignette demonstrates that structure and function are reciprocally interrelated. The patient's musculoskeletal system was compromised due to rheumatoid arthritis that affected the liver's function and in turn we observed elevated liver enzymes, anemia, fatigue, and weight loss. This demonstrates how the body works as a unit relying on multiple interrelated systems. Furthermore, impressing the importance of observing patients in a wholistic method rather than just focusing on symptoms of a single organ system.
Results: Patient is a 41-year-old Hispanic female. Clinical presentation and pathology results demonstrated post-infantile giant cell hepatitis.
Conclusion: Our case report aims to bring forth a vignette of PIGCH to spotlight this ill-defined disease and highlight some of the proposed causes, treatments, and laboratory markers. Management of PIGCH largely depends on the underlying mechanisms; thus, treatment is often etiology specific. Patient is currently being followed by a hepatology specialist and managed with ursodeoxycholic acid and prednisone.

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Post-Exertional Vestibulocular Markers in Pediatric Patients with Concussion and Exercise Intolerance (EI)

Alicia Chen, Tyler Marx, Kate Paulsen, Arvind Balaji, Brett Dusenberry, James Dill, Cindy Ong, Leslie Streeter, Leila Samsam, Jaden Bailey, and Jon Minor
Mentor: Dr. Mo Mortazavi, MD

Context: Millions of pediatric-aged patients suffer from a concussion insult yearly, with 15% leading to persistent post-concussion symptoms (PPCS) [1]. King-Devick (KD), force plate (FP) and the near point of convergence (NPC) tests are vestibulocular biomarkers that can measure a patient's post-concussion recovery. Previous studies have shown significant correlations of exercise tolerance with the vestibulocular screen (VOMS), NPC, KD times, and force plate sway velocities [2,3,4,5].
Objective: To further determine if pediatric patients with exercise intolerance (EI) will not only have more severe abnormal VOM biomarkers on presentation compared to exercise tolerant (ET) patients, but we also aim to assess whether post-exertional VOM markers are exacerbated in the EI group.

Methods: This was a retrospective cohort study of 132 pediatric patients aged 6-21 years old, who had 250 total clinical visits for concussion symptoms between 5/1/20 to 4/30/22. Clinic visits were broken down by gender with 76 males and 173 females in our cohort. Each patient went through a standardized exercise tolerance testing protocol at every visit. Buffalo treadmill concussion test (BCTT) was utilized through linear maximal exertion with the HIIT-MD (high-intensity interval training with multi-directional) protocol for those tolerating max linear exertion. Exercise tolerance was determined by a concussion specialist evaluating for onset of symptoms and/or signs of cardiovagal dysautonomia utilizing a standardized 5-step testing protocol. EI etiologies included symptom provocation (sxs), autonomic dysfunction (dys), vestibular provocation (vest), and/or visual provocation (vis). VOM markers including KD, NPC, and force plate sway velocities were tested before and after the exertional test with pre/post changes (delta) documented.
Osteopathic Significance: Effective treatment of concussions requires a multi-aspect approach to have patients successfully return to learn and play protocols, highlighting the 4^{th} osteopathic tenet of understanding the body and its capacity and interrelationships.
Results: EI incidence was 40% amongst all trials, and more common in females (75%) than males (25%).
The mean Pre-FP for EI was 0.94 and for ET 0.82, with a p-value of 0.20. The mean sway velocity change (delta-FP) for EI was 0.40 deg/sec vs. 0.05 deg/sec for the ET group (p-value of 0.002). The mean comprehensive sway velocity post-exertion (Post-FP) for EI was 1.34 deg/sec vs. 0.87 deg/sec in the ET group (p-value of 0.004).
The mean Pre-NPC for EI was 13.97 cm and for ET 13.75 cm, with a p-value of 0.92. The mean Post-NPC for EI was 16.35 cm and for ET was 14.36 cm, with a p-value of 0.32. The mean delta NPC for EI is -1.86 cm and for ET -0.91 cm (p-value of 0.38).
The mean Pre-KD for EI was 40.20 sec and for ET 44.71 sec, with a p-value of 0.16. The mean Post-KD for EI was 40.25 sec and for ET 42.10 sec with a p-value of 0.54. The mean delta KD for EI was 0.18 sec and for ET 1.71 sec, with a p-value of 0.08. Pre/post-exertional KD testing did not show a significant difference between the two groups in this pediatric cohort.
There was significance between the EI and ET in the Post-FP as well as in the delta FP. The EI group showed no significance between the Pre- and Post-KD, along with Pre- and Post-NPC compared to ET.
Conclusion: Post-exertional vestibulocular makers may be a critical part of EI evaluation and should be tested post-exertion to objectively assess exercise tolerance. Worsening vestibular and visual markers may help identify subclinical EI, as well as help target the underlying cause of EI. Utilization of FP as a biomarker may be a more accurate measurement of neurological function, especially compared pre/post-exertion following a concussion. This marker should be encouraged in post-concussion patients and started as close to the time of insult for prime effectiveness. The NPC and KD tests may not be the best gage of recovery due to our findings. Further testing on larger populations is needed and all prior studies done on this marker should be compared to rule out any abnormality in our cohort. It is also seen that pre-exertion values in all biomarkers show no significance between the EI and ET groups. At rest, patients can perform VOMS testing regardless of their exercise tolerance. This means physical exertion is when neurological symptoms are more likely to arise.
Overall, our data can be used to improve concussion protocols, such as return to learn and return to play, allowing patients to have optimal recovery and minimizing neurological damage by following an appropriate treatment timeline. Future studies should aim for a more equally

divided population by gender to avoid biased.

