The Office of the VP for Research & Biotechnology presents
The eighth annual
Student Technology and Research Symposium 2015
Tuesday, August 4
8 a.m. - 5 p.m. lunch: 11:45 a.m. rsvp

Swift Hall (Rodney Weinberg/URC Building)
8:00 a.m. to 5:00 p.m.
The 2015 Summer Grant Fellowships Research Presentations
The Student Technology and Research Symposium (STRS) is the culmination of a year’s worth of professional education and a summer of research under the direction of a principal investigator.

Students featured in this program have worked diligently to research problems affecting the modern world. The subjects of research span across the disciplines. All research at WesternU falls into one of five research clusters: Integrative Neurobiology, Molecular & Metabolic Diseases, Infectious Diseases & Immunology, Evolutionary Biology, and Lifestyle Medicine.

Research is part of teaching our healthcare professionals how to find the facts that lead to wise healthcare decisions. Nothing can replace the experience of first-hand knowledge of the experimentation, study, literature searching, and review of the methods used to create scientific conclusions.

To this end, we celebrate our eighth year of Summer Grant Fellowships at Western University of Health Sciences. This year we would like to acknowledge the following sources of financial support to the student: Summer Student Research Fellowships (supported by WesternU), The National Institutes of Health (NIH), Tri City Mental Health Grant – Urban Mission Project, Kure It Rivals United Team Science Award; Hyundai Hope On Wheels Scholar Research Grant; St. Baldrick’s Foundation Scholar Award, and The Morris Animal Foundation.

Tom Phillips, DVM  
Managing Director, WesternU Ventures,  
STRS Program Coordinator

Steven J. Henriksen, PhD  
Vice President, Office of Research & Biotechnology
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Shana Feinberg, College of Osteopathic Medicine, Graduation Year: 2018, Advisor’s Name: Dr. Jesus Sanchez, Advisor’s College: College of Osteopathic Medicine, Source of Funding: None, Evaluating the Effect of Osteopathic Manipulative Techniques on Diabetic Foot Ulcers: Wound Healing, Blood Flow, Neuropathy and Inflammatory Biomarkers, Author List: Shana M. Feinberg, BS, Jesus Sanchez, DO, MA, Michael Seffinger, DO, Vishwanath Venketaraman, PhD

Hannah Kang, College of Osteopathic Medicine, Graduation Year: 2018, Advisor’s Name: Dr. Airani Sathananthan, MD, MSHS, FACE, FACP, Advisor’s College: College of Osteopathic Medicine, Internal Medicine, Source of Funding: Summer Student Research Grant STRS, Patients’ Perception of Hypertension, Author List: Hannah Kang, BS, Airani Sathananthan, MD, MSHS, FACE, FACP

Matt McKee, Jon Wheelwright, Bernard Roscoe, Jay Anderson & Sean Wooding, College of Osteopathic Medicine – Northwest, Graduation Year: 2018, Advisor’s Name: Dr. Goering, Advisor’s College: Osteopathic Medicine, Source of Funding: Summer Grant, A heritage project: Developing protocols for ultrasound-guided needle aspiration of donor cadaver patient’s joints to educate medical students and analyze synovial fluid for biomarkers of inflammation correlating to leg length inequality., Author List: Matt McKee, Jon Wheelwright, Bernard Roscoe, Jay Anderson, Sean Wooding, Dr. Kisby & Dr. Goering

Tanya Duong, Graduate College of Biomedical Sciences, College of Dental Medicine, Graduation Year: 2015, 2019, Advisor’s Name: Dr. Pen-Jen Lin, Advisor’s College: Graduate College of Biomedical Sciences, Source of Funding: WesternU Startup Fund, The Role of BiP and PDI in the Endoplasmic Reticulum Associated Degradation of Misfolded Proinsulin, Author List: Tanya Duong, Anna-Marie Kelemen, Pen-Jen Lin

Danny Plyler, Ankur Gill, College of Podiatric Medicine, Graduation Year: 2018, Advisor’s Name: Dr. David Shofler, Advisor’s College: College of Podiatric Medicine, Source of Funding: WesternU Summer Research Grant, Medi-Cal Managed Care: Coverage of Podiatric Medicine in the State of California, Author List: Danny Plyler BS, Ankur Gill BS, David Shofler DPM

Assal Nour, College of Podiatric Medicine, Graduation Year: 2018, Advisor’s Name: Dr. Shofler, Advisor’s College: College of Podiatric Medicine, Source of Funding: Summer Research Fellowship, WesternU, Antibiotic Treatment for Diabetic Foot Osteomyelitis: A Systematic Review, Author List: Assal Nour

Peyman Danesh, College of Podiatric Medicine, Graduation Year: 2018, Advisor’s Name: Dr. Jennifer D’Amico, Advisor’s College: College of Podiatric Medicine, Source of Funding: None, Incidence of Depression Among Students of Podiatric Medicine, Author List: Peyman Danesh BA, Jennifer D’Amico DPM

Tina Nikoomanesh, Graduate College of Biomedical Sciences, College of Dental Medicine, Graduation Year: 2015, 2018, Advisor’s Name: Dr. Melanie Goldfarb, Advisor’s College: John Wayne Cancer Institute of St. John’s Hospital, Source of Funding: Summer research fellowship, Emotional Distress in Young Surgical Cancer Patients, Author List: Tina Nikoomanesh BA; Dr Melanie Goldfarb MD, FACS
Rachel Williams, College of Veterinary Medicine, Graduation Year: 2017, Advisor’s Name: Dr. Dominique Griffon, DMV, MS, PhD, DECVS, DACVS, Advisor’s College: College of Veterinary Medicine, Source of Funding: WesternU Summer Research Grant, Factors influencing cosmetic results of surgical wound closure in dogs and cats, Author List: Dr. Wanda Gordon-Evans, Dr. Dominique Griffon, Rachel Williams

Zachary Gustin, College of Podiatric Medicine, Graduation Year: 2018, Advisor’s Name: Dr. Jonathan Labovitz, Advisor’s College: College of Podiatric Medicine, Source of Funding: Summer Grant, Effect of Hospital Characteristics on Cost of Diabetic Foot Care, Author List: Dr. Jonathan Labovitz DPM, Dr. David Shofler DPM, Zachary Gustin podiatric medical student

Jeffrey Lo, College of Graduate Nursing, Graduation Year: 2017, Advisor’s name: Jan Boller, Ph.D, RN, Advisor’s College: College of Graduate Nursing, Source of Funding: TriCity Mental Health Grant – UrbanMission Project, UrbanMission Nutritional Education and Wellness, Author List: Jeffrey Lo, B.Sc., Jan Boller, Ph.D, RN

Chris Tatum, College of Podiatric Medicine, Graduation Year: 2018, Advisor’s Name: Dr. David Shofler DPM, Advisor’s College: College of Podiatric Medicine, Source of Funding: Summer Grant, Developing a Charcot Foot Radiograph Repository, Author List: Chris Tatum BS, Dr. David Shofler DPM

Allison Salinger, College of Veterinary Medicine, Graduation Year: 2018, Advisor’s Name: Dr. Faramarzi, Advisor’s College: College of Veterinary Medicine, Source of Funding: Morris Animal Foundation, The development of the distal limb in the foal, Author List: Alli Salinger BSc, Babak Faramarzi DVM, MSc, PhD

Daniel Gutman, College of Veterinary Medicine, Graduation Year: 2017, Advisor’s Name: Dr. Susana Tkalcic, Advisor’s College: College of Veterinary Medicine, Source of Funding: Summer Research Fellowship, Volcanic Gastric Ulcers in California Sea Lions, Author List: Tkalcic, S., Gutman, D., Palmer, L.

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Matt Cikra and Michael Read, CPM, Graduation Year: 2018, Advisor’s Name: Dr. Janelle Green, Advisor’s College: CPM, Source of Funding: Student Summer Research Grant, Does Depression Affect Diabetic Foot Ulcer Healing: A Systematic Review, Author List: Incomplete

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Stephanie Campbell & Meghan Blanchet, College of Podiatric Medicine, Graduation Year: 2018, Advisor’s Name: Dr. Rebecca Moellmer, DPM, Advisor’s College: College of Podiatric Medicine, Source of Funding: Western University of Health Sciences, Transmetatarsal
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Madison McKay, College of Veterinary Medicine, Graduation Year: 2018, Advisor’s Name: Dr. Babak Faramarzi, Advisor’s College: Western University College of Veterinary Medicine, WesternU Summer Grant Fellowship, Quantitative Analysis of the Equine Distal Limb, Authors: McKay, M. and Faramarzi, B. ..........................................................63
Kayla Ross, College of Veterinary Medicine, Graduation Year: 2017, Advisor’s Name: Dr. Joe Bertone, Advisor’s College: College of Veterinary Medicine, Source of Funding: Summer Student Fellowship Grant, Equine Sidewinder Syndrome: A Review of Twenty-Eight Cases Author List: K. Ross, C. Fenger, J. Bertone ..........................................................64
Emily J. Trumbull, Veterinary Medicine, Graduation Year: 2017, Advisor’s Name: Malika Kachani, DVM, PhD; Hannah Mirrashed, PhD, Advisor’s College: Veterinary Medicine, Source of Funding: Summer Research Fellowship, Toxoplasma gondii Antibody Seroprevalence in Veterinary Students at Western University of Health Sciences, Author List: Emily Trumbull, Hannah Mirrashed, Cesar Ochoa, Jessica Coote, Kevin Barber, James Reynolds, Helen Engelke, Malika Kachani ................................................................................................................64
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(Gorby) Divvijot Singh, Student’s College: College of Pharmacy, Graduation Year: 2018, Advisor’s Name: Dr. Nazarian, Advisor’s College: College of Pharmacy, Source of Funding: WesternU Pharmaceutical Sciences, Pain Evoked Neuronal Activation In the Amygdala and the Bed Nucleus of Striata Terminalis of Formalin Treated Rats., Author List: (Gorby) Divvijot Singh BS, Arbi Nazarian PhD, Alex Armendariz BS ........................................................................67
Danny Wang, College of Podiatric Medicine, Graduation Year: 2018, Advisor’s Name: David Shofer, DPM, Advisor’s College: College of Podiatric Medicine, Source of Funding: TBD, Investigating the Use of Prolia (Denosumab) in the Treatment of Acute Charcot Neuroarthropathy: A Phase 1 Trial, Author List: Danny H. Wang BS, Jessica Lin BS, Kevin Ragothaman BS, David Shofer DPM ........................................................................68
Dana Lin, College of Podiatric Medicine, Graduation Year: DPM 2018, Advisor’s Name: David Shofler, DPM, Advisor’s College: College of Podiatric Medicine, Source of Funding: Western University of Health Sciences Summer Research Fellowship, The combine effects of social history, culture, and social support in diabetic foot care management among Latinos, Author List: Dana Lin, BS, David Shofler, DPM ..........................................................70

Michael Czulinski, College of Osteopathic Medicine, Graduation Year: 2018, Advisor’s Name: Dr. Glen Kisby, Advisor’s College: College of Osteopathic Medicine, Source of Funding: COMP Start-up Funds, Effect of environmental chemicals on tau and synuclein expression in human neural stem cells, Author List: Michael E. Czulinski, Morgan Florek..........................71

Summer Research Grants, Project Summaries

Christi Waer, Graduate College of Biomedical Sciences, Graduation Year: 2016, Advisor’s Name: Dr. Jill Lewis, Advisor’s College: College of Dental Medicine, Source of Funding: CDM Start-up Funds and Western University of Health Sciences Intramural Research Grant, Exploring a Novel Combination Treatment for Oral Squamous Cell Carcinoma: Effects on Cellular Signaling and Proliferation, Author List: Christi Waer, Zohra Tumur, Carlos Guerra, Bradley Henson, Jill Lewis

Despite advances in treatment options for patients with head and neck squamous cell carcinoma (HNSCC), the morbidity and mortality of the disease continue to affect many patients around the world. Many current treatment methods do not take into account the biologic behavior of individual tumors, and current treatment approaches are known for their toxic and disfiguring effects. Active components of natural foods and other noninvasive treatment methods, such as photodynamic therapy, are increasingly receiving attention for their chemopreventive and chemotherapeutic potential in a wide variety of cancers. Rosmarinic acid (RA), a phenolic compound in various herbal plants, is well recognized for its anti-oxidant and anti-inflammatory properties. It has also been shown to have growth-inhibitory and anti-inflammatory effects in colon, skin, breast and ovarian carcinomas, but the mechanisms underlying these effects are poorly understood. Even though the chemopreventive role of RA in HNSCC has been described in 7,12-dimethylbenz(a)anthracene-induced hamster buccal pouch carcinogenesis, a chemotherapeutic role for RA in the treatment of HNSCC has not yet been reported. Blue light (400-500 nm) has been shown to have selective toxicity for tumor cells through NRF2-mediated up-regulation of phase 2 genes that neutralize oxidative stress and metabolize electrophiles. Recent studies in our laboratory have shown that blue light treatment of A431 epidermoid carcinoma cells leads to decreased proliferation and levels of oxidized proteins in vitro and reduced tumor growth in vivo, however no other studies exist to date that explore the potential therapeutic effects of blue light in HNSCC. Importantly, the combination of these two treatment methods as a non-invasive anti-tumor treatment is completely novel. Hence, the hypothesis of this proposal is that combined rosmarinic acid and blue light treatment (RA-BL) will work synergistically or additively to significantly decrease cell proliferation, migration/invasion, oxidative stress and cell survival in HNSCC cells. Oral carcinoma is in a group of head and neck cancers that only have an estimated 50% survival rate at 5 years. These forms of cancer can be easily treated in early stages with chemotherapeutic drugs, like Cisplatin. Unfortunately, most of the diagnoses are in late stages when the cancer has already metastasized to neck lymph nodes. Patients who recover from a primary tumor have a 20 times higher risk of developing a second cancer due to the tumors’ aggressive nature. There are many contributing factors to oral cancer growth, but early detection is the best way to control the progression of the disease. Rosmarinic acid (RA) and blue light have both shown antioxidant, anti-inflammatory and anticancer benefits in various research studies leading to a great potential for these compounds to be considered as therapeutics in the future. The idea of creating an adjuvant cocktail with almost no side effects is extremely appealing, especially for chemoprevention. The creation of a reliable cocktail combination could lead to a lower dose of chemotherapy being administered and the possibility of reduced chemotherapeutic side effects.

This idea has led to our hypothesis that predicts RA combined with blue light will work synergistically or additively to significantly decrease cell proliferation, migration/invasion, oxidative stress and cell survival in HNSCC cells. Cell signaling studies are utilized to help understand the mechanisms behind the treatment. Proliferation was examined through the ERK pathway. AKT was used to assess the level of apoptotic signaling. Assessment of cell signaling molecules will give way to understanding which proteins are up regulated and down regulated. By understanding these protein signals we will be able to identify other proteins within correlating pathways either upstream or downstream from the signals we have already verified. This will hopefully lead to an outstanding new discovery of the mechanisms behind combination cocktails like blue light and RA.
**Study Methodology:** 1. **Cell Culture:** HNSCC cells were grown in DMEM with 10% FBS and Pen-Strep and incubated at 37°C with 5% CO₂. Cells were sub-cultured to 70% confluency and were plated into Corning 24-well plates at a density of 150,000 cells/well. Once attached, cells were serum-deprived for 2h in DMEM with Pen-Strep prior to treating with blue light (60 J/cm², 2 min). Cells were incubated for 1h at 37°C with 5% CO₂ then treated with RA (80 μg/ml in serum-free DMEM). One hour after RA treatment, cells were harvested in a cocktail of RIPA buffer, HALT Phosphatase and Protease inhibitors and proteins were quantified with the Bradford Protein Assay. 2. **Western Blot Analysis:** Proteins (25 μg) from each sample were electrophoresed on 8 or 12% SDS-PAGE gels and transferred to PVDF membranes. Membranes were blocked for 1 hr at room temperature prior to addition of primary antibodies (Erk, p-Erk, Akt, P-Akt, GAPDH) and incubated overnight in 4°C. Secondary anti-rabbit or anti-mouse IR-conjugated antibody was added at a dilution of 1:10000 for 1hr at room temperature. Proteins were visualized on the Li-Cor Odyssey Imaging System and quantified using ImageJ Software. All protein specific bands were normalized to GAPDH loading control. 3. **Statistical Analysis:** GraphPad Prism Software was used to determine the ordinary one-way ANOVA statistical significance with a p<0.05.

**Results:**

![Graph showing P-AKT, T-AKT, and GAPDH levels across different conditions](image1)

Figure 1

![Graph showing P-ERK, T-ERK, and GAPDH levels across different conditions](image2)

Figure 2

**Conclusions:** Blue light upregulated the activation of Akt and Erk possibly due to an increase in ROS.

Activation of Akt and Erk were decreased with rosmarinic acid’s antioxidant effect creating a decrease in cell proliferation and apoptosis.

Akt/Erk activated returned to control levels possibly keeping the proteins in a controlled state to limit cell proliferation with the combination treatment.

**References:**

Laura Capelle and Eileen Yang, College of Optometry, Graduation Year: 2017, Advisor’s Name: Dr. Joshua Cameron, Advisor’s College: College of Optometry, Source of Funding: College of Optometry, *Compound A2E’s Impact on Zebrafish with AMD*, Author List: Laura Capelle BA, Eileen Yang BS, and Joshua Cameron, PhD, FAAO

Age-related macular degeneration (AMD) is the leading cause of blindness and vision loss affecting millions of Americans, often 60 years and older. AMD causes permanent vision damage because it attacks the macula of the eye, where our sharpest central vision occurs. There are many possible factors that may cause AMD and one is thought to be associated with retinal lipofuscin. A2E is the most abundant compound in retinal lipofuscin, which is considered toxic to retinal pigment epithelial cells (RPE). In this study we looked at how pre-treatment of Zeaxanthin will protect against A2E vs. no pre-treatment. We investigated the effects of the compound A2E on retinal toxicity and its relationship to AMD progression by assessing vision function and OCT imaging.

**Study Methodology:**

Zebrafish care: Zebrafish are maintained under standard conditions at 28.5 degree C on a 10 hour dark – 14-hour light cycle. Other parameters are maintained in accordance to the guidelines established by Institutional Animal Care and Use Committee of Western University of Health Sciences.

Intraocular Injections: Fish are sedated using 160mg/L of tricaine and placed under a dissecting microscope for injections, which were performed at the dorsal-most aspect of the cornea, into the aqueous humor, using pulled glass capillary needles. Two groups of Zebrafish, each contained 6 fish, were studied: one group with Zeaxanthin pre-treatment followed by A2E compound and the other group had A2E compound. These injections were in one eye, allowing the fellow eye to be the control. The A2E concentration was 200μM.