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Clinical Efficacy of transcutaneous auricular vagus nerve stimulation (taVNS) in Plaque Psoriasis

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Mentor: Dr. Harald M. Stauss

Context: Plaque psoriasis affects over 10 million Americans [1]. This study explores the clinical efficacy of transcutaneous auricular vagus nerve stimulation (taVNS) as a novel non-invasive treatment that may be used in conjunction with standard of care treatment for plaque psoriasis. The use of taVNS in healthy patients has been shown to activate anti-inflammatory pathways [2,3,4]. The activation of these pathways can possibly reduce inflammation, which is an underlying mechanism in plaque psoriasis.
Objective: To determine whether the cutaneous manifestations of plaque psoriasis can be improved by using taVNS to activate anti-inflammatory pathways, in addition to standard of care treatment.
Methods: Research subjects must be 18 years of age or older with a diagnosis of plaque psoriasis and their symptoms should not be 100% improved by current treatment methods. Research subjects cannot be pregnant or wish to be pregnant, have epilepsy, vestibulocochlear nerve damage, cardiac conditions, have hearing problems or tinnitus. We plan to enroll a total of 50 patients,

which is based on a power analysis. The study has been approved by the IRB Committee of Burrell College (#0090_2021) and all study participants provided written informed consent. Psoriasis patients were recruited from the community through local advertisement and working with local dermatologists. All participants are required to self-administer taVNS or sham taVNS treatment for 30 minutes daily, for 3 months. Throughout these 3 months participants are required to report to five in-person visits. Visit 1 on day 0, will consist of assessing potential exclusion criteria through Qualtrics survey, training in operation of taVNS, daily diary keeping, and assessment of baseline parameters such as BMI, height, weight and blood pressure will be taken. An ECG will be conducted on the first visit to assess the effect of taVNS on autonomic nervous system function. Venous blood samples will also obtained to assess immune cell function through flow cytometry and cell culture experiments. Psoriatic skin lesions are assessed using the Psoriasis Area and Severity Index (PASI). A PASI score of 5-10 is defined as moderate disease and a PASI score of 10 or higher is considered severe disease. Visit 2 on day 7 consists of the same procedure in addition to discussing potential adverse effects experienced or questions related to taVNS use. Visit 3-5 are held at one-month intervals and consist of the same activities as visit 2. Immune function is assessed by measuring plasma cytokine concentrations. In addition white blood cells are isolated and the relative abundance of different cell populations are determined by flow cytometry. Finally, white blood cells are cultured and incubated in the presence of lipopolysaccharide (LPS) or placebo and release of cytokines is measured in the cell supernatant.

Osteopathic Significance: This study attempts to use the body's own structure (vagus nerve) to improve symptoms of plaque psoriasis. TaVNS can provide non-invasive treatment that allows the body to heal without the use of additional oral or topical pharmacologic agents.

Results: The study is ongoing and as of now, two patients are enrolled in the study. The first patient had a PASI score of 9.9 during the initial visit. During visit 3 (after one month of daily taVNS application), the PASI score decreased to 3.4. This patient also reported using lower amounts of the topical steroid, which was a part of their normal standard of care. This case report suggests that the taVNS was improving the efficacy of the topical steroid. The second subject enrolled in the study had a baseline PASI score of 6.2. At the second visit (after one week of daily taVNS application) the PASI score decreased slightly to 5.3. Both patients noted mild irritation of the ear where the electrode of the taVNS was placed but agreed that the discomfort was not significant enough to cause concern. This issue was addressed by alternating the stimulation site on the right and left ear on subsequent days. At the time of writing, data from the flow cytometry and cell culture experiments do not indicate any systematic changes in different cell populations or LPS-stimulated cytokine release.

Conclusion: It is too early to draw conclusions from this study due to the low number of enrolled participants. However, taVNS has been reported by the current participants to improve their symptoms and reduce the amount of medication needed to alleviate symptoms. This subjective observation is in line with the marked improvement of the PASI score in the first patient at one month into the study. The modest improvement of the PASI score in the second patient at one week into the study, suggests that an improvement in skin lesions with taVNS may need a minimum of one week or longer to be apparent. The current participants in the study have reported that they have established a routine of using the taVNS daily and it does not cause any inconvenience. The main obstacle this study is facing is the recruitment of participants for the study. Previous studies that have investigated psoriasis in the Las Cruces area have recruited subjects through extensive advertising and offering financial compensation to participants. This

could be a possible avenue for improving the recruitment of participants. Efforts to recruit participants will continue until the target of 50 participants is met.

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CT Scans of Lumbar Vertebrae Do Not Demonstrate Fryette Mechanics

Dillon R Haughton
Mentors: Dr. Ryan Rimer, Dr. Harald M. Stauss, and Dr. Adrienne Kania

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Context: The VerSe dataset [1,2,3] provides an open-source resource of lumbar vertebrae imaging. From this data I was able to generate accurate 3D models of vertebrae which maintained their orientation within living human patients [4,5]. Using the natural symmetry of these vertebrae, three planes of orientation of each vertebra could be quantified, providing excellent data to illustrate whether Fryette mechanics is indeed evident in human vertebrae.
Objective: To determine if Fryette Mechanics could be readily appreciated in radiography.
Methods: Using nothing but open-sourced python modules and software, I was able to analyze 58 CT scans form the VerSe testing data. Three planes of symmetry were calculated for each vertebra, each one representing either flexion/extension, side-bending, or rotation [6,7,8,9]. These planes were accurately determined for 256 lumbar vertebrae out of 285. The average flexion/extension

was calculated to be -3.2±10.2 degrees, with negative values representing extension. Vertebrae within the range of the standard deviation were categorized as neutral, values outside this range were categorized as flexed when positive, extended when negative. Positive values of rotation and side-bending faced right, while negative values faced left. The number of vertebrae exhibiting Fryette mechanics, classified as being neutral with opposing rotation/side-bending as well as flexed/extended with same side rotation/side-bending, were calculated, and compared to those that did not satisfy these criteria [10].
Osteopathic Significance: Validation of Fryette mechanics is necessary as osteopathic physicians continue to utilize these principles in their treatment of patients.
Results: For 256 vertebrae, 45 were flexed with 35 matching Fryette's criteria (77.7%). 28 were extended with 11 matching Fryette's criteria (39.3%). 183 were neutral with 108 matching Fryette's criteria (59%). Only 154 vertebrae out of 256 demonstrated Fryette mechanics (60.2%) overall.
Conclusion: Fryette mechanics was appreciated in a majority of lumbar vertebra in flexion and extension, though this clearly does not constitute absolution of Fryette's description of lumbar movement. Possible explanations for this could be the pathologic specimens within the database, skewing the results. Future studies on this topic will also have to identify a quantitative description of neutral, and non-neutral mechanics to verify my definitions are in fact the definitions we establish when diagnosing patients.

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Reduction in Circulatory B Cells and Natural Killer Cells by Occipito-Atlantal Decompression, Transcutaneous Auricular Vagus Nerve Stimulation, and Splenic Pump Interventions

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Context: Although the impact of anti-inflammatory biologic drugs has been profound, these drugs are costly and are associated with adverse side effects. The long-term goal of our study is to develop a cost-effective and non-invasive approach to treat inflammatory diseases. Currently there is little research regarding the effect of occipito-atlantal decompression (OA-D), transcutaneous auricular vagus nerve stimulation (taVNS), and splenic pump on the number of immune cells in circulation.

Objective: The objective of our study is to examine the immunomodulatory effects of OA-D, taVNS, and splenic pump. OA-D and taVNS have been suggested to activate the cholinergic anti-inflammatory pathway through stimulation of the parasympathetic nervous system [1-3]. We hypothesized that OA-D or taVNS can reduce the number of circulating inflammatory immune cells, potentially through activation of the vagus nerve. In addition, we hypothesized that the splenic pump can augment the effect of OA-D or taVNS via translocation of immune cells from the spleen to the systemic circulation.

Methods: This study was approved by the IRB at Burrell College of Osteopathic Medicine (IRB# 0054_2019) and all participants provided written informed consent. The participants were healthy adults of both genders. Exclusion criteria included: pregnancy; any medical condition that affects the autonomic nervous system or the immune system (e.g., autonomic neuropathy, autoimmune diseases); current drug or alcohol abuse.

At the beginning of the study, the subjects filled out a Qualtrics questionnaire to verify eligibility. A dice was used to randomly assign subjects to different groups: OA-D (n=1), taVNS (n=3), splenic pump (n=4), OA-D+splenic pump (n=5), taVNS+splenic pump (n=3), or control (n=11). Height and weight, systolic and diastolic blood pressure, heart rate, and temperature were determined. For subjects in the OA-D group, they also underwent an osteopathic screening. For OA-D, the osteopathic physician or trained medical student placed their finger pads at the junction of the occiput

and superioposterior cervical region. This was standardized to deliver 5N of pressure and was applied for 10 minutes. For taVNS (EMS 7500), a current of 2 mA, 10 Hz, and 300 μs pulse width was applied via electrodes to the ear for 10 minutes. For the control intervention, no treatment was performed. The splenic pump was performed at a rate of 30 pumps per minute and a pressure of 2-4 mmHg for 10 minutes. The pressure used was determined by the placement of a partially inflated blood pressure cuff behind the left lower rib cage. At the end of the study, blood samples were collected by a phlebotomist. The number of immune cells was detected through flow cytometry.

Flow cytometry data were analyzed using a one-way analysis of variance (ANOVA) in WinStat [4]. A post-hoc Fisher test was used if the ANOVA revealed statistical significance to compare effects of the different interventions with the control group. Results were considered significant at $P<0.05$ and considered trends at $P<0.15$.

Osteopathic Significance: Our study suggests that splenic pump and OA-D can be used as an adjunctive therapy to anti-inflammatory treatments. Because both techniques are taught to medical students, they can be easily incorporated into any osteopathic physician's practice.

Results: This study consisted of 17 subjects with a total of 27 interventions performed (some subjects had multiple interventions performed). The independent variable was the type of intervention the subject received. The dependent variable was the relative number of helper T cells, cytotoxic T cells, B cells, monocytes, and natural killer cells (percent of total immune cells). The results showed differences in both natural killer (NK) cells and B cells in some treatment groups compared to the control (B cells $0.857\pm0.110\%$; NK cells $1.398\pm0.199\%$). TaVNS was found to reduce the number of B cells ($0.358\pm0.068\%$, $p<0.05$) and NK cells ($0.855\pm0.153\%$, $p<0.15$). The splenic pump alone reduced the amount of B cells ($0.477\pm0.169\%$, $p<0.05$) and NK cells ($0.826\pm0.164\%$, $p<0.10$). The combination of OA-D and splenic pump was found to reduce B cells ($0.560\pm0.115\%$, $p<0.10$) and NK cells ($0.686\pm0.146\%$, $p<0.05$). The taVNS trial done with the splenic pump revealed a decrease in helper T cells ($1.867\pm0.663\%$, $p<0.15$), B cells ($0.367\pm0.094\%$, $p<0.05$), and NK cells ($0.640\pm0.288\%$, $p<0.05$).