Optokinetic response (OKR): The baseline absolute spatial acuity of zebrafish is determined by observing the OKR. This is done by anesthetizing the fish with tricaine and immobilizing it by placing it on a platform and pining pieces of foam around it. Then the fish is placed in a custom built OKR recording device consisting of a rotating drum and a microscope that is equipped with a camera system. OKR measurements were taken using a range of black and white spatial frequency grating patterns. The absolute spatial acuity was determined by increasing the frequency until the OKR was no longer observed. To obtain visual acuity in cycles per degree (cpd), trigonometric analysis was utilized:  

\[ \text{cpd} = \frac{1}{2\arctan(H/(2a))} \]

Where a is the distance from the center of the lens to the grating and h is the length of one
cycle of the smallest grating at which OKR was observed. Visual acuities were examined at 0, 1, 2, 3, and 4-week intervals after the injections. **Optical Coherence Tomography (OCT):** Fish are sedated using 160mg/L of tricaine and placed on a foam block, wrapped in a wet paper towel. OCT angle and pachymetry measurements were taken of the right and left eyes. We then measured the thickness of the retina. **Results:**
The average visual spatial acuity results appear to not have a significant difference between the two groups of fish. The average retinal thickness between both groups also appears not significant. Our results are still being analyzed, to be completed by next week.

**Conclusions:**
It was expected that due to the accumulation of A2E, the group of zebrafish only injected with A2E would display damage to the RPE and thinning of the cellular layers of the retina compared to the group that received pre-treatment Zeaxanthin. Because of the progressive nature of AMD, it is possible that the timeframe was not sufficient enough to observe any obvious changes. Our sample size of fish was also small due to deaths early in the study.

**References:**
Bone has natural ability to regenerate which is limited if a bone defect becomes too large. A model called the critical size defect (CSD) has been developed to study new treatments. Twenty six male Sprague Dawley rats received a unilateral middiaphyseal 5mm defect in the femur, which was treated with a novel bioactive elastin-like recombinamer (ELR) membrane. Femurs were stabilized using a RatFix 8 hole plate on the dorsolateral surface using self-cutting locking screws. In comparison to empty defect group without ELR membrane and demineralized bone matrix groups we hypothesize that ELR membrane treated groups will have increased bone regeneration. Final results will be observed in histological analysis and CT imaging in 6 weeks’ time. Results will be highly useful for evaluating the future system of ELR membranes as a treatment in non-unions across species.

BACKGROUND: Bone has natural ability to regenerate which is limited if a fracture becomes too large. A model called the critical size defect (CSD) has been developed to study the resulting non-unions. Such a defect is defined as unable to heal without intervention and incapable of healing within the lifetime of the animal (1). The current “gold standard” for treatment of such non-unions is autologous bone grafting, which has multiple disadvantages such as restricted availability, donor site pain and increased risk for infection (2). In order to alleviate those disadvantages we evaluated bone regeneration using bioactive elastin-like recombinamer (ELR) membranes (Fig 1) in the CSD femur model of rats. Elastin-like recombinamers are novel protein-based polymers that contain additional bioactive sites (3). The particular ELR membrane material (HAP) tested was inspired based on the slatherin-derived protein sequence DDDEEKLRRIGRFG. Slatherin is normally found in saliva and plays a role in the nucleation and growth of hydroxyapatite in the oral cavity (4). HAP ELR membranes have been previously shown to promote mineralization and osteoblastic differentiation in vitro as well as increase ossification in orthotopic rat calvarial critical-size defects but have not been attempted in the long bone of a rat (3). Our study assessed the ELR membrane stability and efficacy using surgical, histological and CT imaging approaches. The current step wise surgical technique of the CSD model in a rat described in Poser et al. had to be modified in order to investigate a membrane based treatment protocol. Here we will describe the process of surgical progressions, techniques and modifications for this experimental study in further detail. METHODOLOGY: The latest standardized 5 mm CSD creation described in Poser et al. in 2014 in rats describes the surgical procedure as follows. Rats are positioned in lateral recumbency with the leg facing upwards. A lateral approach is made between the greater trochanter and the knee joint. The intermuscular plane between the M. vastus externus and the M. biceps femoris is separated. The plate i

Next holes are predrilled using a 0.65 mm drill bit in the plate. Standardized 5 mm defects are created using a 0.22 mm Gigli wire saw guided by the sawing device of the jig. After defect sawing, the jig and bone piece were removed. The fresh defect is then flushed with sterile lactated Ringer’s solution. All wounds are closed in two muscle layers with a subcutis and intracutaneous 5-0 Vicryl rapide sutures.

In contrast to this standardized technique the surgical protocol that was used for creation of a 5 mm CSD during the pilot study was as follows. The animal is placed in ventral recumbency and the left femur is aseptically prepared. A curved skin incision is made from tail base to the stifle. The M. quadriceps and M. biceps femoris are separated to

Two towel clamps for small animals are used to position the plate on the femur. The plate (RatFix plate, 8 hole) is fixed to the dorsolateral surface of the femur using two self-cutting locking screws. Screw holes are predrilled using a 0.79 mm Drill Bit. The screws are used to guide the jig over the plate. The two inner screws are then inserted in the same fashion and a 5 mm defect is created in the center of the plate by two osteotomies using a 0.44 mm Gigly hand saw. The jig and the center screws are removed. The bone fragments rotate laterally so that a membrane can be wrapped around the bone (Fig 2). In the first pilot study cyanoacrylate glue (epiglue) was used to close the membrane in place. In the
second pilot study the cyanoacrylate glue was not used. The remaining screws are then inserted, tightened and sheared off. The fascia and the skin are closed both in a continuous pattern with #4-0 Vicryl rapide.

The following modifications and progressions have additionally been added to the surgical protocol used during the final study. The jig is removed completely in many cases in order to complete the osteotomies with the Gigly hand saw. After the membrane is in place two sterile needles are placed into the second most distal screw holes to hold the screw positions due to rotational forces. Lastly several membranes were sutured closed as seen in Figure 3c with 8-0 Vicryl suture material.

Non-surgical protocols in the study include taking weights of the rats 3 days post operatively, 1 week post operatively, 2 weeks post operatively and biweekly thereafter. Radiographs will be taken post operatively and 2 weeks post operatively. Euthanasia is planned to be done 6 weeks post operatively. Post mortem microCT scans will be performed for all animals assessing bone volume density (BV/TV). Rat femur samples will also be sent out for Giemsa staining.

RESULTS:
There were three pilot rats initially planned prior to the study to assess the integrity and sizing of the ELR membranes on rat femurs at the CSD. One was euthanized during surgery due to the ELR membrane being too small. At post-mortem examination one membrane was fully wrapped around the femur and one was unwrapped (Fig 2 a,b) although still on the femur. Another pilot rat was added to assess the larger ELR membrane, which was wrapped around the rat femur one week post-mortem (Fig 2c).

In the main study 15 rats were operated. One was euthanized due to misplacement of the plate on the femur. 14 of the 15 were in the membrane control group and received the ELR membrane (Fig 3a). One received a negative control with no membrane (Fig 3b). Post-operative radiographs confirm correct plate and screw placement as seen with post-operative radiographs (Fig. 3).

CONCLUSIONS:
In the pilot study the three rats used concluded that the ELR membranes needed to be a minimum of 8x15mm in order to properly fit the CSD of the rat femurs. It was also decided that the cyanoacrylate glue (epiglue) that was in the protocol would not be used during the study due to its polymerizing nature in water.

In the main study several surgical modifications were undertaken in order to best fit the various circumstances. The jig was removed completely in many rats in order to complete the osteotomies with the Gigly hand saw due to the large bone size in many of them. Due to the pliable and delicate nature of the membrane the most difficult part of the procedure was after the membrane was fitted in the CSD and the remainder of the screws needed to be fitted into the remainder of the holes. The solution was found by using sterile needles as place holders. Lastly several membranes were sutured closed as an attempt to provide extra stability.

In conclusion, the ELR membrane is a promising novel bone regenerative technology with the proper additional surgical approaches that ensure its stability in the long bone.

Figures

Fig. 1. ELR membrane
Fig. 2. 
(a) Pilot rat 1, 1 week post mortem: Membrane in place, wrapped around femur; epiglue was used (b) Pilot rat 2, 1 week post mortem: Membrane in place, not wrapped around femur; epiglue was used (c) Pilot rat 4, 1 week post mortem: Larger membrane used. Membrane in place, wrapped around femur; epiglue was not used.

Fig 3. (a) Empty rat femur, negative control group (b) ELR membrane, membrane control group (c) ELR membrane, stitched with 8-0 Vicryl suture

Fig. 4. Lateral post-operative femur radiographs, 8 hole RatFix plate on the dorsolateral surface with 6 screws

References


BACKGROUND HIV-1 is the most common strain of the Human Immunodeficiency Virus (HIV), a highly mutable retrovirus that weakens the immune system by specifically infecting CD4+ T lymphocytes. As an obligate parasite, the RNA retrovirus integrates its intermediate reverse transcribed DNA into the genome of a host CD4+ cell and replicates new virions by using the host cell machinery. As the infection progresses, HIV can drastically deplete CD4+ T cell levels, preventing the ability of the immune system to defend against infections and diseases. This final stage of HIV infection is a profound immunodeficiency called associated with acquired immunodeficiency syndrome (AIDS). Infection by HIV-1 leads to an increase of a variety of cytokines including Interleukin-16 (IL-16). IL-16 has been found to inhibit HIV-1 infection in acutely infected CD4+ T cells by regulation of viral transcription. However, studies have yet to thoroughly investigate the factors that influence an increase in circulating IL-16 levels throughout the course of HIV-1 infection and how these levels of IL-16 influence HIV mediated disease progression. This research focuses on novel findings that may explain the increase in IL-16 levels. We hypothesize that IL-16 production is stimulated by recognition of a certain part of the HIV virion upon infection. Our long-term goal is to identifying the mechanism of IL-16 induced antiviral immunity and how to trigger this immunity. Understanding how we can utilize this process can be significant in developing new therapies in the long term that are based on the ability of IL-16 to slow HIV infection.

STUDY METHODOLOGY Cell Culture: CEM cells were grown in RPMI with 5% FBS and 1% Pen-Strep and incubated at 37°C. At 70%-80% confluency, the cells were harvested and centrifuged to remove the supernatant and replaced with fresh serum-free RPMI media. Cells were plated into 6-well plates at a density of 1x10^6 cells/ml. HIV lysate and Lipofectamine solution with prepared RNase A, Ribonuclease H, or Proteinase K were added dropwise into each well and incubated for 36 hours at 37°C. After incubation, the cells were harvested and the supernatant was collected for ELISA analysis of IL-16. RESULTS ELISA analysis of isolated supernatant collected from successfully transfected CD4+ T cell line, CEM, detected a marked mean increase in IL-16 production from 1 μL to 10 μL of transfected HIV lysate. The HIV Lysate represents the components of a virion and are transfected in to simulate the earliest events post infection. Next, HIV lysate was treated with RNase A, Ribonuclease H, or Proteinase K. ELISA analysis of the HIV lysate isolated by Proteinase K showed values for IL-16 production below the level of detection, as opposed to HIV lysate treated with RNase A or Ribonuclease H, which both showed release of IL-16. The experiment was replicated with similar results. CONCLUSION Successful transfection of CEM cells displayed a marked increase in IL-16 levels in the supernatant, verifying previous studies that exposing CD4+ T Cells to HIV induces IL-16 production. Next, identification of the component of the HIV virions that was responsible for IL-16 release was performed by isolating component of either HIV-1 RNA or proteins. Each isolated part of HIV was transfected into cultured CEM cells and the resulting supernatants were analyzed by ELISA. Cells transfected with HIV lysate treated with RNase A, which degrades RNA, or Ribonuclease H, which degrades DNA:RNA duplexes, both showed detectable levels of IL-16 production. However, cells transfected with HIV lysate pre-treated by Proteinase K, which degrades proteins, showed no detectable levels of IL-16 production. This indicates that IL-16 production is stimulated by the recognition of HIV proteins. Currently, luciferase assays in HEK 293T cells are being used to monitor which specific protein produced by HIV signals to turn on IL-16 transcription.
Background: Natural Killer (NK) cells are innate immune cells that mediate spontaneous cytotoxicity against tumor and virus-infected cells, and represent a very promising source for adoptive cellular approaches for cancer immunotherapy. Extensive research has been conducted, including clinical trials, attempting to harness their immune properties. Gene modification of NK cells can direct their specificity and enhance their function, but the efficiency of gene transfer techniques is very limited. Our group has published a protocol for generation of human NK cells from gene-modified hematopoietic stem cells (HSC) isolated from umbilical cord blood. Generation of mature NK cells from HSC provides the opportunity of generation of younger NK cells and expansion of specific gene-modified clones starting from a smaller number of previously isolated and cryopreserved initial cells, with the added advantage of generation of multiple batches from the same donor. Chimeric antigen receptors (CAR) are engineered fusion proteins that combine the antigen specificity of antigen-binding moieties of monoclonal antibodies and intracellular activation motifs capable to activate immune cells. Preliminary evidence suggests that NK cells with specificity directed by CAR may have enhanced cytotoxicity. CD19 is a suitable target because it is only expressed in B cells and not on HSC and it is present in the majority of leukemias and lymphomas. As observed in rituximab-treated patients, ablation of B cells is compatible with life through regular infusions of immunoglobulin. The goal of this project is to develop a protocol with maximal generation of mature CAR-expressing NK cells for immunotherapy of hematological malignancies.

Significance and Innovation of Research: The significance of this project is the development of a protocol that moves towards clinical translation and large-scale good manufacturing practice (GMP) compatibility, maximally generating mature functional CAR-expressing NK cells. Instead of using umbilical cord blood, G-CSF mobilized peripheral blood stem cells (PBSC) were utilized because of availability of a larger quantity of primary human HSC, increasing safety and efficacy for clinical applications. Additionally, changes in culture media conditions based on available literature and larger well sizes were evaluated in order to develop a protocol capable of generating a larger number of cells. Heat-inactivated human AB serum and serum free media were tested to determine the cell yield for large-scale GMP-compatible protocol.
**Study Methodology:** A lentiviral vector co-delivering CD19-specific CAR and enhanced green fluorescent protein (EGFP) was used for gene modification of primary human HSC isolated from mononuclear cells collected by apheresis from anonymous donors treated with G-CSF. Gene-modified cells were then co-cultured with OP9-DL1 stromal cells over 40 days in six different culture media conditions for evaluation. Culture medium “A” was based on our previously published protocol and consisted of alpha-Minimum Essential Medium (alpha-MEM) enriched with 20% of heat-inactivated fetal bovine serum and recombinant human cytokines: SCF (5ng/ml), Flt3L (5ng/ml), IL-7 (5ng/ml), and IL-15 (10ng/ml). Culture medium “B” had AIM V enriched with 10% of heat-inactivated human AB serum and recombinant human cytokines: SCF (5ng/ml), Flt3L (5ng/ml), IL-7 (20ng/ml), and IL-15 (50ng/ml). Culture medium “C” had the same conditions as medium “B” excluding the human AB serum. After 10 days of culturing these cells, IL-2 (10ng/ml) was added to these three media conditions (“plus” conditions) creating six different experimental arms. Flow cytometry was used for detection of the EGFP (evaluation of transduction efficiency) and of NK cell differentiation surface markers.

**Results:**
The experiment ran smoothly with no incidents, accordingly to proposed timeline. Each condition started off with 1.24 million cells. For medium “A-” conditions, we obtained cell counts of 24.1 and 45.8 million cells on day 13 and day 24, respectively. For medium “B-” conditions, we obtained 31.6 and 100.4 million cells, and for medium “C-” conditions, we obtained 55.4 and 87.0 million cells on respective days 13 and 24. For medium conditions containing IL-2, “A+” presented 23.7 and 49.8 million cells, “B+” 35.2 and 79.3 million cells, and “C+” had the highest yield of 60.5 and 117.6 million cells (95-fold expansion), on days 13 and 24 respectively (Figure 1). On days 13 and 24, a third of cells were collected for further activation and expansion using gene-modified K562 cells, still in progress.

Transduction efficiency is defined by flow cytometric detection of EGFP which shows documented successful transduction of HSC. The different culture media conditions seemed to affect the proliferation of gene-modified cells. The percentage of EGFP (viable cells) in the different conditions were 50.5 for “A-”, 31.3 for “B-”, 53.1 for “C-”, 53.0 for “A+”, 33.6 for “B+”, and 54.2 for “C+.” CD56 is the earliest marker to be expressed in NK differentiation, and its expression was detected in all culture media conditions already on day 13. The percentages of viable cells expressing CD56 were 0.2 for “A-”, 0.2 for “B-”, 0.5 for “C-”, 0.3 for “A+”, 0.2 for “B+”, and 0.6 for “C+” on day 13. On day 24, the percentages of viable cells expressing CD56 changed remarkably between the various conditions: 0.1 for “A-”, 6.4 for “B-”, 3.7 for “C-”, 0.1 for “A+”, 3.7 for “B+”, and 3.2 for “C+.” More detailed flow cytometry analysis for NK cells differentiation markers documented that on days 13 and 24 the HPSC had not yet significantly differentiated into CD56+CD16+ mature NK cells.

![Cell counts in millions without IL-2 added to A, B, and C medium.](image1.png)  
![Cell counts in millions with IL-2 added to A, B, and C medium.](image2.png)

**Conclusions:** Our preliminary results confirm that development of clinically-relevant numbers of gene-modified NK cells from human HSC is feasible. The results strongly suggest that higher doses of cytokines IL-15 and IL-7 successfully increase the generation yield of NK cells. The absence of serum did not decrease the yield but there were less viable CD56+ NK cells. Furthermore, addition of IL-2 on day 10 shows promising results in creating a greater cell yield. PBSC showed folds of expansion and NK cell differentiation comparable to umbilical cord blood. These
findings suggest that it is possible to use PBSC and serum-free media for large scale GMP compatibility and clinical translation. Further evaluation needs to be done to eliminate the need of culture with OP9-DL1 murine stromal cells.

References:

Jonathan Yaghoubian, M.S., College of Osteopathic Medicine of the Pacific, Graduation Year: 2018, Advisor’s Name: Sebastien Fuchs, M.D., Ph.D., Advisor’s College: College of Osteopathic Medicine of the Pacific, Biomedical Science , Source of Funding: The Intramural Fund, Role of Spty2d1 and TM6SF2 in Lipid and Cholesterol Metabolism, Author List: Jonathan Yaghoubian, M.S., John Burke, Sebastien Fuchs, M.D., Ph.D.

The intent of this project is to determine the roles and functions of Spty2d1 and TM6SF2 proteins in vivo in genetically modified mice. These proteins were identified by genetic studies in human patients. Isoforms of these genes are associated with abnormal lipid (and other metabolic) profiles. Tissue culture studies suggest that these proteins play a role in maturation of lipoproteins through a process which remains highly unknown. These findings have led to the creation of Spty2d1 and TM6SF2 gene inactivated mouse strains. Studying heterozygous (expected 50% protein expression) and homozygous animals (no protein expression) to determine the effects of these gene modifications on plasma insulin levels and lipid panel which may include LDL, HDL and triglyceride levels. These studies are designed to demonstrate the role for these proteins in lipid and energy homeostasis. This will allow for a more comprehensive understanding of the lipoprotein production pathways. The current research suggests that essential steps (i.e. maturation) of the production of these lipid/protein particles occur intracellularly—specifically in the endoplasmic reticulum and Golgi apparatus. Identifying specific details of this pathway could lead to the development of drugs that can be used to target dyslipidemias in patients who have or are at risk for atherosclerosis, stroke, diabetes, myocardial infarction, arterial disease, obesity or metabolic disease.