Conclusion: Our hypothesis was partially proven correct as there were significant reductions in some circulating immune cells when OA-D or taVNS was performed. Contradicting our hypothesis, the splenic pump was shown to also decrease the number of immune cells in circulation. The relative number of B cells decreased significantly in the taVNS, splenic pump, and taVNS+splenic pump groups. Our results demonstrated that taVNS and splenic pump therapies may be beneficial to patients with B-cell mediated autoimmune diseases such as systemic lupus erythematosus or Hashimoto's thyroiditis [5]. Moreover, taVNS and splenic pump may be indicated in patients with inflammatory conditions treated with biologic drugs, because the reduction of B cells may also reduce antibodies against biologic drugs and, therefore, prevent treatment failure that frequently develops after chronic treatment with biologic drugs. Another interesting finding is that the number of NK cells was significantly decreased by OA-D+splenic pump or taVNS+splenic pump. Although NK cells are important for fighting viral infections and tumors, some studies suggest that NK cells play a role in the pathogenesis of certain autoimmune diseases including ankylosing spondylitis and psoriasis [6-7] One study shows that peripheral NK cells are abnormally elevated in patients with active rheumatoid arthritis [8]. Therefore, it is possible that the combinations of OA-D plus splenic pump or taVNS plus splenic pump can exert some anti-inflammatory effects depending on the types of diseases. Some limitations of this study include the low number of subjects in some experimental groups giving the results a low statistical power. The age (mean =

60.3 years) of our tested subjects also does not represent the age of the population of Las Cruces (mean = 32.3 years) [9] or the United States (mean = 38.2 years) [9] as the average age of our subjects is much higher.

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Optimization of the Osteopathic Suboccipital Decompression Technique for Induction of Parasympathetic Activation

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Context: Pressure-focused techniques have been at the forefront of many studies regarding osteopathic manipulative treatment (OMT) and autonomic nervous system (ANS) response [1,2]. Studies thus far have demonstrated physiologic effects OMT exerts on the ANS [1,3]; however, literature to-date is lacking in terms of effects of pressure modulation [4] as well as assessment of specific pressures [5]. This study seeks to reduce knowledge gaps in pressure differentials with the suboccipital decompression technique.

Objective: This study seeks to ascertain whether a relationship exists between the extent of palpatory pressures utilized in suboccipital decompression and the degree to which the parasympathetic response is activated. Results of this study would help to inform osteopathic physicians the ideal palpatory pressures to use in the suboccipital decompression technique in order to induce a parasympathetic response.

Methods: Four healthy adults received three rounds of suboccipital decompression of varied pressures (3N, 5N, or 7N) in a randomized fashion over three 6-minute periods. Randomization was obtained via random number generation (1-6) of which each number corresponded to a specific sequence of pressures. Eligibility was determined by screening for the following exclusion criteria: medical history of ANS dysfunction (i.e. diabetes mellitus, chronic headaches), use of pharmacological agents affecting the ANS, consumption of alcohol/tobacco/cocaine within 48 hours prior, or consumption of food or caffeine 2 hours prior. After consent was obtained, the following were measured and recorded: height, weight, blood pressure, heart rate, mean arterial pressure, and an estimated VO2 max. An osteopathic screening examination of the T1-4 vertebrae and occipitoatlantal junction was performed, followed by muscle energy technique and facilitated positional release, respectively, if somatic dysfunction was present. Subjects were then attached to a three-lead EKG and monitored throughout the remainder of the protocol. A 30-minute baseline recording was obtained, followed by a baseline pupillometry measurement and Valsalva maneuver. Following a 5-minute rest period, the first round of suboccipital decompression was performed for 6 minutes, in which palpatory pressure was tracked using Loadpad finger pad sensors [6] and were on average within a range of $\pm$ 0.5N from the target value. Upon completion of the decompression technique, a pupillometry measurement and Valsalva maneuver were performed, followed by a 5-minute rest period. The above was repeated for a total of 3 rounds, each with a different designated palpatory pressure. The protocol concluded with a 30-minute recovery period, after which a final pupillometry measurement and Valsalva maneuver was obtained. The study is to be performed for a total of 24 participants; preliminary EKG data analysis was completed via the Hemolab software [7] to ascertain variables including heart rate and heart rate variability, and then averaged between subjects to see a generalized trend in the data.

Osteopathic Significance: The research study focuses on utilizing the suboccipital decompression technique which works to increase vagal tone, showing the power that structure and function holds on the therapeutic response of the technique.

Results: Limited results are available due to the ongoing nature of the study. Data from 4 subjects in the experimental group has been given preliminary analysis in the way of averages to identify

potential trends. To determine autonomic nervous system interaction, mean heart rate and heart rate variability (HRV) were retrieved from EKG data via the Hemolab software. In terms of heart rate, high (7N) pressure produced an average of 69.74 bpm, medium (5N) pressure produced an average of 69.43 bpm, and low (3N) pressure produced an average of 69.64 bpm (N=4). Next, when evaluating heart rate variability, high pressure had a variability of 34.31 ms, medium pressure had a variability of 42.65 ms, and low pressure had a variability of 35.88 ms (N=4). No further analysis or significance testing was completed due to the small sample size, but in the future, pressure groups will be compared with each other in addition to a sham protocol. Pupillometry evaluation and analysis will also be included in future analyses to provide further evidence of autonomic nervous system response.

Conclusion: Overall, no significant conclusions can be drawn from the study due to inadequate sample size and subsequent lack of data collection and analysis. In terms of basic trends, the medium pressure manipulation is demonstrating the greatest decrease in heart rate and increase in heart rate variability compared to the other pressure variables. The trend of these findings allude that the medium pressure may be eliciting the greatest parasympathetic response when compared to the other pressure variables, but no direct conclusions can be drawn from this due to lack of significance testing. The study's current limitations are its sample size which will be corrected through further data collection in the coming weeks. Once done, thorough data analysis and significance testing will be completed to solidify conclusions regarding the study.

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Placental Exosomal Response to Endocrine Disrupting Chemicals

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Context: Endocrine disrupting chemicals (EDCs) are substances found in the environment and everyday products that interfere with hormone pathways [1,2]. Exosomes are extracellular vesicles secreted by the placenta involved in intercellular communication and transferring of molecular cargo to other tissues [3]. The adverse effects of EDCs on placental function can be explored by characterizing placental exosomes and their cargo [4].

Objective: To isolate exosomes from placental cells and profile the molecular cargo, specifically micro-RNA expression (miRNA), protein content, and inflammatory markers in response to EDC exposure. We sought to characterize how EDCs alter placental reactive oxygen species (ROS) generation and inflammation to identify mechanisms of placental dysfunction that relate to maternal-fetal pathologies.

Methods: The immortalized extra villous trophoblast cell line, JEG-3, was used as a model for human placental cells. Dose response curves in JEG-3 cells were determined for each EDC using a colorimetric XTT viability assay. Cells were treated in triplicate with 10 doses of di(2-ethylhexyl)phthalate (DEHP), bisphenol A (BPA) or inorganic arsenic (iAs) and viability was measured at 24- and 48-hour time points. Hydrogen peroxide (H2O2) and estradiol (E2) served as positive controls for exosome isolation. JEG-3 cells were exposed to EDCs at 2 doses with viability greater than 80% on dose exposure experiments and relevance to human exposure levels for 48 hours. Exosome isolation was performed per instructions provided by kit. Cells were incubated with EDCs for 48-hours in exosome-free media and supernatant with exosomes was collected. Media was centrifuged at 2000g for 30 minutes to remove any debris and the supernatant transferred to a new conical. Samples were mixed with exosome isolation reagent at 2:1 ratio and incubated at 4°C overnight. Incubated samples were centrifuged again at 4°C and the resulting exosome pellet was resuspended in 50 μL of 1x PBS and stored at –20°C for further analysis. Whole cell lysates were also collected for future analyses per standard protocol. Trypsinized cells were counted and washed twice in ice cold PBS. Samples were incubated on ice in lysis buffer containing 1mM PMSF protease inhibitor and 0.5 mL of pre-aliquoted 1 μg/mL aprotinin and 0.5 μg/mL leupeptin and then recentrifuged to remove debris. Supernatant with clarified whole cell lysate was stored at –20°C for further analyses. Exosomal protein cargo was quantified using the BCA assay per BCA assay kit protocol. Each sample was incubated in the working reagent provided with the kit at 37°C for 2 hours and absorbance was measured at 562 nM for each sample. Absorbance at 562 nM of bovine serum albumin (BSA) serial dilutions were used as standard for calculating final protein concentrations for each condition.

Osteopathic Significance: The allostatic load imposed by endocrine disrupting chemicals is an understudied phenomenon that impacts both maternal and fetal health. Mother and fetus are reciprocally intertwined systems that utilize the placenta as mediator. Thus, understanding how

placental exosome signaling may be altered by EDCs and the self-regulatory processes this may interfere with, is highly relevant to osteopathic philosophy and practice.
Results: BPA 24- and 48-hour treatments had an IC20 of 150 μM and 55 μM, respectively. Dose curves for both treatments were non-linear with average viability less than 100% at the lowest dose. A sharp decline in viability was seen after IC20 on 24-hour treatment and a more gradual decrease was seen in the 48-hour treatment group after IC10 of 5 μM. 24- and 48-hour DEHP treatment dose curves were non-linear like BPA. However, both DEHP treatment groups showed an average of 100% growth or greater with a sharp increase at approximately 7 μM and a subsequent sharp decrease in viability at doses greater than approximately 170 μM. IC20 was approximately 550 μM for both treatments of DEHP. Both DEHP and BPA required extremely high doses to decrease viability. 24- and 48-hour treatments for iAs had linear trends when compared to DEHP and BPA and had a smaller average IC20 at approximately 4 μM for both treatments. A gradual decline in viability was observed at doses greater than 0.9 μM iAs and 1 μM iAs at 24- and 48-hour time points, respectively. For the positive controls, H2O2 and E2, viability was decreased and increased, respectively at the single dose tested. Using dose response data and published human exposure data, 2 doses per EDC were selected for exosome isolation studies. Exosomes were isolated after 48-hour treatments of 0.1 μM and 2 μM of iAs, 0.1 nM and 10 μM of BPA, or 10 nM and 100 μM of DEHP. Following standardization of data, exosomal protein content was highest in the samples treated with 1 mM H_2O_2, followed closely by untreated and 1 nM E2-treated cells.
Conclusion: Exosomes from EDC-treated JEG-3 cells were isolated as well as their corresponding whole cell lysates. Total exosomal protein content was quantified. We found that among the total protein concentration of different doses of EDCs, 2 μM iAs was higher than 0.1 μM iAs, 10 μM BPA was higher than 0.1 nM BPA, and 10 nM DEHP was higher than 100 μM DEHP, while the estradiol and hydrogen peroxide controls were higher than each EDC tested. In the future we aim to quantify the concentration of exosomes and further characterize the changes in inflammatory markers and apoptotic markers in exosome cargo. As exosomes are mediators of intercellular communication, the changes in these markers can provide insight on how EDC exposure impacts placental cell function.