Purpose of the Research or Description of the Problem: Specific aim 1: One specific aim of this project is to study a set of genetically altered mice that have Spty2d1 and TM6SF2 genes inactivated and are fed a normal diet. Examining their lipid panel as well as plasma insulin levels will elucidate any metabolic issues, relating to lipid metabolism, caused by the absence of these two genes under normal diet conditions. Specific aim 2: Another specific aim of this project is to study the same set of genetically altered mice while they are fed a high fat diet. Examining the same parameters described above, may provide more information as to the functions of these two genes. Study Methodology: Mouse models for each gene (Spty2d1 and TM6SF2) have been produced using a specific approved breeding protocol and are currently being bred at Western University of Health Sciences. Mice of both genders and all three genotypes (wild type, heterozygote knock-out, and homozygote knock-out) will be produced and used in the cohort
studies described below. Managing these colonies, collecting biopsies, isolating DNA, and performing genotype studies using PCR will all be performed at Western University of Health Sciences. **Specific Aim 1: Plasma Lipid and Insulin Studies** — Blood will be collected retro-orbitally, between the ages of 8 to 20 weeks. Four hours prior to collection, the animals will be transferred to a new cage without access to food but with access to plenty of water. After the collection of blood, the mice will have immediate access to food again. This will be the standard fasting procedure. At most, four collections will be performed per animal, and no less than 2 weeks will be allowed for recovery between collections per eye. Collections will alternate eyes. The mice will be anesthetized with isoflurane inhalation and proparacaine will be administered to the eye. Residual bleeding will be stopped by digital pressure with a sterile gauze. Dr. Fuchs will perform this procedure and train me, as he is fully trained with years of experience in this technique. Approximately 200 microliters of blood will be collected each time and since the mice weigh over 20 grams, the volume collected will be less than one percent of the animal’s body weight.

The blood will be collected on heparin to prevent coagulation and spun to collect the plasma. The plasma will be frozen at -80°C until LDL, HDL and triglycerides can be measured. Insulin will be measured by ELISA following the manufacturers’ instructions (ALPCO). Mouse lengths and weights will also be measured weekly for comparison and body mass index (BMI) equivalent calculations. BMI will be calculated based on the formula:

$$\frac{Weight \ (g)}{(Snout \ to \ Anus \ Length \ (mm))^2}$$

The snout to anus length is measured while the mouse is anesthetized with isoflurane, to allow for accurate measurements. **VLDL and Chylomicron Studies** — To study very low density lipoprotein (VLDL) and chylomicron production, the mice will be fasted overnight in a fresh cage (to prevent ingestion of feces) without food, but they will have access to water. In the morning, the mice will be intraperitoneally injected with Pluronic F-127 at 300 mg per kilogram of body weight in 100 microliters of sterile saline solution which will block triglyceride lipolysis. Then the mice will receive 20 microliters per gram of body weight of vegetable oil by gastric gavage. Four samples of 20-30 microliters of blood will be taken at the tail in 30 minute intervals. For the first blood sample (30 minutes after the vegetable oil administration) the mice will be anesthetized with isoflurane, then the tail tip will be amputated and blood will be collected. All subsequent samples will be collected without anesthesia, as bleeding can be resumed by simply removing the dried scab and allowing for the collection of one to two drops of blood. After the final blood collection (120 minutes after vegetable oil administration) the mice will be transferred into their cage with regular access to food and water.

The VLDL and chylomicron studies will be performed in tight collaboration with Dr. Peterfy’s group. **Specific Aim 2:**

**Long Term Effect of a High Fat/Cholesterol diet on Lipid Profiles** — “Lipid tolerance” will be evaluated in these two strains of mice using a different cohort of animals. This cohort will be fed a high cholesterol (0.2%) diet also known as Harlan chow TD.88137 (“Western Diet” given at 4.5 kcal/g) for 14 weeks starting at six weeks of age. During the final eight weeks of this diet, plasma will be collected following the protocol described in the section above. Upon conclusion of the 14 week diet, the mice will receive a lethal injection of intraperitoneal ketamine/xylazine. Their blood will be collected under deep anesthesia via transthoracic cardiac puncture and their livers will be harvested for histology, biochemistry and enzymatic analysis.

The blood will be treated as in the same protocol as specific aim one. One lobe of the liver will be collected, dehydrated and paraffin embedded for histological studies. Another part of the liver will be frozen at -80°C until further studies can be conducted. **Results:** At this point in the experiment it is very difficult to have gathered any significant results. There are many variables at play and there has not been sufficient time to have gathered enough data to plot and analyze. Figures 1, 2, 3, and 4 show the current weights recorded for SPTY week 9, SPTY week 11, TM6 week 11 and TM6 week 13, respectively.

Fig 1
**Conclusions:** Currently, no conclusions can be made about the function of SPTY or TM6 genes. What can be observed based on the graphs above, is that SPTY and TM6 male knock-outs are not as viable as the other gender/genotype combinations, however we are still breeding more mice to be able to gather more data. The project seems to have launched quite nicely and will most probably take another 6 months before any true conclusions can be made.

**References:**
4. From noncoding variant to phenotype via SORT1 at the 1p13 cholesterol locus”, Musunuru et al. Nature 466, 714-719 (05 August 2010).

Roman Pan, College of Veterinary Medicine, Graduation Year: 2018, Advisor’s Name: Dr. Hrvoje Smodlaka, Advisor’s College: College of Veterinary Medicine, Source of Funding: Western University of Health Sciences Summer Research Fellowship, Anatomical Properties of the Northern Elephant Seal Ear, Author List: Dr. Wael Khamas, Dr. Hrvoje Smodlaka, Dr. Lauren Palmer, Roman Pan

BACKGROUND: Besides vision and vibrissal tactile senses, auditory abilities are one of the major senses used by marine mammals to navigate the depth of the ocean. In modern times, northern elephant seals (NES; Mirounga angustirostris) are exposed to negative anthropogenic and environmental stimuli. Most notably, deep ocean floor sound exploration and use of military sonars are linked to mass stranding of marine mammals, mostly cetaceans. Such use of sound exploration underwater is documented to cause acoustic trauma in marine mammals resulting in rupture of middle and inner ear structures. While overstimulation with sound can cause loss of inner and outer hair cells and degeneration of cochlear nerve fibers in mammals, anatomical adaptations of the ear in NES were poorly documented. To define their anatomical adaptations and to establish a norm for future comparison with pathologically affected ears, it is essential to evaluate the ear anatomy of the NES. Material and Methods: In this study, all samples were obtained from Marine Mammal Care Center in San Pedro, California. We collected three M. angustirostris weaner (6-11 months of age) skulls. The full ear, from petrous temporal bone to the external acoustic meatus, was excised. A stud of cranial nerve VII and VIII were left on the petrous temporal bone for tracing purposes. All tissues were processed following standard histological procedures, and slides created from Leitz 1512 microtome were stained with Hematoxylin and Eosin, or Masson’s trichrome stain. The prepared slides were then photographically documented. Quantitative analysis of cochlear nerve fibers was performed with Nikon DS-5M camera head on Nikon DS E200 Eclipse microscope. The cochlea was exposed using cutting burrs for morphometric measurements.

RESULTS: The external acoustic meatus of M. angustirostris was a collapsed structure. We suspect a cerumen plug may be in the lumen, but it was likely removed during tissue processing. A cartilaginous ring was also present, but it does not hold the lumen open. The tympanic membrane had four layers, with middle two layers being a striated collagenous structure. The entire auditory tube was composed of collagen fibers, and lined with ciliated pseudostratified columnar epithelium. This epithelium continues to cover the entire tympanic cavity. In the submucosa of the middle ear lies a cavernous sinus from the medial-rostral aspect to the caudal-lateral aspect. Such cavernous sinus, even in its relaxed state, was 13 mm thick. When the middle ear was further dissected, the stapes was found fully embedded in this cavernous tissue. The cochlea was measured to be 11.0 mm in its basilar width, 5.0 mm in its apical width, 6.0 mm for its axial height, 2.5 mm wide for its basilar coil, 1.5 mm wide for its apical coil, and 33.0 mm for its total length. The cochlear nerve was calculated to have an average of 60,364 ± 55 axons and each of the fibers to have an average diameter of 4.62 ± 0.99 μm.

CONCLUSIONS: Anatomical properties of the northern elephant seal (Mirounga angustirostris) ear were obtained from three weaners. The acoustic meatus was a fully collapsed structure. Although a cartilaginous ring was present, it does not maintain an open lumen. Structure of tympanic membrane, having four layers instead of three, was different compared to terrestrial mammals. We believe that the two layers of striated collagenous structure allow better compliance of the tympanic membrane during deep diving. This may be a special adaptation to withstand dramatic
pressure and structural changes in the middle ear cavernous sinus. Their auditory tube lacks a cartilaginous core that was described in other seal species. Nonetheless, the epithelium was similar to other mammals consisting of ciliated pseudostratified columnar cells. Such respiratory-like epithelium continues from the auditory tube to cover the entire tympanic cavity, which differs again from terrestrial mammals. One possible function of such trait may be to expel seawater entering the tympanic cavity caused by swallowing reflex. In the submucosa of the middle ear lays a cavernous sinus as thick as 13 mm at the medial rostral to caudal lateral position. However, *M. angustirostris* lacks a cavernous tissue in the external acoustic meatus as described in other seal species. Such cavernous sinus can engorge to occupy almost the entirety of the middle ear air space, and the stapes was completely embedded inside the cavernous tissue. Because seals use bone conduction to hear, the cavernous sinus potentially eliminates the difference in transduction impedance between skull/water and air space. We believe such adaptation grants *M. angustirostris* better hearing sensitivity when diving. The massive cochlea of *M. angustirostris* grants them more space in the spiral ganglion canal to house more spiral ganglion cells. This grants *M. angustirostris* higher number and complexity of neuronal pathways to process acoustic stimuli. A similar result was also measured in the cochlear nerve, which contains 60,364 neuronal fibers. Each of these fibers has an average diameter of 4.62± 0.99 μm. Both of these numbers are two times higher than in humans.

REFERENCES:

Understanding the anatomy of the rabbit skull is essential for answering questions about their lifestyle and locomotion. The rabbit skull is highly transformed, exhibiting a significant degree of facial tilting. The extent of dorsal arcing of the skull roof and associated facial tilt is typically minimal among mammalian skulls. In the rabbit, however, expansion and flexion of the supraoccipital bone on the dorsal skull facilitates the ventral flexion of the facial region. The degree to which rabbits tilt their face shows strong correlation in their locomotor style. Osteological transformation of the supraoccipital bone is intimately related to facial tilting; which is associated with configuration of the deep posterior triangle muscles, and the superficial extensor muscles. Here we investigate the relationship between soft tissues structures in the ear and sub-occipital area to the osteological transformations of the rabbit skull. We used both gross-dissected and iodine enhanced, Ct-scanned rabbit head and necks to discern anatomical relationships.

**Purpose of the Research or Description of the Problem:** Canine, feline and other common domesticates species have published cross-sectional anatomy atlases for computed tomography and gross anatomy. Barone et al. (1972) published “Atlas of the rabbit anatomy”, which represents the most detailed anatomical survey of the rabbit (*Oryctolagus cuniculus*). Even so, as illustrated by Alessandro Zotti (Zotti et. al 2009), better detailed descriptions of the head and neck anatomy of the rabbit is needed. To date, no studies have investigated the head and neck anatomical landmarks in detail. Furthermore currently available anatomic literature for rabbits carries minimal thoracic and abdominal cross-sectional drawings (Barone et. al., 1973). Other research has also shown developmental changes to have an affect in vertebral bones and locomotion; these were emphasized by the curvature of the spine in relationship to the cranium (Jones 2013). A new staining technique for computed tomography was recently developed; this utilizes iodine for muscle staining, which allows for greater contrast in imaging. The purpose of this study is to utilize this technique and gross anatomical dissection to reconstruct a 3D model of the neck and ear muscles for head tilt analysis.

**Study Methodology: Gross anatomy & Digital imaging and fixation** The research was conducted at WesternU for duration of 6 weeks. Four adult rabbits (*Oryctolagus cuniculus*) were obtained from ENasco Biological Supply. The dissection was guided by employing the “Atlas of the rabbit anatomy” (Barone et. al., 1973). Two rabbits were skinned by applying a small and shallow incision in the abdomen towards the head. The superficial fascia was separated with surgical scissors (straight, s/s) and skin was disposed. The specimens were dissected using micro dissection tools and photographic images were obtained using a Nikon D90 camera at different time intervals. The images were then segmented and analyzed using ImageJ software.

Two more rabbit specimens were stained using 15% potassium-iodine solution (KI); consisting of 85% distilled water, 10% KI and 5% Iodine, mixture first explained in (Cox, 2015). CT imaging was preformed in a GE/Phoenix Vtomex S CT scanner with a DXR250 detector for digitally imaging construction. Tissue staining and CT scanning took place at different time intervals, 1st at week two, 2nd at week three and finally at week four. Different fixation times allowed for the enhancement of future protocols on large specimens such as the rabbit, which have not yet to be documented. These results will help minimizing tissue shrinkage due to excessive staining time. CT scan was conducted at Aerospace Corporation, in El Segundo, Ca. The microCT scan was obtained using 200 kV (kilovolts), 80 uA (micro-Amps) X-ray tube power. The detector timing was set to: 500 msec per frame capture, 8 frame averages per image. The diameter included 2000 images for 360 degrees of rotation and an image every 0.18 degrees of rotation, with a total scan time of 153 minutes. Images were inspected and used for digital reconstruction.

**Results:** Partial results of our study are shown as the matched photographs presented in Fig. 1 & 2. Images are displayed so that the right side of the animal corresponds to the reader’s right side. Fig. 4 & 5 display a CT images at week 2 & 3 of staining. We expect better absorption of potassium iodine in all CT images at week 4 yielding improve
segmented images. The protuberancia occipitalis externa shown in fig. 3 is a major attachment site for the deep posterior muscles of the neck. Muscles such as M. Oblicus capitus cranialis, M. Rectus capitus dorsalis major & M. Obliquus capitis caudalis. We expect both, manual and automated muscle segmentation of the occipital area to show identical attachment and insertion points. We also expect morphological changes in the protuberancia occipitalis externa to be explained by the development of the deep neck muscles leading to the bases for facial tilt and locomotion in this species.

Fig. 1. Superior view of the superficial muscles of the ear and head. (A) M. Interscutularis; (B) M. Frontoscutularis; (C) M. Scutoauricularis; (D) M. Cervicoscutularis; (E) M. Cervicoauricularis Superficialis

Fig. 2. Superior view of the deep muscle of the ear and head; (F) M. Parietoscutularis; (G) M. Cervicoauricularis Medius; (H) M. Cervicoauricularis Profundus

Fig. 3

(K) Protuberancia occipitalis externa; (L) M. Oblicus capitus cranialis; (N) M. Rectus capitus dorsalis major; (M) M. Obliquus capitis caudalis

Fig. 4

Fig. 5
**Figures:**

- **Fig. 4**: CT cross-sectional imaging at week 2.
- **Fig. 5**: CT cross-sectional imaging at 3-week staining.

**Conclusions:** Studying facial tilt is a new approach in understanding the locomotion of the rabbit. The current advances in iodine fixation and CT imaging allows for new and improved methods to understand soft tissue anatomy. Correlation between the facial tilt and locomotor styles among leporids (rabbits and hares) has been reported in recent studies (Kraatz, 2015), and understanding the anatomical basis of this correlation is important to discern its function. Transformation and expansion in the protuberance occipitalis externa is believed to be the basis for greater facial tilted, which is intimately related to the posterior triangle of the neck. In comparison to other mammals, the proportion of the muscles found in the posterior triangle of the rabbit is more significant than those found in the human body; likely due to the ability of these animals to hold, stabilize and rotate their necks. We expect our Ct-model segmentation will improve the understanding of locomotion in species such as the rabbit; leading to an improved and enhanced understanding of the head, neck and ear region of the rabbit.

**References:**


Margaret Kim, College of Osteopathic Medicine, Graduation Year: 2018, Advisor’s Name: Sebastien Fuchs, Advisor’s College: College of Osteopathic Medicine, Source of Funding: Principal Investigator, *AcSDKP wound healing*, Author List: Margaret Kim, John Burke, Sebastien Fuchs

The tetrapeptide AcSDKP (N-acetyl-seryl-aspartyl-lysyl-proline) has been hypothesized to be important factor in controlling fibrosis. AcSDKP is inactivated by ACE (Angiotensin Converting Enzyme), which has two catalytic domains the N-terminal and C-terminal. The N-terminal catalytic domain, is the sole domain that can cleave AcSDKP (1). Therefore, in N-KO (N-terminal knockout) mice which have the N-terminal domain inactivated, AcSDKP is accumulated, not degraded. This is observed in N-KO mice, which provides a good model to study AcSDKP in vivo. Studies have previously shown that increased AcSDKP concentration reduced fibrosis in the lungs of mutant mice treated with bleomycin compared to WT (wildtype) mice with the same treatment conditions (1). WT mice that were treated with bleomycin also had reduced fibrosis in the lungs when administered injections of exogenous AcSDKP (2). Morphologically, N-KO mice had lung tissue that was histologically similar to uninjured lung via Masson’s Trichome and H&E staining compared to bleomycin-treated WT mice (2). N-KO mice also had reduced markers of inflammation, reduced lung hydroxproline content and reduced weight loss when treated with bleomycin compared to similarly treated WT mice. These findings suggest that AcSDKP has a role in mediating fibrosis. A previous study has shown that exogenous AcSDKP in a mouse skin flap model showed increased survival of ischemic skin flaps (6). This project seeks to analyze the effects of increased AcSDKP concentration and its possible effect on fibrosis and healing after skin injury. In this study, N-KO and WT will be injured via freezing using liquid-nitrogen cooled-probe (Cry-Ac TrackerCam® Brymill) on the dorsal skin. Wounds will be analyzed for morphological differences and durations in wound healing. Our hypothesis is that AcSDKP could ameliorate the wound healing process and possible scar reduction. This would be important information for dermatologists that use this device in human treatments.

**Purpose of the Research or Description of the Problem:** Assess the differences in wound healing of the dorsal skin between the control and N-KO mice after injury with liquid nitrogen-cooled probe. The probe is placed on the skin for
20 seconds for both the 10mm and 6mm injuries. Healing will then be assessed by morphological differences and histological differences in the time it takes for the wounds’ closure and analyzed by ImageJ for any differences between WT and KO mice. Once the 6mm wound closes, the 10mm wound will be collected for histological analysis. In particular, the degree of inflammation, rate of wound closure and collagen deposition will be determined. 