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Potential Implications of Restraint Stress and Estrogen Modulation on Food Addiction

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Context: Obesity rates are high in the U.S., with greater rates of severe obesity in women [1,2,4]. Women suffer more from food addiction and craving, which has been associated with increased activity in the insula of the brain [3,4,5]. Heightened responses to stress in women impact food intake and predispose for addiction [3,4,5]. The hormonal profiles of women likely play a role, and the fact that responses to food cues vary throughout the menstrual cycle suggests that estrogen plays a role in the modulation of food cravings [5].
Objective: To determine the effects of estrogen and stress on neuronal activation in the insular cortex of the brain in adult female rats. We hypothesized that exposure to acute restraint stress would increase Fos protein expression in the insular cortex of intact females, and this effect would habituate with chronic restraint stress exposure. We further hypothesized that ovariectomy (OVX) would abolish the acute stress response, which would be restored following estradiol replacement.
Methods: All animal procedures were conducted at the University of Texas at El Paso, in accordance with the Public Health Service Guide for the Care and Use of Laboratory animals and under a protocol approved by the Institutional Animal Care and Use Committee. Female Sprague Dawley rats were individually housed in a climate-controlled vivarium with food and water available *ad libitum*. In adolescence, prior to the initiation of estrus cycling, the rats underwent OVX or sham surgery, with different groups receiving subcutaneous implants of pellets of vehicle or low- or high-dose 17β-estradiol. As adults, the rats were exposed to emotional stress in the form of acute or repeated restraint by placement in a plastic restraining device for 30 min/d for 1 or 14 consecutive days, respectively. Control rats were exposed to open restraining devices daily, but never restrained. Post-stress, the rats were transcardially perfused with ice-cold 4% paraformaldehyde and brain tissues collected and sectioned on a freezing tabletop microtome. Free-floating coronal sections of 30 μm thickness were stained immunohistochemically for Fos protein using a peroxidase method and mounted on gelatin-coated slides. Sections were photographed on a light microscope coupled to a digital imaging system, and analyzed using ImageJ software. Counts of positively-stained cells were taken from 4-9 sections throughout the rostrocaudal extent of the insular cortex. Average counts were calculated and compared across treatment groups by t-test, with $p \leq 0.05$ considered significant.
Osteopathic Significance: Osteopathic medicine focuses on the intrinsic healing capacity of the body. It is important to understand how hormones and stress affect obesity, along with diet and behavior, to improve outcomes without surgery or medications.
Results: Acute restraint stress increased the number of neurons expressing Fos in the insular cortex of intact adult female rats (50.9 ± 4.5), compared to Controls (69.7 ± 10.0), but this effect was non-significant (p=0.07). Repeatedly stressed animals did show a significant habituation of the acute stress response (29.0 ± 7.5; p=0.02 vs. Control, p=0.01 vs. Acute). OVX abolished the responses to both acute (39.4 ± 3.9) and repeated (44.1 ± 5.3) stress without changing the basal level of insular cortex Fos expression in Control animals (39.2 ± 5.0; p=0.1). Low dose replacement with estradiol did not rescue the stress response; data from animals replaced with a higher dose of estrogen have not yet been analyzed.
Conclusion: Our data suggest that estrogen plays a significant role in mediating the responses to

acute and repeated stress in the brain, particularly in the insular cortex which has an extensive role in food processing and is involved in addiction. Given that estrogen replacement in OVX rats did not restore normal Fos expression, it is possible that progesterone is another critical factor. The low number of animals per treatment group in our study so far limits our confidence in these results. Additional work will expand the number of sections analyzed for each animal, and total counts rather than average counts per rat will be compared. However, disruptions in estrogen secretion have been seen in both overweight and underweight individuals, highlighting the importance of this hormone in feeding and metabolism. By understanding the effects of stress on a brain region associated with food and other addictions, as well as the modulating effects that estrogen has on the stress response, we could develop treatment plans which might aid patients in choosing more nutritious foods, consuming less, and improving pathologic outcomes.

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Effects of Early Life Stress on the Development of Anxiety in Adulthood

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Context: Adverse childhood experiences (ACEs) include intense, frequent, or sustained stress exposures that occur in early life [1]. Without a supportive adult or environment to buffer their effects, ACEs predispose an individual to behavioral and mental health disorders as well as other chronic health problems later in life [2]. Greater exposure to adversity is correlated with poorer outcomes. This is due, at least in part, to the dysregulation of metabolic, immune, and neuroendocrine function [3].

Objective: We used neonatal maternal separation as an ACE model in rodents, to determine if this early life stressor would lead to the development of anxiety-like behavior in adults. Both sexes of rats were tested, since anxiety is more prevalent in human females [4]. An increased understanding of how ACEs affect behavior may improve our ability to screen for early life adversity and help us treat patients with ACE-related conditions more effectively.

Methods: All animal procedures were conducted at the University of Texas at El Paso, in accordance with the Public Health Service Guide for the Care and Use of Laboratory animals and under a protocol approved by the Institutional Animal Care and Use Committee. Wistar rat litters were born in a climate-controlled vivarium and either maintained as controls (Con) or subjected to neonatal maternal separation (MatSep). Con pups were reared normally, with regular handling, until weaning on postnatal day (PND) 21. Pups in MatSep groups were physically separated from their mothers for 3 hrs/day on PND 2-14, then weaned on PND 21. Post-weaning, all rats were housed in pairs and allowed to grow to adulthood with access to food and water and no other disturbance. Adult male (n=16) and female (n=24) Con and MatSep rats were tested for basal anxiety-like behavior in an open field apparatus. Experimenters placed individual rodents into the open field and video recorded their behavior for 5 minutes. Blinded observers documented the animal behaviors including: defecation, rearing, grooming, freezing, and locomotion. Time spent in the periphery versus the center of the apparatus was also measured. Behavioral data were grouped by sex and treatment, and statistically compared by t-test with significance determined at the $p \leq 0.05$ level.

Osteopathic Significance: Understanding how self-regulating body systems are disrupted by early life stress supports rational treatment and is essential in addressing root causes of toxic stress-related health issues. This work will inform screening and prevention methods.

Results: Behavioral analysis was completed for a total of 40 adult rats in the following groups: Con male, n=10; MatSep male n=6; Con female n=9; and MatSep female n=15. Of the behaviors analyzed, only three statistically significant differences were found. The number of grooming events was reduced in MatSep compared to Con females (MatSep average = 1.73; Con average = 2.67; p=0.03). A similar relationship was noted in the males, with the MatSep group grooming less often (average = 0.5) than the Con group (average = 1.5) (p=0.02). The amount of time spent grooming, however, did not differ significantly across groups. Of particular note was the finding that locomotor behavior was significantly decreased in MatSep males (average = 210.8 lines crossed) compared to Con males (average = 261.6 lines crossed) (p=0.01). No differences were seen in rearing or freezing behavior, and very little freezing behavior was seen by any of the rats in this study. Number of defecations was equivalent in all animals. The amount of time any of the rats spent exploring the center of the open field versus the perimeter of the apparatus also was unchanged

by MatSep.

Conclusion: Some of our results indicate an increase in anxiety-like behavior in our study. This is consistent with previously published studies on child neglect or parental separation that demonstrate a link between ACEs and the development of anxiety in adolescents and adults [5]. Surprisingly, the male rats in our experiment showed slightly more anxiety-like behavior than the females after exposure to MatSep. This model of early life stress is similar to attachment disruption between a child and their primary caregiver, an ACE category in humans that has been shown to be predictive of child emotional or behavioral problems [6]. It should be noted that we evaluated basal anxiety-like behavior in animals that experienced the early life stress of maternal separation many weeks before the behavioral tests were done. It will be interesting to determine the response of these animals if another stressor is applied before the behavioral test. Future work in our laboratory will test anxiety-like behavior in MatSep rats subjected to a 30-minute restraint stress prior to the open field analysis. In addition, the effects of MatSep on adolescent and aged rat behavior will also be tested.

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Parasympathetic Activation During Exercise Recovery and in Response to Transcutaneous Auricular Vagal Nerve Stimulation is Directed Towards Different Target Organs

Brian Beyer, Kevin Dwyer, Jordyn Mullins, Anjali Patel and Cody Sheppard
Mentors: Dr. Pedro Del Corral and Dr. Harald M. Stauss

Context: Lifestyle changes that increase parasympathetic activity (PSA), notably exercise, reduce cardiovascular mortality, the number one killer in the US [1]. Some patients, including those with advanced heart failure are unable to utilize exercise [2] to enhance PSA. Heart rate variability (HRV) studies suggested that transcutaneous auricular vagus nerve stimulation (taVNS) increases PSA [3]. However, newer studies failed to confirm these studies [4]. Thus, alternative methods to assess PSA are needed.

Objective: The aim of this study was to identify reliable methods to assess changes in PSA in response to taVNS and to contrast these changes to those elicited by aerobic exercise. To achieve this aim, we tested the hypothesis that HRV, phase 4 of the Valsalva maneuver, the cold face test, and the pupillary light responses are equally capable of assessing PSA following aerobic exercise [5,6] and that taVNS results in similar changes in PSA than that observed following aerobic exercise.

Methods: Subjects were young (22-30 years old) and generally healthy.

Exercise Protocol: Baseline heart rate (HR) was measured for 20 minutes with a Garmin HR Monitor that provides beat-by-beat HR values. After assessing baseline PSA by HRV, pupillometry, VM, and cold face test, subjects started a 30-min exercise intervention during which blood lactate concentration and Borg's Rate of Perceived Exertion was determined at 5 and 25 minutes into exercise. PSA was assessed at 5, 20, 40, and 60 minutes post-exercise using the same techniques as baseline. 2 female and 10 male subjects were part of the study. The exercise intervention used a cycle ergometer at 70% of the % estimated maximum HR (208-0.7*age bpm).

TaVNS Protocol: Baseline HR was measured for 20 minutes using an EKG. Thereafter, baseline PSA was assessed by HRV, pupillometry, VM, and cold face test, followed by a 15-min taVNS intervention. Ten minutes into the taVNS intervention PSA was assessed as before. Immediately after taVNS, pupillometry was conducted and 15 min post taVNS PSA was assessed again as before. Seven male and four female subjects were included. TaVNS was done with a Transcutaneous Electrical Nerve Stimulator (TENS 7000, 2-3 mA, 10 Hz, 300 ms).

Pupillometry: Outcome parameter: percent change (constriction) in pupillary diameter in response to light flash (Neuroptics PLR-3000 pupillometer).

VM: Exhalation into a mouthpiece while maintaining 40 mmHg pressure for 15 seconds. Outcome parameter: HR decline during phase 4.

Cold Face: A frozen towel was placed on the face for two minutes. Outcome parameter: difference between maximum and minimum HR response.

HRV: Outcome parameter: Root Mean Square of Successive Differences (RMSSD, HemoLab software), which largely depends on PSA.

Statistics: Data were analyzed by one-way ANOVA for repeated measures followed by Fisher post-hoc tests (WinStat). Significance was assumed for $P<0.05$. Data are presented as means±SEM.

Osteopathic Significance: Osteopathy is based on the principle that the body is capable of self-healing. Low PNSA has been implicated with higher cardiovascular risk. Enhancing PNSA by taVNS may lower cardiovascular risk through activation of self-healing mechanisms.