**Study Methodology:** WT and ACE KO mice are injected with a ketamine/xylazine solution in saline i.p. at a concentration of 125/12.5 mg/kg for anesthesia. Mice are shaved and then both 6mm and 10mm probes are cooled to -10°C and then placed on dorsal skin for 20 seconds on both flanks of the back. After injuries, mice are injected with 0.05 mg/kg buprenorphine as an analgesic subcutaneously. Wounds are analyzed for size, development of necrotic tissue damage visually via ImageJ and once the 6mm wound closes, the 10mm wounds will be collected for histological analysis for inflammatory markers and morphological differences. **Results:** Initial experimental results showed that 20 seconds of probe application was the best for wounding. In these mice, time points of 5, 10, 15, 20, and 25 seconds were performed and analyzed for wounding. 20 seconds had sufficient inflammation after the first day and necrotic tissue formation after 3-4 days post injury. The lower time points did not have necrotic tissue formation or the wounds were not as robust. The development of the wounds have been monitored for closure and one tissue sample has been collected 10 days after injury which is being prepared for histological analysis.

**Fig 1**

![Graph showing 10 mm wound healing over time for WT and KO mice](image)

- WT1
- WT2
- WT3
- KO2
- KO3

Wound size vs. day
**Conclusions:** The first trial with WT and KO is currently in progress and wounds are under analysis for size and any visible morphological differences. Histological samples will be collected when the smaller wounds have closed.

**References:**


**Shana Feinberg, College of Osteopathic Medicine, Graduation Year: 2018, Advisor’s Name: Dr. Jesus Sanchez, Advisor’s College: College of Osteopathic Medicine, Source of Funding: None, Evaluating the Effect of Osteopathic Manipulative Techniques on Diabetic Foot Ulcers: Wound Healing, Blood Flow, Neuropathy and Inflammatory Biomarkers, Author List: Shana M. Feinberg, BS, Jesus Sanchez, DO, MA, Michael Seffinger, DO, Vishwanath Venketaraman, PhD**

Type II diabetes mellitus (T2DM) is a condition in which individuals have elevated blood glucose, and if the blood glucose level is not controlled, it can lead to serious health consequences, including diabetic foot ulcers (DFUs). DFUs can result from T2DM because the high glucose circulating in the blood of these individuals influences the cytokine TNF-α release. In healthy individuals, the role of TNF-α is to produce inflammation, as a contributor to regulating the immune system, but in T2DM patients, TNF-α release has been found to be increased chronically, leading to inflammation and poor wound healing. If TNF-α can be reduced and regulated in T2DM individuals with DFUs, then it is likely that the inflammation would decrease and wound healing will improve.

**Purpose of the Research or Description of the Problem:**

In this study, we wanted to evaluate the use of Osteopathic Manipulative Technique (OMT) as a therapeutic intervention and observe its effects on T2DM individuals with DFUs, using TNF-α as an objective marker for inflammation decrease. Based on research literature, there is increasing evidence that OMT has positive effects on reducing TNF-α, decreasing pain, inducing blood and lymphatic flow, and improving mood. The goal of this project is to determine the influence of OMT on wound healing and inflammation in T2DM patients with DFUs and to monitor other effects of OMT that may improve these patients’ conditions. This is a proof of concept study, so the purpose is to determine the correlation between OMT and patient outcomes. If there is a significant correlation, then a randomized clinical trial may be conducted with the following subject populations: T2DM with DFU, T2DM without DFU, and healthy patients. In addition, this follow-up study will compare the significance of OMT in these subjects and the degree of benefit on wound size, infection, and inflammation markers.

**Study Methodology:** Upon IRB approval, this study will consist of adult patients from Western University Foot and Ankle Center in Pomona, CA, both male and female, with T2DM and DFUs. Blood samples will be collected from all patients before, during, and after OMT treatment, and these samples will be analyzed using ELISA, which will test cytokine levels of TNF-α, as well as the following cytokines: TGF-β, IL-1, and IL-6. In addition, the wound size and healing process will be monitored before, during, and after OMT treatment by measuring the wound and taking pictures.
Results: As the IRB approval for this study is still pending, preliminary and research design planning was the emphasis of this project during this period of time. In preparation of this project, I researched several books and articles (see references) and discussed them with my mentor and learned essential lab skills, such as conducting ELISA, lab safety, centrifugation, and preparing samples. I also became certified in NIH lab safety and NIH working with human subjects, and learned the process of IRB review, as well as the process of making changes to IRB recommendations. As soon as IRB approves this project, the plan is to move forward with this study and to begin the subject recruitment process, followed by collecting baseline measurements.

Conclusions: During this project, it was concluded that OMT may be a beneficial intervention for T2DM individuals with DFUs, based on research literature, and this idea will be investigated upon IRB approval. This is a proof of concept study, so the purpose is to determine the correlation between OMT and patient outcomes of T2DM patients with DFU. If there is a significant correlation, then a randomized clinical trial may be conducted with the following subject populations: T2DM with DFU, T2DM without DFU, and healthy patients. In addition, this follow-up study will compare the significance of OMT in these subjects and the degree of benefit on wound size, infection, and inflammation markers.

References:


Hannah Kang, College of Osteopathic Medicine, Graduation Year: 2018, Advisor’s Name: Dr. Airani Sathananthan, MD, MSHS, FACE, FACP, Advisor’s College: College of Osteopathic Medicine, Internal Medicine, Source of Funding: Summer Student Research Grant STRS, Patients’ Perception of Hypertension, Author List: Hannah Kang, BS, Airani Sathananthan, MD, MSHS, FACE, FACP

Hypertension is the greatest risk factor for cardiovascular diseases and other comorbidities. Despite the availability of effective medications, hypertension remains ineffectively managed in the United States. Disparities in hypertension control are multifactorial. Studies have shown that patients’ hypertension control and use of medication treatments are influenced by factors including patients’ beliefs, which may be influenced by the patients’ ethnicity and socioeconomic status. We will evaluate the patients’ perception of healthy and non-healthy blood pressure levels and factors that affect patients’ choice of treatment to manage their blood pressure.

Purpose of the Research or Description of the Problem: The goal of this study is to assess the perception of patients in the Pomona community about their blood pressure and their knowledge regarding symptoms, causes, and treatments of hypertension. This pilot study will assist in understanding the barriers that restrain patients from achieving their blood pressure goals. Furthermore, this study will help progress future programs, which allow patients to manage and understand their hypertension, and prevent the progression of complications from hypertension.

Study Methodology: All subjects were selected from the Western University of Heath Sciences Medical Center (Patient Care Center) in Pomona, CA.

After the IRB granted Informed Consent was provided and signed, participants were given a questionnaire specifically asking about the participants’ perception about their ideal blood pressure, awareness, and knowledge about hypertension-related medical issues.

Patients’ demographic information including age, gender, ethnicity, educational level, occupation, and annual income was surveyed.

Chart Review: Blood pressure was retrieved from the patients’ chart/ check-in form from the current day visit. The chart was also reviewed to see if the patient carried a diagnosis of hypertension.

Data Analysis: Information collected from all subjects was analyzed using Microsoft Excel. Only a preliminary analysis has been done so far.

Results:

Upon observation of the characteristics of patients recruited at the Patient Care Center, the majority (69.2%) was female compared to that of male (30.8%). Most of those patients (29.9%) fell into age range 18-39 years. 46.2% of the patient population was Hispanic with 22.2% being White/ Caucasian. Most (59.8%) admitted to having hypertension. Level of education among the patient population was diverse, with 24.8% having less than high school education, 25.6% with college graduate education, and the rest of the recruited population in between those extremes.
When assessing hypertension knowledge of recruited patients, the majority (79.5%) believed that high blood pressure (BP) was “extremely” dangerous to a person’s health, while the next highest subgroup (10.3%) believed it was “somewhat” dangerous. 82.1% of the subjects also believed that lowering high BP would improve health. By inquiring patients on normal BP levels, 58.1% thought the systolic (top) number should be less than 140, while 54.7% thought that the diastolic (bottom) number should be less than 90. Most of the patients (49.6%) believed that both top and bottom numbers of BP were important; one number not dominating the other in significance. When asked about the 3 main organs at risk from high BP, 100% believed that the heart was one of the top 3 organs. The next highest subgroups of patients, 50.4% and 42.7%, believed that the kidney and brain were among the top 3 organs affect, respectively.

By examining attitudes and perceptions of hypertension, it was discovered that 47.9% of subjects believed that high BP is a life-long disease, while 37.6% did not. The majority (96.6%) also believed that changing lifestyles help to lower BP, and 65.8% believed that high BP is an avoidable part of aging. When asked to choose the single most important factor in controlling BP, 37.9% chose exercise, while the following 20.7% and 18.1% of patients believed that taking medications and changing the diet, respectively, were important factors.

![Fig 1](image1)

**How dangerous is high BP to a person's health?**

<table>
<thead>
<tr>
<th>% of Patients</th>
<th>100</th>
<th>79.5</th>
<th>20</th>
<th>10.3</th>
<th>5</th>
<th>2.6</th>
<th>1</th>
<th>7.7</th>
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</thead>
<tbody>
<tr>
<td>Extremely</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Somewhat</td>
<td>28.2</td>
<td>38.5</td>
<td>3.4</td>
<td>1.7</td>
<td>4.3</td>
<td>0.9</td>
<td>2.6</td>
<td>25.6</td>
</tr>
<tr>
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<td>42.7</td>
<td></td>
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<td></td>
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<td>0</td>
<td>1</td>
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</tbody>
</table>

![Fig 2](image2)

**3 main organs at risk from high BP?**

<table>
<thead>
<tr>
<th>% of Patients</th>
<th>100</th>
<th>28.2</th>
<th>38.5</th>
<th>50.4</th>
<th>4.3</th>
<th>0.9</th>
<th>2.6</th>
<th>25.6</th>
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<tbody>
<tr>
<td>Heart</td>
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<td>Lung</td>
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<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td>3.4</td>
<td>1.7</td>
<td>4.3</td>
<td></td>
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</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
<td>50.4</td>
<td></td>
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</tr>
<tr>
<td>Stomach</td>
<td></td>
<td></td>
<td></td>
<td>3.4</td>
<td>1.7</td>
<td>4.3</td>
<td></td>
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</tr>
<tr>
<td>Spleen</td>
<td></td>
<td></td>
<td></td>
<td>50.4</td>
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</tr>
<tr>
<td>Pancreas</td>
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<td></td>
<td>4.3</td>
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<tr>
<td>Brain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.3</td>
<td>0.9</td>
<td>2.6</td>
<td>25.6</td>
</tr>
<tr>
<td>Skin</td>
<td></td>
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<td></td>
<td></td>
<td>0.9</td>
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<tr>
<td>Colon</td>
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<td></td>
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<td>2.6</td>
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<tr>
<td>Eyes</td>
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<td></td>
<td></td>
<td>25.6</td>
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</tbody>
</table>
Conclusions: In efforts to address the barriers that prevent patients from achieving their blood pressure goals, a pilot study that examines patients’ perception and knowledge of hypertension was conducted in Pomona, CA. Upon recruiting 117 adult patients at the Western University of Health Sciences, Patient Care Center, the characteristics of the patient population was analyzed.

The majority of the subjects appeared to possess general knowledge on hypertension, with most acknowledging that high blood pressure (BP) is “extremely” dangerous to a person’s health, and most correctly stating that lowering high BP would improve health. Interestingly however, a significant percentage of patients were unfamiliar with normal blood pressure levels. Furthermore, our results indicate that patients are generally unaware that systolic BP is important in high BP.

These findings suggest the need for education of patients on the importance of blood pressure levels and elevation of systolic BP in relation to cardiovascular disease. This pilot study may help guide future efforts from the physician and other health care providers as to patient education about blood pressure and the significance of elevated levels.

References:

for the presence of inflammatory markers. LLIs will be measured radiographically and compared to each metabolomic profile in order to determine the effect LLI had on the composition of synovial fluid and joint health. The procedural protocol will be used to train other medical students and assess the most effective way to teach ultrasound guided joint aspirations. **Conclusion:** With the metabolomic results we hope to determine if those with an LLI have an increase in inflammatory biomarkers. We also hope to determine the most effective teaching method in performing ultrasound guided aspirations. **Purpose of the Research or Description of the Problem:** Ultrasound is becoming more commonly used in the clinical setting, yet formal training for physicians is not as common place. One procedure gaining prominence is ultrasound guided needle aspirations and injections of musculoskeletal structures (12). Ultrasound guided needle techniques once practiced allow for increased accuracy, reduced aspiration attempts and allows the clinician to account for anatomical variation. By providing medical students access and practice in these techniques early in their training it is believed that they will be more competent in its use as physicians. We aim to create a training protocol to be used to train our classmates and future cohorts in conducting direct ultrasound guided needle aspirations of the knee joint on Donor Cadaver Patients (DCP) (1, 4).

Leg Length Inequality (LLI) is believed to be linked to a variety of biomechanical and musculoskeletal ailments. It has been shown that LLI can be measured accurately using a simple tape measurement however radiographic imaging remains the gold standard in determining LLI (10). Both gait asymmetry and loads across the joints of the lower limb have been shown to be affected by LLI (7,13). It has been found that a difference in leg length can also cause low back pain and increased time away from work (11). This seemingly benign issue can have a large impact on a person’s life, and given the number of miles traveled in a lifetime with LLI, an association has been found that chronic joint disease such as osteoarthritis (OA) can develop (5).

Inflammatory joint diseases such as OA do cause measurable changes to the tissue and fluids surrounding them (6). Synovial fluid analysis has proven to be a viable way to determine if a joint is in a diseased state and is not limited to OA (8,9,17). What if a physician was able to analyze the synovial fluid of a patient with a known LLI and determine the severity of damage and the appropriate therapy by using the less invasive ultrasound guided needle aspiration? The ability to determine biological age by using biomarkers is now possible (14). This may allow a physician to make clinical judgements using metabolomics and imaging. We aim to develop protocols for educating medical students as well as collecting, storing, and analyzing synovial fluid from DCP and comparing this to their radiographically measured leg length inequality.

**Study Methodology:** **Aspirating Synovial Fluid for Analysis** To aspirate synovial fluid from the knee joint, a 22 gauge needle was inserted either superolateral or superomedial to the patella. All knees were bent from 50 to 60 degrees and ultrasound guidance was used to be able to accurately access the joint. Once the sample was obtained it was immediately placed on ice to preserve the protein and cellular integrity, and transferred to a centrifuge chamber where it was spun for 5 minutes at 10,000 rpm with the temperature at 4C. The supernatant containing the proteins was transferred to a cryofreeze tube where it and the pellet containing the cells were flash frozen using liquid nitrogen. Both the pellet and the supernatant were then stored at -80C. From here the cells will be sent to have a metabolomics profile ran and the results will be analyzed for inflammatory cytokines: Interleukin (IL)-1α, IL-1β, IL-1RA, IL-4, IL-6, IL-6Ra, IL-7, IL-8, IL-10, IL-13, tumor necrosis factor (TNF)α, Interferon (IFN)γ, oncostatin M (OSM), leukemia inhibitory factor (LIF), adiponectin, leptin, monocyte chemotactic factor (MCP)1, RANTES, basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF), vascular growth factor (VEGF). Determining the penetration of embalming fluid into the synovial capsule Pre-embalmed samples will be taken and compared to post-embalmed samples obtained from the same joint at different intervals to determine if the embalming process could contaminate the synovial fluid. A color gradient of 0-5 will be pre-determined with 0 indicating a clear sample and 5 indicating a significant color change.
Figure 1: Sunrise view showing articular cartilage of femur with synovial sac of the knee joint directly above.

Figure 2: Image of the 22 gauge needle piercing the synovial sac of the left femoral condyle.

Conclusions: When metabolomic analysis is conducted on the samples currently stored at -80°C, we expect to see a rise in inflammatory markers consistent with the severity of the LLI of the DCP. Analysis will occur after a sufficient number of samples have been collected which may take several generations of research teams. Setting this up as a heritage project will allow ongoing sample collection and analysis. It is uncertain at this time how much embalming fluid enters the joint space and is extracted with the synovial fluid. As the research progresses we would like to determine if the embalming process changes the synovial fluid. In regards to LLI, the current imaging study will help to identify anatomical discrepancy but will not allow for recognition of functional/physiological LLI. This does provide an advantage as it would be unknown how long a functional LLI existed whereas an anatomical LLI was probably in existence since the patient reached mature height. It should also be noted that inflammation can be caused by other pathological processes, and the DCP’s past medical history is not currently available for evaluation. We will attempt to account for this by comparing inflammatory biomarkers from both knees. As longer legs sustain greater loads with LLIs, we expect to see an increase in inflammatory cytokines in the knee of the longer leg (7).

References:

The accumulation of misfolded proinsulin has been shown to be a leading cause of pancreatic beta cell failure, a well-known characteristic of diabetes mellitus. As misfolded proinsulin accumulates, it essentially clogs the ER resulting in ER stress and toxicity. If the misfolded proinsulin is not degraded efficiently, this may lead to beta cell dysfunction and eventual cell death, a leading cause of diabetes [1, 2]. However, misfolded proteins are actually a common phenomenon within cells, but can be detrimental upon aggregation. In order to prevent this subsequent aggregation, the body has evolved a method to help clear misfolded proteins. This process is called Endoplasmic Reticulum Associated Degradation (ERAD), where misfolded proteins are retrotranslocated from the ER lumen to the cytoplasm for degradation by a proteasome. Thus, as misfolded proteins begin to accumulate in the ER lumen of pancreatic beta cells, ERAD is upregulated in order to help clear these proteins [1,4].
Many lumenal proteins have been implicated to aid in the ERAD of misfolded proinsulin, specifically, binding immunoglobulin protein (BiP) and protein disulfide isomerase (PDI). Both BiP and PDI have been shown to be upregulated in the Akita mouse, a diabetic mouse model. BiP aids in initial folding and also helps bind misfolded proteins to facilitate refolding to reach its proper conformation [1, 3]. PDI has been shown to have multiple roles, such as a chaperone and an ER retention factor. Furthermore, PDI has the ability to aid in proper disulfide bond formation and also to isomerize non-native disulfide bonds which is critical for proper proinsulin folding [2,4].

To date, the actual mechanism of ERAD for misfolded proinsulin is not well understood, but both BiP and PDI are believed to facilitate ERAD of non-glycosylated proteins, such as proinsulin. This study will specifically examine PDI’s role in the ERAD pathway and determine whether PDI is sufficient or needs to act in concert with BiP for efficient ERAD of misfolded proinsulin.

**Purpose of the Research or Description of the Problem:** The accumulation of misfolded proteins within cells has been associated with many human diseases such as diabetes, cirrhosis, and several neurodegenerative diseases. Aberrant proteins aggregate within the endoplasmic reticulum (ER) of cells, interfering with cell function and eventually leading to tissue and organ damage. Specifically with diabetes, the accumulation of misfolded proinsulin causes ER stress and eventual beta cell failure. By increasing ERAD levels within pancreatic beta cells, this minimizes aberrant protein aggregation and the likelihood of beta cell failure, thus having a therapeutic effect [1,2]. This study aims to identify ER lumenal proteins that can potentially be targeted to enhance ERAD of misfolded proinsulin in the future.

**Study Methodology:** For this study, we used a reconstituted ER microsome system where we resuspended pelleted ER membrane with our defined lumenal contents: PDI and mutant proinsulin. These reconstituted ER microsomes were placed in a cytosolic medium containing rabbit reticulocyte lysate, which contains the components necessary for ERAD. Upon increasing the temperature from 4 to 30 degrees Celsius, ERAD is initiated. Theoretically, as time elapses, mutant proinsulin exits the ER microsome and is degraded by the proteasome. At time points 0, 20, and 40, samples were taken and ERAD is halted by adding SDS and boiling. The samples were ran on a western blot and proinsulin proteolysis levels were quantified by measuring signal intensity and averaged over two experiments to determine rate of mutant proinsulin proteolysis. Proinsulin proteolysis in natural ER microsomes was used as a standard for “efficient” ERAD. We repeated the same experiment with both BiP and PDI within the reconstituted microsomes to compare PDI’s efficiency alone or in concert with BiP.

**Results:** After calculating the natural logarithmic fraction of the remaining proinsulin and plotting each set of data, we found that with reconstituted ER microsomes containing only PDI and mutant proinsulin had a linear regression slope of $-2.2 \times 10^{-4} \cdot s^{-1}$ (Figure 1). In comparison to natural ER microsomes which had a slope of $-6.9 \times 10^{-4} \cdot s^{-1}$. These two rates showed that PDI alone had a proinsulin proteolysis rate slower than that of a natural ER microsome, meaning another protein must be involved to facilitate ERAD. We then looked at another potential candidate that aids in ERAD, BiP. We wanted to see the degradation rate of BiP and PDI in concert. With microsomes containing both BiP and PDI, the slope was $-1.5 \times 10^{-3} \cdot s^{-1}$ (Figure 2), considerably faster than PDI alone and a natural microsome.
**Figure 1.** Graph of natural logarithmic fraction \( \ln(\frac{P}{P_0}) \) of remaining proinsulin versus time (sec) from proteolysis assay with PDI alone over two experiments.

**Figure 2.** Graph of natural logarithmic fraction \( \ln(\frac{P}{P_0}) \) of remaining proinsulin versus time (sec) from proteolysis assay with both BiP and PDI over four experiments.

**Conclusions:** PDI alone in reconstituted microsomes had a slower proinsulin proteolysis rate compared to that of natural ER microsomes. From this we concluded that other player(s) is (are) involved in facilitating the ERAD of mutant proinsulin. With both BiP and PDI, proinsulin proteolysis rate was faster than both the natural ER microsome and PDI alone in the reconstituted microsome. Elevated BiP and PDI concentrations compared to that of a natural microsome may account for the faster proinsulin degradation rate. Future adjustments of protein concentration will be done for more accurate results. We also hope to further examine PDI’s proteolysis rate by extending the reaction time from 40 to 60 minutes in order eliminate any inconsistency. Overall, from this experiment we can conclude that out of the hundreds of lumenal proteins, both BiP and PDI are critical for efficient ERAD of misfolded proinsulin.

**References:**

An Independent Physicians Association, also known as an Independent Practice Association (IPA), is a physician organized, self-directed entity which serves as a middle man, contracting between physicians and managed care organizations such as an HMO through per capita, flat retainer, or fee-for-service plans, with capitation remuneration representing roughly ¾ of California IPAs. An IPA may be primary care, single-specialty, or multi-specialty. Because of the Federal Trade Commission Act, IPAs are not used for collective bargaining but instead focus on implementing healthcare solutions on a regional basis and influencing positive political change. While data is somewhat limited as to the effectiveness of an IPA, some studies have shown greater than 50% decreases in in-hospital admissions, hospital days, and pharmaceutical costs compared to conventional care. An IPA Medical Director is generally in charge of physician recruitment. Because the IPA system has shown the positive potential in regional healthcare communities, it may further benefit by the inclusion of cost-effective Doctors of Podiatric Medicine (DPMs).

There are currently around 130 IPAs in California. The current Medical Director of each IPA was emailed a survey through Qualtrics to determine their understanding of and attitude toward DPMs. The survey includes questions such as the type of IPA with which they contract, the number of years the IPA has been in operation, the size of the IPA in physicians, and enrollees, whether or not outpatient podiatric services are covered, what types of podiatric care is available (diabetic, trauma, wound care, etc.), and general attitude toward podiatrists as far as skill and cost-effectiveness.

**Purpose of the Research or Description of the Problem:** The purpose of this research was to determine Independent Physician Association Medical Directors’ perception of Doctors of Podiatric Medicine in California.

**Study Methodology:** All Independent Physician Associations in California were identified and contact information gathered. Each group was then contacted with the goal of obtaining their respective medical director’s email. Once all emails were gathered, a survey was sent out to the medical directors.

**References:**

Assal Nour, College of Podiatric Medicine, Graduation Year: 2018, Advisor’s Name: Dr. Shofler, Advisor’s College: College of Podiatric Medicine, Source of Funding: Summer Research Fellowship, WesternU, *Antibiotic Treatment for Diabetic Foot Osteomyelitis: A Systematic Review*, Author List: Assal Nour

Osteomyelitis is a frequent complication of diabetic foot ulcers, particularly in neuropathic patients. Traditional management of the condition includes surgical versus conservative approaches. Conservative management involves long-term antibiotics delivered either intravenously or orally. While numerous clinical studies have described outcomes of patients with diabetic foot osteomyelitis (DFO), there has been no systematic collection of data performed. The purpose of this project is to perform a systematic review of the literature and a meta-analysis of the collected data. By doing so, the goal is to determine the outcomes of antibiotic treatment for DFO with increased statistical confidence. Secondary data will be used to determine if specific antibiotic protocols were associated with improved treatment outcomes and if specific locations of the foot or ankle were more or less responsive to antibiotic treatment.

Peyman Danesh, College of Podiatric Medicine, Graduation Year: 2018, Advisor’s Name: Dr. Jennifer D’Amico, Advisor’s College: College of Podiatric Medicine, Source of Funding: None, *Incidence of Depression Among Students of Podiatric Medicine*, Author List: Peyman Danesh BA, Jennifer D’Amico DPM

1st, 2nd, 3rd and 4th year Podiatry students at Western University of Health Sciences College of Podiatric Medicine were asked to complete a survey to determine the prevalence of depression among the CPM student population. The survey is based on the depression scale made by the Center for Epidemiologic Studies Depression Scale – Revised (CESDR) scale which measures symptoms of depression in nine different groups as defined by the American Psychiatric Association Diagnostic and Statistical Manual, fourth edition. Each of the questions have a response value associated with them which are summed to find the total CESDR score. The responses that show statistical significance will be compared to analyze which demographics might cause a higher incidence of depression. All demographics will be compared to each other using IBM SPSS Statistics. The aim of the research is to find out the depression rate in the Podiatry students at Western University of Health Sciences College of Podiatric Medicine and try to find the underlying factors such as the demographics that might cause depression in these students. Over 25% of the students have at least subthreshold depressive symptoms. Additionally, people that have been diagnosed as depressed or currently diagnosed as depressed were found to have major depressive episodes. Using the results from the survey, the future goal would be to come up with a special program that helps these students overcome depression by taking the underlying factors into consideration.

**Purpose of the Research or Description of the Problem:**

Medical students, such as podiatry students, experience depression, burnout and mental illness at a higher rate than the general population. Research has shown that burnout and depressive symptoms have led to thoughts of suicide. There are many factors that contribute to depression in addition to the stressful environment of medical school. Some studies have shown that the prevalence of stress and depression varies between different stages of education, such that first year medical students indicated the highest level of stress and depression. In addition, some studies have shown that there are gender differences in the prevalence of stress and depression, such that women reported more stress and depression than their male peers. Knowing that medical students have a higher risk of suicide, and higher rates of burnout than their peers in other fields, it is important to evaluate the incidence of self-reported depression among podiatry students. Furthermore, the research should investigate which demographics might put podiatry students at a higher risk of depression and what can be done for the students to minimize the depression and hence, reduce or even eliminate the thoughts of suicide.

**Study Methodology:** The survey was made accessible for the Podiatry students at Western University of Health Sciences College of Podiatric Medicine through Qualtrics, an online survey software. The survey was completely
anonymous and voluntary and no compensation was awarded to any of the students completing the survey. In order to access the survey, the students must accept that they have read, understood and agree to the consent form. The survey begins with demographic and background questions such as age, gender, race, class year, current relationship status, number of children, and whether they have been diagnosed or felt depressed and if they have ever sought or are receiving treatment for depression. The rest of the questions are from the CESD-R, The Center for Epidemiologic Studies Depression Scale Revised, which measure symptoms of depression in nine different groups such as sadness, loss of interest, appetite, sleep, concentration, guilt, tired, movement and suicidal ideation. The survey consists of statements such as “my appetite was poor,” or “I felt depressed,” to which the student would pick how often they have felt this way on a scale of “not at all or less than 1 day last week” to “nearly every day for 2 weeks” or “daily.” Each statement has response value associated with it such as “Not at all or less than one day” is given a value of 0 to “nearly every day for 2 weeks” which is given a value of 4 with the other responses in the middle given values between 1 to 3 depending on how often. The total CESDR score is calculated as a sum of responses to all of the questions and the determining categories range from “meets criteria for major depressive episode” to “no clinical significance.” The data is then compared with all of the demographics to evaluate if there are any demographics that are statistically related to the frequency of depression.

**Results:** Out of the 88 students that completed the survey this year, 64 had no clinical significance, 15 had subthreshold depression symptoms, 1 had probable major depressive episode and 8 met the criteria for major depressive episode. After analyzing and using correlations, there was a slight correlation of .121 of women being more likely to be depressed than men. Class year was found to be significant with a p value of .012 showing the correlation that higher upperclassmen are less likely to be depressed. Also, relationship status was significant with a p value of .007 showing that people involved in a committed relationship/married were less likely to be depressed than a single student. The questions “have you ever been diagnosed as “depressed?” and are you currently diagnosed as “depressed?” both had a p value of .000.

![Fig 1](image-url)

**Conclusions:** They study shows that 10% of the CPM students at Western University of Health Sciences have either had a major depressive episode or were probable to have had one. Additionally, another 17% have subthreshold depressive symptoms. These results show that over 25% of the CPM students have symptoms that might eventually reach the threshold of depression. The correlation of class year showing the upper classmen having a much lower incidence of depression shows that as students transition to their rotations, they’re less likely to be depressed if they made it this far. Additionally, students in a serious/committed relationship are less likely to be depressed since they have a partner in their relationship by their side. These results can be shared with upcoming students to show how prevalent depression is and which demographics have shown to decrease their likelihood of depression.

**References:**


Tina Nikoomanesh, Graduate College of Biomedical Sciences, College of Dental Medicine, Graduation Year: 2015, 2018, Advisor’s Name: Dr. Melanie Goldfarb, Advisor’s College: John Wayne Cancer Institute of St. John’s Hospital, Source of Funding: Summer research fellowship, *Emotional Distress in Young Surgical Cancer Patients, Author List: Tina Nikoomanesh BA; Dr Melanie Goldfarb MD, FACS*

**Background:** Emotional distress can impact quality of life in cancer survivors, especially younger patients. However, few large studies have explored factors associated with the development of emotional distress in adolescent and young adult (AYA: ages 15-39) cancer survivors that have had surgery as part of their cancer treatment.

**Methods:** AYA cancer survivors that had surgery as part of their cancer treatment were identified in the 2010 LiveStrong survey. Only patients that responded yes or no to all questions about depression, anxiety, and mood swings were included. Logistic regression analyses examined the sociodemographic and treatment factors associated with development of emotional distress.

**Results:** Of 1,104 AYA survivors, most were white (87.8%), female (60.8%), married (63.2%), insured (95.3%), and had survived at least 5 years (70.3%). Depression was reported by 58.5%, mood swings by 47.2%, and anxiety by 59.7% of patients; 37.3% of patients experienced all 3 elements of emotional distress. On multivariate logistic regression, unmarried (OR=1.68, CI:1.24-2.27) females (OR=1.89, CI:1.41-2.52) that received chemotherapy (OR=1.37, CI:1.03-1.82) had a greater likelihood of experiencing depression. Unemployed (OR=1.93, CI:1.09-3.40) females (OR=1.74, CI:1.30-2.34) within the first 5 years of treatment (OR=1.42, CI:1.04-1.94) were more likely to report mood swings. Anxiety was more likely to be reported by females (OR=1.41, CI:1.04-1.90) with a household income <$40,000 (OR=1.95, CI:1.24-3.05)that received immunotherapy (OR=6.56, CI:1.51-28.53) or radiation therapy (OR=1.52, CI:1.12-2.04).

**Conclusions:** Emotional distress is reported by many AYA surgical cancer survivors. Females are more likely to experience all elements of distress whereas type of adjuvant therapy, marital status, employment, income, and survivorship time only impact specific components of distress. Clinicians caring for these patients should have a heightened awareness and inquire about distress symptoms in at-risk AYA survivors.

**Fig.1 Multivariate logistic regression model of emotional distress in surgical cancer patients controlled with type of cancer and age (AYA)**
Cosmesis is often the only outcome that owners are able to visualize and understand in pets. The information gleaned from human medicine, regarding factors affecting cosmesis, is conflicting for many of those factors (Smith et al 2010, Sharma et al 2014, Sanni, Dunning 2007). For example, some studies state that staples result in improved cosmetic outcomes, while others found no difference of suture superiority (Sharma et al 2014, Angelini et al 1984, Mullen et al 1999). When it comes to cosmesis, literature in veterinary medicine is lacking and many principles are adapted from human medicine (Pavletic et al 2010). Since this study has not been performed in veterinary medicine, there is no validated cosmetic measure. Once the visual analog scale is validated for use in veterinary medicine, it could be invaluable to future study designs.

**Purpose of the Research or Description of the Problem:** This study chose to address two aims: 1) to compare the evaluation of cosmetic results of incisions by lay evaluators, owners, and veterinarians using a visual analog scale after suture removal, and at 8 weeks post-operatively. 2) to elucidate the risk factors for bad cosmetic outcomes following surgery including age, species, location, type of surgery, closure time, length of incision, surgeon, infection, inflammation, dehiscence, suture material, suture pattern, length of hair coat, and underlying systemic disease.

**Study Methodology:** For the first aim, 100 patients were admitted into the study. Photos of the incisions were taken after suture or staple removal, and at eight weeks post-operatively. Owners were asked to score their satisfaction with the appearance and the surgery at 2 weeks and 8 weeks after surgery. The photos were evaluated by 3 veterinarians and 3 lay people.

For the second aim, demographic data was gathered on the same patients admitted for the first aim. This data included age, species, location, type of surgery, closure time, length of incision, surgeon, infection, inflammation, dehiscence, suture material, suture pattern, length of hair coat, and underlying systemic disease. Digital images with calibration were taken immediately after surgery, after suture removal, and 8 weeks after surgery. These images were sent to three blinded evaluators who scored the photographs according to a scoring system from a previously published protocol (Sylvestre et al 2002, Smeak 1992) that looked at attributes such as swelling, dehiscence, erythema, and discharge.

**Results:** Initially, 104 patients have been recruited for this project; several have been lost to follow up. Ages ranged from 15 weeks to 15 years. Surgery time varied from 20 minutes to nine hours and five minutes. A variety of closure
materials were used such as PDS, prolene, monocryl, and staples. Out of the 104 patients enrolled, 89 have been seen for their two week recheck. Only two complications have been seen. Now, we are collecting the final data points for the 8 week recheck on all patients. Many different surgeries were performed, and some of the most common were TPLOs, OHEs, and exploratory laparotomies. Preliminary results show that surgery performed by a veterinary surgeon results in higher outcome scores than by a general practitioner. More results need to be collected and interpreted before a definitive consensus can be drawn. Results are needed from laypersons and veterinarians to determine whether expert opinion correlates with owner opinion in order to validate the visual analog scale used in this study.

**Fig 1**

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**Conclusions:** We expect to see several different factors influencing cosmesis. Surgery performed by a veterinary surgeon should result in better cosmetic outcomes than the same surgery performed by a general practitioner. Increased surgery time and closure time should impede healing and lead to worse cosmetic outcomes. For the same reason, it is hypothesized that incisions closed with suture will have worse scores than those with staples. For the second aim, we believe that layperson opinion will correlate with that of expert opinion and the visual analog scale will be validated for use in veterinary medicine.

**References:**


5. Jul;42(2):199-203


Zachary Gustin, College of Podiatric Medicine, Graduation Year: 2018, Advisor’s Name: Dr. Jonathan Labovitz, Advisor’s College: College of Podiatric Medicine, Source of Funding: Summer Grant, *Effect of Hospital Characteristics on Cost of Diabetic Foot Care*, Author List: Dr. Jonathan Labovits DPM, Dr. David Shofler DPM, Zachary Gustin podiatric medical student.