Results: Autonomic Response to Exercise: Compared to baseline, a shift in autonomic balance towards sympathetic dominance was observed during exercise (VM: 43±5 bpm vs. 52±5 bpm, P<0.05; pupil response: 40.5±1.8% vs. 45.9±1.5%, P<0.05; RMSSD: 0.42±0.05 ms vs. 2.22±0.26 ms, P<0.05; cold face test: not performed during exercise). Compared to baseline, autonomic balanced switched to parasympathietc dominance at 60 minutes post-exercise, as indicated by an increase in RMSSD (2.78±0.52 ms vs. 2.22±0.26 ms, P<0.05). At this time point (60 min post-exercise) neither the pupillary light response, the VM, nor the cold face test detected an increase in PSA compared to baseline.

Autonomic Response to taVNS: No change in autonomic tone was detected during the application of taVNS by any of the applied methods. However, a shift in autonomic balance towards parasympathetic dominance was observed at 30 minutes following taVNS compared to baseline (cold face test: -10.2±1.9 bpm vs. -3.4±1.6 bpm, P<0.05; pupil response: -47.6±1.6% vs. -45.3±1.5%, P=0.06, VM: not significant). However, consistent with a recent meta-analysis [4], the HRV parameter RMSSD was not able to detect this increase in PSA following application of taVNS.

Conclusion: In summary, all employed autonomic function tests, including the Valsalva maneuver, the pupillary light response, and HRV reliably detected the increase in sympathetic nervous system activity during exercise. However, the post-exercise increase in PSA that is well documented in the literature [9], was only detected by the HRV parameter RMSSD, but not by any of the other autonomic function tests, including the Valsalva maneuver, the pupillary light response, or the cold face test. In contrast, the increase in PSA following application of taVNS was only detected by the cold face test and the pupillary light response, but not by the HRV parameter RMSSD. The lack of RMSSD to detect the increase in PSA following taVNS is in line with a recent meta-analysis [4].

These findings suggest that parasympathetic activation during recovery from exercise affects different target organs than parasympathetic activation in response to taVNS. Following exercise, parasympathetic tone seems to be directed towards the cardiovascular system and specifically the heart, whereas parasympathetic activation in response to taVNS seems to be directed towards other organ systems, such as the oculomotor system, the trigeminal nerve that mediates the cold face response, and possibly other systems, such as the immune system, that were not assessed in this study.

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Awards

The first prize winner will receive a travel award (up to $2,500) to present their data at a national conference selected by the winner in consultation with the mentor.

Eligibility for Poster Competition

SRE Projects: Only abstracts resulting from Summer Research Experience (SRE) projects (one abstract per SRE project) were considered.

Timely Submission: Only abstracts that were submitted on or before the announced deadline were considered.

Adherence to Abstract Guidelines: Only abstracts that adhered to the instructions included with the abstract template, including maximal number of characters, were considered.

Judging Criteria

Abstract Scoring: The judges scored the abstracts based on the criteria used for the American Osteopathic Association OMED conference, which assess the Context, Research Question/Hypothesis, Research Methods, Data Analysis/Results, and Conclusion Sections.

Poster Presentation Scoring: The judges will score the poster presentations according to the Poster Presentation Rubric listed below.

Finalist Presentation Scoring: Based on the abstract scoring and poster presentation scoring, the judges will select five finalists. During the finalist presentations, the judges will consider the depth of knowledge of the presenters and the readiness of the research study for presentation at a national conference.

Poster Presentation Rubric

Standards	Exemplary (5-4)	Satisfactory (3-2)	Unacceptable (1-0)
Content	Strong material. Well summarized. Clearly shows development of study or research. Material appears to accurately support purpose of study, hypothesis, or research question. Strong conclusion and implications presented.	The content was adequately presented but support for the study, research hypothesis, or question(s) is somewhat general. Conclusion and implications were reasonable.	Connection not found between poster content and purpose of study, research hypothesis/question(s), method, conclusions, or implications.

Depth of knowledge	Demonstrates substance and depth; is comprehensive; shows mastery of material, main points were clearly presented.	Covers topic; shows marginaladequate mastery and is objective; main points were adequately presented.	Does not give adequate coverage of topic; poor mastery of subject, main points were poorly presented.
Organization of content	Presentation is strongly ordered and easy to follow; visual elements (if any) are clearly arranged and synchronized with presentation.	Presentation order and clarity is of acceptable quality; slightly difficult to follow; visual elements (if any) are somewhat arranged and synchronized with presentation.	Presentation order and clarity of transitions is of poor quality or below; visual elements (if any) may be difficult to follow or out of synch with the presentation.
Delivery and clarity of presentation	Has natural delivery; modulates voice; is articulate; projects enthusiasm, interest, and confidence.	Has appropriate pace; has few distracting mannerisms; is easily understood.	Is often hard to understand; has voice that is too soft or too loud; has a pace that is too quick or too slow; demonstrates several distracting mannerisms.
Ability to respond to questions	Demonstrates full knowledge of topic; explains and elaborates on all questions.	Shows ease in answering questions but does not elaborate.	Demonstrates little grasp of information; has undeveloped or unclear answers to questions.

Teams of Judges

There will be two teams of judges. Each team of judges consists of two faculty judges and a student judge. The student judges have been selected based on prior year's participation in the Summer Research Experience and other research-related credentials, such as participation in the Distinction in Research Program. Each judge will score all eligible abstracts. Each poster presentation will be judged by one team of judges.

Team No.	Faculty Judge 1	Faculty Judge 2	Student Judge	Posters	Time
1	Dr. Cindy Funk	Dr. Gabor Szalai	Cristina Lee	P01-P05	9:30-11:10
2	Dr. Marc Benson	Dr. Scott Ochs	Dillon Haughton	P06-P10	10:10-11:50

Author Index

Equipped with his five senses, man explores the universe around him and calls the adventure Science.

Edwin Powell Hubble (1889 - 1953)

AUGUST 13, 2022

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