**Background:** Diagnosed cases of diabetes cost the United States an estimated $245 billion in medical services and lost productivity in the year 2012 according to the American Diabetes Association. The most common lower extremity conditions that are associated with diabetics include neuropathy, ulcers, infection, and arterial disease. It is common for these conditions to require patient hospitalization. Factors that are analyzed in this study include hospital ownership, teaching status, city population, medical service study area(MSSA), and number of beds. Previous studies have attempted to estimate the cost of care for these patients, but few have described which factors tend to lead to increased cost and utilization of resources. By identifying these factors, health care facility planners and administrators may be
better informed in their attempts to provide quality care while maximizing the efficiency of resource use.  **Study Methodology:** This paper presents an analysis of factors associated with cost and resource utilization for the inpatient treatment of subjects with lower extremity complications of diabetes mellitus (DM) within CA hospitals. Subjects were selected from OSHPD public discharge data files between the years 2010-2013. We extracted all patient files with a primary diagnosis or one of the first five (5) secondary diagnoses of DM. These patients were divided based on their ICD-9 codes into 5 cohorts: "Peripheral neuropathy", "Peripheral arterial disease", "Foot ulcer", "Foot Infection", and ">1 of any of the divided cohorts". Microsoft Excel was used to calculate the Elixhauser co-morbidity index, which was used to quantify and control for the severity of each subject’s illness. Excel was then used to identify characteristics of the hospital at which each subject received treatment. These characteristics were defined as the following: Owner (private, non-profit, district, city/county, unknown), Teaching Hospital (yes, no), City Population (>50000, 2500-50000, <2500), MSSA (urban, urban center, rural), and Number of Beds (small, medium, large). SPSS was then used to run a Pearson correlation between each independent variable and both the Estimated Cost and the Length of Stay of each subject. Variables that had a significant correlation were analyzed with a linear regression to determine how strongly they influence cost and length of stay. Finally, multivariate linear regression models were constructed to determined the combined influence of all 5 variables, while controlling for each subjects comorbidity index score.  **Results(Charts):** Ownership status was significantly correlated (p-value < .01) with cost and length of stay in all 5 cohorts. The averages of the linear regression coefficients for ownership between the 5 cohorts were $5431.031 and 2.3 days, demonstrating higher cost and longer LOS for publicly owned facilities. Teaching hospitals were correlated with higher cost in 4 out of 5 cohorts, and with shorter LOS in only 2 cohorts. The averages of the linear regression coefficients for teaching facilities were $13034.28 and 2.18 days of stay. Hospitals in geographic regions defined as urban by MSSA criteria were correlated with higher cost in all 5 cohorts, but no such correlation was found with LOS. The MSSA linear regression model produced coefficients of $10142.20 and 0.74 days of additional stay. Hospitals in cities with populations of >50,000 were correlated with higher cost in 4 out of 5 cohorts, and no significant correlation was found with LOS. The population linear regression model produced coefficients of $5014.06 and 0.83 days of additional stay. Hospitals with higher bed counts were correlated with higher costs in all 5 cohorts, and showed correlation with LOS in only 2 cohorts. The linear regression models for bed count produced coefficients of $8942.96 and 0.27 days of additional stay. Interpretation of data from the multivariate linear regression analysis models has not yet been finalized.  **Conclusion:** In summary, our preliminary data indicates strong correlations of all 5 characteristics with cost of treating diabetic patients with lower extremity complications. Higher cost was associated with the following: publicly owned hospitals, teaching hospitals, MSSA designated urban regions, heavily populated cities, and larger hospitals with higher bed counts. There are many possible explanations for these results. Large urban hospitals often function as teaching and research centers. These facilities may offer patients more advanced, and expensive, diagnostic and treatment options. The correlation of these characteristics with length of stay was much weaker. Only hospital ownership had a significant impact, with publicly owned facilities having a longer LOS. Further research is needed into other potential drivers of cost and utilization such as patient demographics, behavioral health, and procedures preformed during acute stay.  **References:**


Jeffrey Lo, College of Graduate Nursing, Graduation Year: 2017, Advisor’s name: Jan Boller, Ph.D, RN, Advisor’s College: College of Graduate Nursing, Source of Funding: TriCity Mental Health Grant – UrbanMission Project, UrbanMission Nutritional Education and Wellness, Author List: Jeffrey Lo, B.Sc., Jan Boller, Ph.D, RN

**BACKGROUND:** Sustainable health improvement is an on-going topic of concern amongst local community leaders and healthcare practitioners, as incidences of poor nutrition continue to rise within the underserved rural and urban
populations. A needs assessment and evaluation tool is a necessary first-step to identifying and describing the nutritional deficits affecting the local community.

Poor diet is a common outcome in many low-income, rural, and ethnic communities, particularly amongst disadvantaged groups. As a result, obesity, and other chronic diseases are more prevalent in these populations where there is a lack of access to healthier food options. The retail food chains that surround these communities are less available and therefore healthy food choices are frequently not an option (Zenk et al., 2014). Studies have demonstrated that the availability of better food and beverage choices in inter-community supermarkets allow for broader and healthier lifestyles as compared to the ones provided by convenience and grocery stores (2014). Their findings suggest that socioeconomic disparities in the lower-income population result in fewer healthier alternatives and subsequently poorer eating habits. However, one way to improve the relative availability of healthier food options would be to start the focus on small stores, and by bringing in more local, fresh produce to the area.

A collaborative effort between UrbanMission and the College of Graduate Nursing at Western University of Health Sciences, in the assessment, evaluation, and implementation of a nutritional education and wellness program, will address the special needs of this population. A nutrition education program can have numerous positive outcomes on the local community, such as developing better decision-making skills, improving knowledge of nutrition and dietary habits, nutritional counseling, and in developing healthy behavioral changes. In addition, perceived benefits and barriers are some of the components of the needs assessment and evaluation tool, and by identifying and addressing these concerns, a sustainable health improvement program can be established and tailored to these local communities.

PURPOSE: The purpose of this research investigation is to conduct a preliminary neighborhood survey in conjunction with a community engagement project centered around families and nutrition. The improvement of current nutrition availability for Pomona’s most vulnerable populations is a city-wide project aimed to promote healthy eating, and an active lifestyle. In a 2014 community needs assessment study conducted by Boller, Trudgeon, and Tuason (2014) in conjunction with the Healthy in Pomona initiative, nutritional education was among the top three health priorities identified by Pomona’s residents. This study will inform the development of a nutrition and wellness collaborative program involving UrbanMission, Western University of Health Sciences College of Graduate Nursing, and other community agencies. The project is funded in part by a grant to UrbanMission from TriCity Mental Health. Data gathered from this needs assessment will inform a community-based participatory research project involving the use of social, environmental, and experiential education, and policy strategies to reduce food insecurities among high-risk populations in the City of Pomona.

METHODOLOGY: An initial review of the literature was conducted to identify central concepts related to food insecurities, nutrition availability, and community-based approaches to improve nutrition in underserved vulnerable populations. Based on the initial literature review, five core concepts were identified relating to factors contributing to nutritional health in vulnerable populations (Figure 1).

Fig 1

A second review of the literature was conducted to identify valid and reliable survey instruments to gather baseline assessment data relating to the five central concepts.

RESULTS: While valid and reliable instruments were identified via the literature search, none addressed the five central concepts under investigation in a format appropriate for a community-based participatory action research study. The investigators are developing a brief exploratory survey, which will be submitted for WesternU IRB approval. Surveys will be completed by participants at monthly community dinners at UrbanMission, which are prepared by the
College of Graduate Nursing students. Results from the surveys will be shared with the participants who will work in collaboration with Western University and UrbanMission to improve nutritional health knowledge and access to healthy foods. **CONCLUSIONS:** We expect the outcome of the research project to provide us with a better understanding of the factors that influence a community’s dietary choices; the number of factors can revolve around a systems-level approach, such as health, cost, convenience, taste, and satisfaction; in addition, individual-level characteristics such as knowledge of nutrition, perception of healthy eating, ability to access local resources, attitudes toward improvement and change, and the skills level necessary to make these changes can also influence these decisions. Lastly, the implementation of a nutrition wellness program based on the assessment needs of the community would be established to help promote the positive outcomes of nutrition education and skills-development.

**REFERENCES:**
Boller, J., Trudgeon, R., & Tuason, I. (2014). *Community-based collaborative quality and safety education and practice improvement across the care continuum: Community needs assessment.* Study funded by Western University of Health Sciences Intramural Grant (IMR12330N)


**Chris Tatum, College of Podiatric Medicine, Graduation Year: 2018, Advisor’s Name: Dr. David Shofler DPM, Advisor’s College: College of Podiatric Medicine, Source of Funding: Summer Grant, Developing a Charcot Foot Radiograph Repository, Author List: Chris Tatum BS, Dr. David Shofler DPM**

**BACKGROUND:** Charcot Neuropathy is a very serious condition that can lead to a host of deformities and disabilities for patients. There are various treatment options available to correct this ailment. Deciding which option to use can be a source of frustration for clinicians due to the complexity of this condition. The purpose of this research is to create an online repository containing radiographs of patients with Charcot Neuropathy. These submissions will also contain other patient information such as age, gender, and nature of surgical treatment. It will also follow the course of treatment, from preoperative to postoperative. It is hypothesized that this repository will help clinicians determine the best course of action in treating their patients. A sample page of the repository is depicted below in Figure 1.

**RESULTS:** As of the writing of this abstract, the results consist of the completion of a working online repository. We are now moving into phase 2 of the research, which consists of collecting cases of Charcot Neuropathy from clinicians and loading them into the repository. This will be accomplished by contacting podiatrists and asking them for submissions. There is also a place on the repository that instructs clinicians how to submit cases without having to be contacted by us first. Once we have around 20 cases, the repository will be made accessible to the public and we can start to determine its effectiveness in treating this condition.

**CONCLUSION:** Charcot Neuropathy can be a complicated condition to treat. However, this repository should become invaluable to clinicians treating the condition. Now that development of the repository is complete, case submissions will be obtained. Once we have a sufficient number of cases loaded into the repository, we will then be able to collect data to determine its efficacy.

**Fig. 1:**
Little is known about the development of the distal limb, particularly the foot, in foals. Although much research has focused on the adult horse foot, foal feet cannot be compared as an equal but smaller version. The juvenile form has a different structure, and its rapid growth and radical change of shape hints at a uniqueness that has not yet been studied in full. The goal of this study was to establish a baseline of normal foal foot growth and conformation. This objective was met by quantifying external and internal anatomical characteristics of the foot throughout the first year of development. Radiographs and photographs were taken from both front feet of 9 Arabian foals every 2 months, beginning at birth until 12 months of age. Fifty-eight linear and angular variables were measured using Image J software. The ratio of linear measurements solar width : solar length increased except between 6 and 8 months of age, at which time the ratio decreased. Palmar heel length measurements showed an increasing growth pattern except between 6 and 10 months. Measurements of the distal phalanx were analyzed as a ratio of distal bone length : proximal bone length and increased in value until 6 months, at which point values decreased until 12 months of age. Intermediate and proximal phalangeal joint-to-ground distances followed a pattern similar to that of the palmar heel length. Interestingly, all results illustrated a distinct change in pattern after 6 months of age. It can be speculated that at this specific age, the foot begins remodeling into a mature variation. The sudden lengthening of the solar hoof may explain the shift in hoof shape from circular to oval, while the decrease in palmar heel length and decrease in phalangeal joint-to-ground distances may encourage the foot to break over in a palmar direction. These findings describe when and how the foal foot transforms from a club-like, cylindrical conformation to a more angled, conical shape. This information is useful in discerning physiologic from pathologic changes in the foot of the growing foal.

**Purpose of the Research or Description of the Problem:** A better understanding of foal foot growth is needed in order to differentiate physiological changes from pathological changes. Such pathological changes may present as conformational abnormalities which can be linked to subsequent lamenesses. It is well known that the majority of lamenesses occur in the distal forelimb, and that 70 to 80 percent of these originate from the foot. Proper management of feet during the first months of life can affect development and play an instrumental role in distal limb health and overall athletic capabilities. Ensuring that foot growth is occurring within normal parameters will decrease the likelihood of future lamenesses. The goal of this study was to compose a baseline of normal foal foot growth and conformation from 0 to 12 months of age in order to establish such parameters. This objective was met by quantifying several external and internal anatomical characteristics of the foot. **Study Methodology:** Digital radiographs and photographs were taken from both front feet of 9 foals, beginning at birth until 12 months of age at an interval of every 2 months. Fifty-eight linear and angular variables were measured from dorsal, palmar, lateral, medial, and solar views using Image J software. The following variables were analyzed for growth patterns:
Photograph variables:
*SW: the average of LSW and MSW; measured from the widest part of the hoof to the midline
*SL: the average of LPL and MPL, plus DL. MPL & LPL are measured from the point of the bars to the level of the apex of the frog (TAX)
*PHL: the average of LPHL and MPHL

Radiograph variables:
*PBL, DBL, JGD1, JGD2

Results: The ratio of SW/SL increased with age except between 6-8 months, at which time the ratio decreased (Fig. 1). The PHL measurements showed an increasing growth pattern apart from decreasing between 6-10 months (Fig 2). Measurements of the distal phalanx were analyzed as a ratio of DBL/PBL and increased in value until 6 months, at which point values decreased until 12 months of age (Fig. 3). Intermediate and distal JGD followed a pattern similar to that of the PHL (Fig 4).

Legend
SW= Solar hoof width; SW = LSW + MSW
LSW= Lateral solar hoof width
MSW= Medial solar hoof width
SL= Solar hoof length; SL = [(LPL + MPL) / 2] + DL
LPL= Lateral palmar hoof length (bar to apex of frog)
MPL=Medial palmar hoof length (bar to apex of frog)
DL= Dorsal length of the hoof (tip of frog to toe)
TAX= Transverse axis
PHL= Palmar heel length; PHL = (MPHL + LPHL) / 2
PBL= Palmar bone length of the distal phalanx
DBL= Distal bone length of the distal phalanx
JGD1= Intermediate phalangeal joint-to-ground distance
JGD2= Distal phalangeal joint-to-ground distance
Fig 3

Conclusions: The results of this study quantify major physiologic changes of the foot that occur during the first year of life in the horse. Despite widespread acknowledgement of the importance of good forelimb conformation\(^5\), there are no documented physiologic parameters for distal limb growth in the foal. Data were interpreted in order to illustrate hoof transformation as it morphs from a cylindrical shape to a more conical shape\(^6\), while describing some corresponding phalangeal growth and joint angle alterations.

Based on the findings, the beginning of this transformation is dominantly evident starting at 6 months of age. From a solar aspect, the hoof begins to grow predominantly in a dorso-palmar direction around this time. The lengthening of the solar hoof describes hoof reshaping, while the decrease in PHL and JGD may encourage the foot to break-over palmarly. Farriers strive to adjust break over of the foot\(^7\), thus it is useful to know when this shift should naturally occur.

This time period of crucial change also outlines a distinct relationship between the hoof and the distal phalanx. At 6 months old, the PBL begins to lengthen, while the SL does the same. This suggests that the structure and developmental status of the hoof may be indicative of the internal architecture, such as the distal phalanx. Parallels have been acknowledged between external and internal structures of the hoof\(^8\), but never quantified in respect to development. Collectively, these findings identify an age at which the distal limb begins remodeling from an upright conformation to a more adult form. A better understanding of development allows for better management and prevention of diseases of the foot.

References:


Daniel Gutman, College of Veterinary Medicine, Graduation Year: 2017, Advisor’s Name: Dr. Susana Tkalcic, Advisor’s College: College of Veterinary Medicine, Source of Funding: Summer Research Fellowship, *Volcanic Gastric Ulcers in California Sea Lions*, Author List: Tkalcic, S., Gutman, D., Palmer, L.

The objective of this research is to quantify and qualify the lesions associated with ulcerative gastritis in California Sea Lions (Zalophus californianus) that died or were euthanized due to unsuccessful rehabilitation efforts at Marine Mammal Care Center. The specific aim is to record the occurrence of volcanic gastric ulcers vs. acute bleeding ulcers in the targeted population of pinnipeds that died or were euthanized as a result of their illness or injury between April and August. Since these lesions are most commonly associated with gastric parasitism, the number and type of parasites residing in the stomach will be recorded. This data will contribute to biomedical science regarding the etiology and pathology of gastric ulceration in California Sea Lions. We expect that the most severe ulceration will occur in younger and stressed animals with higher overall parasite ratio (gastrointestinal and other systems) and a longer duration of disease, and/or treatment. The results of this research may identify possible targets for intervention during rehabilitation with the goal of improving health outcomes.

Purpose of the Research or Description of the Problem:
The Marine Mammal Care Center (MMCC) in San Pedro rehabilitates hundreds of California Sea Lions annually. Unfortunately, not all of these animals recover and some will die or be humanely euthanized as a result of their injury or illness. Previous necropsies on carcasses revealed a high incidence of severe, prominent, gastric ulcers, both acute and chronic. Several of the animals had severe parasitic gastrointestinal infections concurrent with one or more
volcanic gastric ulcers. Many of the subjects had multiple systems affected by heavy parasitic load or concurrent pathologies. This focused research should allow gathering of more data and materials to determine the current epidemiological status and confirm the incidence of ulcerative gastritis in different age categories of California Sea Lions, and further correlate its occurrence with nematode burden within the stomach.

According to previous studies, gastrointestinal parasitism represents a large segment of diseases affecting marine mammals, mostly associated with roundworm infestations. Pinnipeds are final (dead-end) hosts for most stomach worms, acquiring them from other aquatic animals (copepods, fish, squid) as parthenic hosts (1). Animals typically present clinically with a severe weight loss, anorexia, and dark feces (melena). Identification of worms is based on the morphology and morphometry of the parasites, and its histopathological appearance (5). Roundworms (class: Nematoda) are the most diverse group of parasites typically present throughout the gastrointestinal tract of susceptible pinnipeds. Common nematode species reported to be associated with ulcerative lesions in the stomach of California sea lions include members of the family Anisakidae (large roundworms; superfamly: Heterocheiloidea, Order: Ascaridida, Class: Nematoda) with Contracaecum spp., Anisakis spp., and Pseudoterranova spp as less represented (3,5). Anisakidae are found in the necropsied marine mammals (pinnipeds and cetaceans) rescued along the Pacific Coast from Alaska to Galapagos Islands and South America (2,4). Pinnipeds, as final hosts for most anisakids, are playing a role in the life cycle and movement of parasites between marine invertebrates, fish, birds, and other aquatic mammals (3).

Ulcer in the alimentary tract is defined as a full thickness loss of mucosa due to necrosis beyond the level of basement membrane that extends through muscular mucosa into submucosa, usually provoking inflammation (7). The term “volcanic” ulcer was introduced into medical terminology in “Protocols for the care of oil-affected marine mammals” by Oiled Wildlife Care Network and UC Davis Wildlife Health Center (6). These descriptive terms align with gross morphology of chronic gastric ulcers, but searching through scientific literature did not reveal more usage of this term.

Study Methodology:
Necropsies were performed at the Marine Mammal Care Center in San Pedro on carcasses of pinnipeds that did not survive the rehabilitation during the period of high morbidity/mortality (April-August). No animals were euthanized for participation of the study. Medical records have been evaluated and necropsy forms prepared with data indicating the date of stranding and death, age, sex, body condition, and size. All animals were examined between 3 hours to 4 days post-mortem, most of which within 24 hours. During the necropsy, stomachs were grossly evaluated, photographed, and documented. Any ulceration was measured, morphologically qualified, and sampled for histopathology. The tissue samples were placed in 10% buffered formalin, transferred to the WesternU, and fixed for a minimum of 18 hours before trimming for lab submission. Gastric parasites were quantified and collected in alcohol for future identification. A complete necropsy was performed on each animal to further identify any concurrent lesions and diseases. Histopathological samples of concurrent pathological lesions have been processed routinely for histopathology, and the hematoxylin/eosin stained 5-micrometer sections will be evaluated morphologically with a veterinary pathologist and the findings will be recorded. All the data will be analyzed statistically in the near future.

Gastritis was grossly evaluated in full by reflecting the mucosal surface and cutting the full length of the lesser curvature of the stomach. Gastritis was deemed positive through diffuse or locally extensive reddening of the mucosal surface (fig. 1). Acute ulceration was confirmed with active bleeding or coagulation within one or more focal areas within the gastric mucosa (fig. 2). And, volcanic ulceration was identified as a bleeding or fibrotic focal area with obvious raised edges above the surrounding mucosa (fig. 3,4). Microscopic identification of the extent of volcanic ulceration is still in process.

Age of individual sea lions was based on body length. Animals under 100 cm of length from nasal planum to tail tip were deemed pups under 1 year of age. Animals of length between 101 cm and 114 cm were designated as yearlings aging between 1-2 years old. Subadults (2-3 years old) were designated as individuals between 115 and 150 cm. Adults (3+ years old) were individuals at lengths greater than 150 cm.

Gastric parasite load was grouped into severity categories based on the number of individual parasites as follows: mild with 1-10 parasites; moderate with 11-25 parasites; and severe with 25+ parasites.
Results: A total of 28 sea lions were available for necropsy and data evaluation. 20 out of 28 individuals had evidence of gastritis, of which 10 had evidence of volcanic gastric ulceration. Age correlation shows that animals under 2 years of age did not have evidence of volcanic gastric ulcers, but individuals 2 years or older were affected by chronic gastric ulceration (Table 1.1).

Only 12 animals were evaluated for parasite load; 9 out of 12 of these animals presented with gastritis. The remaining 16 animals were excluded from analysis because they were in rehabilitation for longer than 48 hours. All animals are dewormed upon intake at the care center, and deworming treatment has potential for affecting an accurate evaluation of parasite burden in individual animals. Animals with no or less than 10 individual gastric nematodes did not have evidence of volcanic gastric ulcers despite 4 out of 5 having gastritis. Of the remaining animals, 4 out of 7 with moderate to severe gastric nematode burden presented with volcanic gastric ulceration; 5 out of 7 had evidence of gastritis.

Body Condition Score was accounted on all necropsied animals based upon sternal blubber thickness, visualization of bony prominences/ribs, and weight to length ratio. 4 out of 6 animals with the lowest body condition score (BCS = 1) showed evidence of gastritis with only 1 animal containing volcanic gastric ulceration. 12 out of 16 underweight animals (BCS: 1.5 - 2) showed gastritis with 5 animals containing volcanic gastric ulceration. 4 out of 6 normal weighted animals (BCS: 2.5 - 3) showed gastritis with all 4 animals containing volcanic gastric ulceration.

Other common parasite infected organs were evaluated relative to incidence of gastritis. 21 of 28 animals contained pulmonary infection, 4 of which had no gastritis, and 9 of which contained volcanic gastric ulceration. 19 of 28 animals contained large intestinal pathology, 4 of which had no gastritis, and 10 of which contained volcanic gastric ulceration. The nasal cavity of 21 animals were examined, and all animals contained mild to severe nasal mite infection.

The length of rehabilitation before mortem was also evaluated with respect to gastritis. 4 out of 6 animals that died within 24 hours of rehabilitation had gastritis, 2 of which had volcanic gastric ulceration. 5 out of 7 animals that died within 1-3 days of rehabilitation had gastritis, 2 of which had gastric ulceration. 6 out of 7 animals that died within 4-7 days of rehabilitation had gastritis, 3 of which had gastric ulceration. 3 out 6 animals that died 8 or more days in rehabilitation had gastritis, 2 of which had gastric ulceration.

Ulcerative Gastritis In California Sea Lions
Conclusions: The incidence of gastritis in rehabilitating California Sea Lions observed in this study is very high at 71%. Reports showed that volcanic ulcers are a chronic condition only found in maturing animals that were at least 2 years of age. There is also evidence that heavier parasite loads are correlated with incidence of volcanic gastric ulcers, but not necessarily gastritis in itself. The data on this subject is suggestive, but more necropsies will need to be performed on animals without rehabilitation to confirm these findings. Contrary to our hypothesis, volcanic gastric ulcers were more common in animals that had higher body condition scores. However, this needs to be further evaluated with respect to age as most of the animals with lower body condition scores are younger. The results may suggest that a chronic, compromised, gastric environment can only be withstood by heartier animals that have a reservoir of stored energy.

Concurrent pathologies of different organs play a role in the ultimate degradation of the body; however, no correlations could be drawn as to which might be the primary parasite. Nasal mites in varying degrees of severity were observed within nasal cavities explored on all subjects; however, it was rarely associated with significant pathogenesis such as mucopurulent discharge or severe rhinitis. Lungworms were observed on many of the subjects and lung pathogenesis was considered to be a primary cause of death in many animals; but, there was not a significant correlation between gastric pathology and the occurrence of severe pulmonary disease. It should also be noted that many of these animals suffered definitive, traumatic causes of death. Parasitic infiltration may have contributed to their overall health status, but were not a significant cause of death. Two cases are used for example, a
pyloric blockage from garbage foreign body ingestion or osteomyelitis from a fractured metatarsus. Both cases, the animals did not have any gastric parasite burden or gastric ulceration. On the contrary, in another case, an acute death (<24 hrs) from a gunshot wound to the head of a subadult animal did have volcanic gastric ulceration and a moderate parasite burden. The severity of traumatic incidences cannot be qualified or quantified, but their parasitic burden and concurrent pathologies are still representative of the natural population. This research is still in progress with histopathological and fine tuned statistical evaluation needing to be conducted.

References:


Patil Injean, COMP, Graduation Year: 2018, Advisor’s Name: James A. McKinnell, MD, Advisor’s College: UCLA, Source of Funding: WesternU Research Committee, Surveillance of VRE and CRE in a Liver Transplant ICU. Author List: P. Injean BS, R. Linfield BA, S. Miller PhD, M. de Shadarevian BS, A. Gregson MD, H. Li PhD, N. Bangayan BS, Z. Rubin MD, T. Kim MS MPH, S. Eells PhD, R. Humphries PhD, J. A. McKinnell MD

Background: Multidrug resistant organisms (MDRO), such as VRE and CRE, are associated with higher hospital mortality and costs1,2 MDRO surveillance can prevent spread of infection through early infection prevention efforts3,4 Optimal surveillance methods remain undefined in terms of convenience, sensitivity, and detection rate. We hypothesized that (i) weekly surveillance will detect more MDRO colonization, acquisition, and spontaneous loss when compared to admission only surveillance, (ii) perirectal swabs are less sensitive than stool in detection of MDRO colonization, and (iii) compliance with perirectal swabs is higher than stool. Method: Perirectal swab and stool samples were collected at admission and weekly from all patients in a liver transplant unit 8ICU for 4 weeks. VRE and CRE were detected using standard chromagar methods. Sensitivity of weekly surveillance was calculated with simultaneous swab and stool specimens. Compliance was defined as sample obtained compared to sample opportunities. Statistical analysis were calculated using Mcnemar’s, Fisher, or Chi square measures of association (Stata).

Result: There were 133 surveillance opportunities among 53 patients. Compliance with the surveillance protocol was >99% for admission and 100% for weekly testing. Swab sensitivity did not differ from stool for VRE (36/38 vs 35/38, p=1) or CRE (6/6 vs. 6/6). Compliance with swabbing was higher than stool (120/133 vs. 80/133, p<0.01). Perirectal swab detected more patients with VRE (57/65 vs. 41/65, p<0.01) but, did not differ from stool for CRE (7/8 vs 7/8). Weekly colonization prevalence ranged from 38% - 67% for VRE and 3% - 13% for CRE. Weekly testing discovered more VRE (2 versus 8) and CRE (0 versus 2) patients compared to admission only. The combination of admission and weekly swabs detected 8 incident VRE colonizations, 2 incident CRE colonizations, and 10 spontaneous losses.

Conclusion: Given the small difference in VRE and CRE sensitivities between perirectal swab and stool cultures, surveillance compliance is the key component. The highest-yield approach would be increasing compliance with swabs, or requesting both a swab and stool sample. The addition of weekly surveillance to traditional admission swabs results in a 5-fold increase in MDRO detection. Weekly surveillance also demonstrates the dynamic nature of MDRO
colonization. Future MDRO surveillance protocols for routine clinical practice or research purposes should include weekly MDRO surveillance.

Fig 1 - Sensitivities of Swab Versus Stool For VRE and CRE

![Sensitivities of Swab Versus Stool For VRE and CRE](image)

Fig 2 VRE and CRE colonization prevalence

![VRE and CRE colonization prevalence](image)

Fig 3 Weekly VRE and CRE incidence with 29 patients present for more than 1 week

![Weekly VRE and CRE incidence with 29 patients present for more than 1 week](image)

References:
INTRODUCTION. The recent proliferate use of video screen monitors (VSMs) with computer and cell phone use has drastically increased in the last decade. The prevalence of myopia in the United States has also increased in the last four decades. The long-term effects on visual strain and myopia related to proliferate VSMs use is an active area of debate and research. Objective of this study is to investigate the affects of VSMs use on vision. METHODS. Literature search was conducted using contemporary ophthalmic journals regarding VSMs use and affects on vision, and on myopia prevalence rates in the US. Data collected on native Ugandan’s (N=23) was randomly collected to ascertain their visual acuity, and to inquire about their daily usage of VSM. Visual prescription data compiled on native Ugandans, who drastically have lower VSM use than adults in the US was compared, to determine a relationship between lower VSM use and myopia prevalence. RESULTS. Literature search revealed no known research comparing developed country vision prescription data to developing country vision prescription data in the context of the affects of VSM. As expected, a self reported survey found that only 1 out of 23 native Ugandan’s uses a computer. Prevalence of myopia rates of Ugandan’s that have a myopia diopter of greater than 1, was found to be 30.4
percent. According to literature search data, current myopia prevalence rates for the US adult population is approximately 42 percent. DISCUSSION. The research results found that individuals in developing countries have lower prevalence of myopia than individuals in developed country. One possible explanation of this phenomenon could be due to the excessive VSM for daily tasks in developed countries, despite better access to health care and nutrition in developed countries. CONCLUSION. The purpose of this study was to collect data on myopia prevalence rates for individuals in a developing country, and to compare those rates to individuals in a developed countries, in order to ask the question, could proliferate VSMs use in a developed country be a possible cause for the increase in the prevalence of myopia. The research showed that despite better access to nutrition and health care, individuals in the United States have a higher prevalence of myopia. The proliferate use of VSMs could be a possible explanation for the disparity of myopia rates between developing countries and the United States.


BACKGROUND: *Mycobacterium tuberculosis* is the causative agent of tuberculosis, a worldwide disease. Phagocytosis by alveolar macrophages in the lung is the initial immune response to the presence of the bacteria, but they escape the cidal effects of macrophages by evading the fusion of the phagosome with the lysosome, a process that is vital for successful activation of killing mechanisms utilized by macrophages (Kindt, Goldsby, Osborne, & Kuby, 2007). Previous studies have shown that enhanced control of *M. tb* by macrophages appears to be highly correlated with glutathione, an antioxidant that is normally responsible for combating oxidative species (Venketaraman et al., 2003). In order to study the effects of glutathione, macrophages will be infected with an attenuated strain of bovine mycobacteria named *Bacillus Calmette-Guerin* (BCG), conjugated to Green Fluorescent Protein (GFP) and treated with a liposomal glutathione formulation. Using fluorescent microscopy and Lysotracker-red to stain the phagolysosome, the co-localization of BCG-GFP and stained lysosomes will allow for a better understanding of the effects of glutathione on phagosome-lysosome fusion in BCG infected macrophages, successfully thwarting the infection.

Purpose of the Research or Description of the Problem: Mycobacterial species survive inside human cells by evading the host defense system. Specifically, the bacteria prevent fusion of the phagosome and lysosome, which allows survival and avoids intracellular destruction usually resulting from phagocytosis by macrophages. Because GSH has been linked to increasing the immune system, we hypothesize that treating human THP cell lines after differentiation into macrophages with GSH will enhance the cells’ ability to destroy phagocytosed BCG bacteria.

Study Methodology: The effect of supplementation with a liposomal formulation of GSH (L-GSH) on differentiated macrophages will be studied by using an attenuated strain of bovine tuberculosis (BCG), conjugated to Green Fluorescent Protein (GFP). BCG-GFP is known to exhibit the same mechanism of evasion as Mycobacterium tuberculosis, making it ideal for studying the effects of L-GSH. BCG-GFP can be studied in vitro using THP-1 cells differentiated into macrophages. THP-1 cells will treated with PMA to differentiate the cells into macrophages. The differentiated THP cells will then be infected with BCG and lysed at 1 hour and 5 days. Viability of BCG cell cultures will be plated to determine CFUs, allowing us to study the effects of GSH. Controls involving no treatment and treatment with a GSH depleting agent, BSO, will serve as controls for our experiment. Furthermore, lysosomes and acidified phagosomes will be visualized using an acidophilic fluorescent dye, Lysotracker-Red. This will allow us to understand the effect of GSH on fusion of the phagosome and lysosome.

Results: We expect the results will yield promising findings. Specifically, we expect to see markedly decreased viability of BCG cultures from wells containing lysed THP cells treated with L-GSH and NAC (a glutathione precursor). In contrast, untreated THP cells and cells treated with a GSH-depleting agent, BSO, should show elevated CFU counts.
Fig 1: In cells supplemented with GSH, we expect increased phagosome-lysosome fusion, leading to increased survival of the THP cell, while decreasing the viability of BCG.

Conclusions: At the completion of our experiment, we expect to better understand the role of GSH on the immune system by understanding the role it plays in enhancing the ability of immune cells to control intracellular infections of BCG, which can be applied to infections of Mycobacterium tuberculosis, an intracellular pathogen.

References:


Stephanie Campbell & Meghan Blanchet, College of Podiatric Medicine, Graduation Year: 2018, Advisor’s Name: Dr. Rebecca Moellmer, DPM, Advisor’s College: College of Podiatric Medicine, Source of Funding: Western University of Health Sciences, *Transmetatarsal Amputations: A Biomechanical Analysis*, Author List: Stephanie Campbell, B.S., Meghan Blanchet, B.S., David Shofter, DPM, Rebecca Moellmer, DPM, FACFAS

Background: The incidence of diabetic wound amputation is prevalent worldwide. Diabetic patients often acquire complications due to their disease that require amputations in order to prevent systemic disease. Generally, the more distal the amputation, the better the outcome and prognosis for the patient. Transmetatarsal amputations (TMA) have therefore become a preferential procedure as opposed to more proximal amputations. However, there is not sufficient research literature discussing what type of parabola shape created across the metatarsals is the most beneficial for the patient in terms of post-operative biomechanics. This study aims to compare different parabolas from transmetatarsal amputation procedures and their effects on plantar foot pressures, postoperative complications, and the presence of abnormalities in the post-operative physical exam. Further, this study aims to fill the literature deficit in order to provide a standard of care. The patients' post-operative parabola and biomechanics are assessed after the transmetatarsal amputation surgery using a Tekscan HR mat, radiographs, and clinical observation. This ten patient
case study will provide clinical support of whether to choose a TMA surgical technique that creates metatarsal parabolas with either the second or third metatarsal as the longest, leading to the most successful patient outcomes.

**Purpose of the Research or Description of the Problem:** Transmetatarsal amputations are a useful tool for diabetic patients in order to prevent local infections from becoming systemic. The purpose of this study is to examine whether there is a relationship between the shape of the post-operative metatarsal parabola and the patient’s resulting plantar foot pressures, post-operative biomechanical complications or abnormalities in their physical exam, such as calluses and/or ulcers. This research will provide insight into specifications for the transmetatarsal amputation surgical technique and will improve its effects on the patient’s resulting post-operative biomechanics. With optimal post-operative biomechanics, the patient will have a lower risk of future proximal amputations, recurrent ulcerations, and other complications.

**Study Methodology:**
Patients will be enrolled from the podiatry clinic at Arrowhead Regional Medical Center in Colton, California over a one-year period. The anticipated number of patients for this project is 10-20.

Inclusion criteria will include:
1.) Transmetatarsal amputation with or without Achilles lengthening performed at least one year earlier  
2.) Patient age greater than 18 and less than 70 years  
3.) English speaking  
4.) Patients must be ambulatory without the aid of crutch, walker, or wheelchair

Exclusion criteria will include:
1.) Patient must not have had a previous cardiac event or angioplasty  
2.) Patients with active foot infections will be excluded from the study

In this study, the metatarsal parabola will be assessed using weight-bearing AP radiographs and plantar foot pressures will be measured using a pressure plate. Ollier’s system as described in “Metatarsal Protrusion Angle: Values of Normality” will be used to measure metatarsal lengths and angles. Patients fulfilling the inclusion criteria, subsequently completing informed consent via the supervising podiatrist, will participate in the study and be compensated monetarily for their participation.

The Tekscan HR Mat is calibrated for each patient following weighing. The patients are weighed during their office visit, without shoes, and the measurement is recorded in the software in order to properly calibrate the mat for each patient doing a step calibration. The two-feet step calibration was chosen in place of the single-foot step calibration with the understanding that the pressure points are monitored as a percentage of distributed weight and as a measure of safety for the patients. The technicians became proficient with the Tekscan digital pressure plate scanner in order to calibrate and record computerized scans of the patient’s sway stance and gait. After calibration, the patient completed two-30 second stance recordings on the mat. The patients began the recording already standing on the mat, eyes open, looking at a fixed point on the wall ahead with arms relaxed at their sides. Between measurements, the patients were asked to step off the mat, and then step back on the mat, and complete a second recording. It was recorded in the Tekscan Research Software that both feet were on the mat, eyes open, with frames of 0.10 seconds for 30 seconds or 600 frames. Per frame, the software further recorded pressure mapping of both feet, the center of gravity over the recording, as well as distributed pressure percentages in the anterior, posterior, right and left portions of the feet, and peak pressure in PSI.

The second phase of study was gait analysis. The parameters inputted during gait analysis were the corresponding foot striking the map during the line of projection with frame setting at 0.0055 seconds. The recording began with heel strike to last contact on the mat. This imprint was recorded as the third step in the patient’s gait cycle. The patient was asked to walk upright looking at a fixed position on either side of the room. The recordings register for approximately seven seconds. Three measurements were taken for both the left and right foot.

**Results:**
Figure 1. Patient 176, 39M, R TMA, Two Feet Standing Pressure Mapping

Figure 2. Patient 176, 39M, R TMA, Graph depicting peak force (lbs) over time (s) during a two feet standing, 30 second period of pressure mapping.

Figure 3. Patient 176, 39M, R TMA, Post-operative radiograph of right TMA.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Longest Metatarsal in Parabola</th>
<th>Location of Peak Force Point- TMA</th>
<th>Avg Standing Peak Force (lbs)</th>
<th>Patient's Weight (lbs)</th>
<th>% Total Weight at Peak Force Point</th>
</tr>
</thead>
<tbody>
<tr>
<td>176</td>
<td>4th</td>
<td>2nd met head</td>
<td>6.471</td>
<td>251.0</td>
<td>2.58</td>
</tr>
<tr>
<td>177</td>
<td>3rd</td>
<td>5th met head</td>
<td>7.025</td>
<td>178.4</td>
<td>3.94</td>
</tr>
<tr>
<td>178</td>
<td>1st</td>
<td>1st met head</td>
<td>4.382</td>
<td>257.8</td>
<td>1.70</td>
</tr>
<tr>
<td>179</td>
<td>3rd</td>
<td>heel</td>
<td>5.866</td>
<td>215.2</td>
<td>2.73</td>
</tr>
</tbody>
</table>
Table 1. Patient information describing parabola shape, peak pressure points of TMA foot, corresponding average forces in pounds of pressure during both 30 second standing trials, and total proportion of total body weight represented at indicated pressure points.

Conclusions: This study is ongoing and thus far, the results have been observed from 4 different patients. Hence, the data can therefore not adequately support any strong conclusions yet; however, the two patients with parabolas exhibiting the third metatarsal as the longest have had the highest pressure points while standing in proportion with their weight and these points were at the fifth metatarsal head in one patient and the medial heel in the other. Neither of the other patients had their second or third metatarsals as their longest in their parabola, so the two parabola shapes cannot be compared until more patients are assessed. Currently, no correlations can be drawn from parabola shapes and the formation of post-operative recurrent ulcerations, or any other complications, from this patient sample.

References:


Sun-Jae Kim, College of Osteopathic Medicine, Graduation Year: 2018, Advisor’s Name: Dr. Ying Huang, Advisor’s College: College of Pharmacy, Source of Funding: WesternU Summer Research Fellowship, Effect of Beta-blocker Carvedilol on drug-resistant BRAF-mutant melanoma, Author List: Sun-Jae Kim, Mandy Liu, Ying Huang

One of the most exciting approach to treat malignant melanoma is the use of drugs that target the cancer-driving BRAF gene, which is mutated in 45% of the melanomas (Glitza, 2014). Vemurafenib is a small-molecule drug that inhibits BRAF and shows improvements in survival of melanoma patients with BRAF mutant tumors (Su, 2011). However, most patients treated with vemurafenib develop resistance (Zhang, 2015). Thus, resistance is a critical clinical problem in the management of melanoma and alternative treatments are urgently required for patients with resistance to BRAF inhibitors such as vemurafenib. The goal of the summer research project is to identify new agents that may overcome the resistance of melanomas to vemurafenib. Recently, Dr. Huang’s lab discovered that a cardiovascular agent carvedilol which belong to the class of β-blockers showed a novel anticancer activity (Chang et al 2015). Although the mechanism for such activity on cancer is unknown for carvedilol, it was examined to determine its capability to overcome the BRAF inhibitor drug resistance. A pair of vemurafenib-sensitive and -resistant melanoma cell lines, A375 and A375RF21, was used in our investigation. First, the response of the two cell lines to vemurafenib and several other inhibitors of the same pathway was examined. The IC50 for vemurafenib was found to be 0.49 μM and > 10 μM in A375 and A375RF21, respectively. Carvedilol was able to increase the sensitivity of both A375 and A375RF21 cells to vemurafenib. The IC50 for A375RF21 was decreased to 0.37 μM in the presence of 10 μM of carvedilol (fold change was ~ 27-fold). Further studies are on-going to examine the effects of other β-blockers. In conclusion, the BRAF inhibitor resistant cell line (A375RF21) was confirmed for the resistance to vemurafenib in comparison with the sensitive cell line A375. Furthermore, combined use of carvedilol and vemurafenib showed improved response in drug-resistant BRAF-mutant melanoma. These results indicate a new approach to overcome resistance in melanoma using FDA-approved β-blockers.

Purpose of the Research or Description of the Problem: Therapies that target the driver oncogene, BRAF, in melanoma have achieved remarkable responses in patients with BRAF-mutant tumor. In fact, treatment with BRAF inhibitor has been shown to increase survival rate by 7.3 months (Robert, 2015). However, as with conventional
chemotherapies, patients often develop resistance to BRAF inhibitors. We hypothesized that a class of cardiovascular drugs, β-blockers (carvedilol as an example), may overcome resistance of melanoma to BRAF inhibitors. The goal of this short-term summer project is to examine the effect of carvedilol on BRAF-resistant melanoma cells. **Study Methodology:**

In order to test this hypothesis, the BRAF inhibitor resistant cell line (A375RF21) was first confirmed for the resistance using sulforhodamine B colorimetric assay (SRB) assay for drug-induced cytotoxicity, in comparison with the sensitive cell line A375. Next, the effectiveness of combined use of carvedilol and vemurafenib on A375 and A375RF21 cells was examined. Although more work must be included to fully develop the mechanism of action, these results may possibly lead to discovery of a new approach to overcome the resistance in melanoma and new anticancer mechanism for β-blockers. **Results:**

1. Growth inhibitory effect of vemurafenib in A375 and A375RF21 cells. As can be seen from Figure 1, vemurafenib treatment on A375 showed IC50 of 0.49 uM. In treatment of A375 BRAF resistant cell line (A375RF21), vemurafenib showed IC50 of >10 uM. The fold change was ~ 20 fold.  

2. Growth inhibitory effect of carvedilol in A375 and A375RF21 cells  

We next tested the effect of carvedilol treatment alone on A375 and BRAF resistant A375. Carvedilol showed similar effects on both cell lines (Figure 2), resulting in IC50 ~ 10 μM.  

3. Effect of carvedilol and vemurafenib combination treatment in A375RF21 cells

The combined treatment of vemurafenib and carvedilol in A375RF21 greatly improved the response (IC50 ~ 0.4 μM) in comparison with the vemurafenib alone (> 10 μM) (Fig 3). The fold change was estimated to > 25-fold (Table 1). This result suggests that carvedilol may increase the sensitivity of BRAF inhibitor-resistant melanomas to the therapy.  

4. Growth inhibitory activity of other inhibitors of RAS-BRAF-MEK pathway in A375 and A375RF21 cells and other cancer cell types without BRAF mutation

We also tested the sensitivity of several cancer cell lines to vemurafenib without BRAF mutations (Table 2). The IC50 values of vemurafenib were all higher than 10 μM. This was reasonable because unlike A375 BRAF pathway mutation, A431, NCI-N87, H460 had mutation in EGFR, HER2, KRAS-PIK3CA pathways respectively.

We next tested several other inhibitors of RAS-BRAF-MEK pathway in A375 (BRAF mutant) and several cancer cells without BRAF mutation. For most drugs, the IC50s were not small except for the MEK inhibitor trametinib (the IC50 was 5 nM in A375 cells) (Table 3). Further studies were on-going to test the activity of trametinib on the resistant cells A375/RF21.

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**Fig 1. Growth inhibitory effect of vemurafenib in A375 and A375RF21 cells**

**Fig 2. Growth inhibitory effect of carvedilol in A375 and A375RF21 cells**

**Fig 3. Effect of carvedilol and vemurafenib combination treatment in A375RF21**

**Fig 4. Effect of carvedilol and vemurafenib combination treatment in A375**
Table 1. IC50 of vemurafenib, carvedilol, and their combination. Growth inhibitory effect of vemurafenib, carvedilol, and combined treatment on A375 and A375RF21 (Measurements in uM).

<table>
<thead>
<tr>
<th>IC50 (µM)</th>
<th>Vemurafenib (Vem)</th>
<th>Carvedilol (Car)</th>
<th>Vem + Car</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>A375</td>
<td>0.49</td>
<td>&gt;10</td>
<td>TBD</td>
<td>TBD</td>
</tr>
<tr>
<td>A375RF21</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>0.4</td>
<td>25</td>
</tr>
</tbody>
</table>

Table 2. IC50 of vemurafenib in non-melanoma cancer cell lines without BRAF mutation (Measurements in uM).

<table>
<thead>
<tr>
<th>Vemurafenib</th>
</tr>
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<tbody>
<tr>
<td>A431</td>
</tr>
<tr>
<td>NCI-N87</td>
</tr>
<tr>
<td>H460</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>IC50 (µM)</th>
<th>Trametinib (MEK inhibitor)</th>
<th>Sunitinib (multi-targeted receptor tyrosine kinase inhibitor)</th>
<th>Sorafenib (BRAF and CRAF inhibitor)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A375</td>
<td>0.005</td>
<td>1.83</td>
<td>&gt;10</td>
</tr>
<tr>
<td>A431</td>
<td>2.4</td>
<td>3.5</td>
<td>&gt;10</td>
</tr>
<tr>
<td>NCI-N87</td>
<td>n/a</td>
<td>n/a</td>
<td>&gt;10</td>
</tr>
<tr>
<td>H460</td>
<td>n/a</td>
<td>n/a</td>
<td>11.33</td>
</tr>
</tbody>
</table>

Conclusions: Our results confirmed that the A375RF21 cell line was resistant to vemurafenib (BRAF inhibitor), consistent with literature report (Wang, 2013). Although carvedilol does not have a potent inhibitory effect on both A375 and A375RF21 cells, it strongly sensitized the BRAF inhibitor resistant melanoma cells to the BRAF inhibitor vemurafenib. Although additional work is needed to determine the reproducibility of these results, the current data indicated that carvedilol may serve as an alternative approach to overcoming of BRAF inhibitor resistance of the melanomas. Furthermore, the effect of other inhibitor of RAS-BRAF-MEK pathway such as trametinib, the MEK inhibitor, may provide additional methods for melanoma treatment.

References:


Aya Fukuma Ozaki, Pharmacy, Graduation Year: 2017, Advisor’s Name: Cynthia Jackevicius, Advisor’s College: Pharmacy, Source of Funding: Western University Summer Student Grant, Cross-Cultural Comparison of Pharmacy Students’ Attitudes, Knowledge, Practice, and Barriers Regarding Evidence-Based Medicine. Author List: Aya F. Ozaki, BScPhm, PharmD Candidate1, Cynthia A. Jackevicius, BScPhm, PharmD, MSc, BCPS-AQ Cardiology, FCSHP, FAHA, FCCP1,2,3,4,5; (1) Western University of Health Sciences, Pomona, CA; (2) VA Greater Los Angeles Healthcare System, Los Angeles, CA; (3) Institute for Clinical Evaluative Sciences; (4) University Health Network; (5) University of Toronto, Toronto, Canada.

**Purpose:** Evidence-based medicine (EBM) is beginning to be adopted into pharmacy education. EBM instruction has had varying penetration in different regions of the world, and the impact of cultural influences on students’ EBM attitudes and knowledge is poorly understood. Therefore, we explored this among pharmacy students in the United States (US) and Japan. **Study Methodology:** A cross-sectional study was conducted using a self-administered survey. Senior students in one pharmacy school in the US and one pharmacy school in Japan were invited to participate in a 33-question survey. Yes/no responses or 4-point Likert scales were used. Categorical data were compared between groups using Chi-square and continuous data were compared using t-tests. **Results:** The survey was completed by 562 students (US: 194/267 (73%); Japan: 368/685 (54%)). Students showed positive attitudes and had general knowledge of EBM in both groups. Most questions about their future use of EBM showed no statistical difference between groups. However, US students had higher usage, self-evaluation of their skills, and frequency of access to EBM resources (p<0.05). Percentages of students that indicated at least some understanding of common EBM terms were: 99%/35% for “number needed to treat”, 99%/58% for “confidence interval”, 99%/71% for “publication bias”, 97%/83% for meta-analysis, and 94%/93% for “odds ratio”, in the US/Japan, respectively. The most common barriers for US students were lack of time (85%), statistical knowledge (64%), and critical appraisal skills (53%), and for Japanese students were lack of training (92%), clinical knowledge (88%), and opportunity (88%). There were 13 barriers identified by >50% of Japanese students, while only 3 barriers identified by >50% of US students. **Conclusions:** EBM was welcomed by students in both countries. However, training and access barriers may have resulted in lower self-evaluation of EBM skills and usage in Japanese students. Future analysis will compare correlations in EBM barriers and practice between countries.

**Fig 1. Proportion of Students Reporting Barriers to EBM Use**
Fig 2. Knowledge of EBM terminology.

Figure 2A.

Students in the US

<table>
<thead>
<tr>
<th>Term</th>
<th>Can explain to others</th>
<th>Know somewhat</th>
<th>Want to know</th>
<th>Don't need to know</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number needed to treat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Publication bias</td>
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<tr>
<td>Confident Interval</td>
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<tr>
<td>Meta-analysis</td>
<td></td>
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<tr>
<td>Odds Ratio</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Fixed event rate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 2B.

Students in Japan

<table>
<thead>
<tr>
<th>Term</th>
<th>Can explain to others</th>
<th>Know somewhat</th>
<th>Want to know</th>
<th>Don't need to know</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number needed to treat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Publication bias</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Confident Interval</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Meta-analysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Odds Ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fixed event rate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Recently, The United States Department of Agriculture reported approximately $1 billion is lost annually due to lameness related injuries in horses. Clinicians rely on measurements of the defect in relation to the surrounding anatomical structures during diagnosis and treatment of musculoskeletal injuries of the distal limb. Therefore, it is critical to have a comprehensive understanding of the normal size of structures of the limb. The aim of this study was to determine morphometric values of soft tissue structures, i.e. superficial and deep digital flexor tendons (SDFT, DDFT) suspensory ligament (SL) and check ligament (CL), and innervation and osseous structures of the distal forelimb, in horses exhibiting no forelimb injuries or lameness.

The distal forelimbs of 16 healthy horses (22 +/- 8.9 years old) were dissected and the width/diameter and length of the SDFT, DDFT, SL, CL, metacarpal bones, 1st phalanx were measured; branching pattern of the palmar nerves were also examined. Data were analyzed and the mean and standard deviation were calculated for each parameter. Horses were euthanized for reasons other than musculoskeletal injuries. This study was approved by IACUC.

Clinicians commonly use diagnostic nerve blocks and morphometric evaluation of the tendons/ligaments to diagnose the distal limb injuries and select treatment options. The results of this study provide valuable data and aids clinicians in better comprehending the dimensional relationship between osseous landmarks and innervation patterns. An understanding of the quantitative anatomy of the equine distal limb will allow implementation of proper diagnosis and treatment of equine musculoskeletal injuries.

**Figures:**

<table>
<thead>
<tr>
<th>Structure</th>
<th>Length (mean ± SD)</th>
<th>Width (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDFT</td>
<td>32.267 ± 1.905</td>
<td>1.393 ± 0.288</td>
</tr>
<tr>
<td>DDFT</td>
<td>31.576 ± 3.149</td>
<td>1.896 ± 0.211</td>
</tr>
<tr>
<td>CL</td>
<td>9.798 ± 1.592</td>
<td>1.931 ± 0.223</td>
</tr>
<tr>
<td>SL</td>
<td>14.590 ± 1.492</td>
<td>3.093 ± 0.461</td>
</tr>
</tbody>
</table>

Table 1: Table of the lengths and of the tendons in the distal limb of the quarter horse. (h1 = 17) Rounded to the nearest hundredth for the value and the SD.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Length (cm)</th>
<th>Width (cm)</th>
<th>Diameter (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metacarpal III</td>
<td>25.511 ± 0.800</td>
<td>3.693 ± 0.287</td>
<td>11.556 ± 0.606</td>
</tr>
<tr>
<td>Metacarpal II</td>
<td>18.260 ± 0.878</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Metacarpal IV</td>
<td>18.159 ± 0.991</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phalanx I</td>
<td>9.161 ± 0.455</td>
<td>3.825 ± 0.192</td>
<td>11.422 ± 0.570</td>
</tr>
</tbody>
</table>

Table 2: Table of the lengths of the bones in the distal limb of the quarter horse. (h1 = 32) Rounded to the nearest hundredth for the value and the SD.

<table>
<thead>
<tr>
<th>Dorsal Branching of Palmar Nerve</th>
<th>Palmar Nerve</th>
<th>Structure</th>
<th>Length (cm)</th>
<th>Width (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dorsal Branch of Palmar Nerve Metacarpal 3 Lateral</td>
<td>Metacarpal 3 Lateral</td>
<td>3.604 ± 1.675</td>
<td>Distance to the Ramus Communicans on Metacarpal 3 Lateral</td>
<td></td>
</tr>
<tr>
<td>Dorsal Branch of Palmar Nerve Metacarpal 4 Lateral</td>
<td>Metacarpal 4 Medial</td>
<td>3.515 ± 1.890</td>
<td>Distance to the Ramus Communicans on Metacarpal 4 Medial</td>
<td></td>
</tr>
<tr>
<td>Dorsal Branch of Palmar Nerve Metacarpal 3 Medial</td>
<td>Metacarpal 3 Lateral</td>
<td>3.021 ± 1.828</td>
<td>Ramus communicans Lateral Metacarpal 4</td>
<td></td>
</tr>
<tr>
<td>Dorsal Branch of Palmar Nerve Metacarpal 2 Medial</td>
<td>Metacarpal 2 Lateral</td>
<td>3.849 ± 2.686</td>
<td>Ramus Communicans Medial Metacarpal 2</td>
<td></td>
</tr>
<tr>
<td>Length Between Dorsal Branch of Palmar Nerve and Ramus Communicans Lateral</td>
<td>-</td>
<td>7.626 ± 2.681</td>
<td>Ramus Communicans Length</td>
<td></td>
</tr>
<tr>
<td>Length Between Dorsal Branch of Palmar Nerve and Ramus Communicans Medial</td>
<td>-</td>
<td>12.992 ± 2.198</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Table 3 and 4: Mean values ± SD for the palmar nerve and its branching patterns in relation to osseous structures.
Equine Sidewinder Syndrome (ESS) presents as a specific neurologic gait that affects hind limb ambulation in horses. Despite being reported by many field veterinarians, this syndrome is unknown to some equine practitioners and has not yet been discussed in the literature. As a result, the presentation and characterization of ESS is not well known and left to the interpretation of individual equine practitioners. The purpose of this study is to develop a working definition of Equine Sidewinder Syndrome through the electronic collection and retrospective analysis of twenty-eight cases provided by U.S. national and international equine practitioners. Pertinent medical records, photographs, videos, diagnostic images, imaging reports, and necropsy reports were reviewed for signalment, history, diagnostics performed, treatments administered, and diagnosis. Horses ranged from seven to 30 years of age, with 15 of 28 horses being over 20 years of age. All horses portrayed a unique pattern of movement in the hind end characterized by lateral propulsive walking with or without propulsive circling, hypotonia in the limb toward the direction of listing and hypertonia in the opposite limb. 16 of 28 cases identified skeletal abnormalities in the lumbosacral spine or pelvis as potential etiologies for ESS. Evidence suggests that improvement to full recovery of neurologic deficits is possible. Therefore, cases of less severe presentation should be afforded a minimum of stall rest as they may recover with or without therapy. In addition, therapy is highly warranted in more severe cases.

Toxoplasma gondii Antibody Seroprevalence in Veterinary Students at Western University of Health Sciences

Millions of people in the United States are infected with the protozoan parasite, Toxoplasma gondii. Toxoplasmosis is a zoonotic disease spread by felines shedding the parasite oocyst. The prevalence of Toxoplasma gondii infections varies greatly geographically. This study aims to quantify the exposure among veterinary students at Western University of Health Sciences. Veterinarians are regularly in contact with felines, the definitive host of the parasite, creating an increased opportunity for exposure. In order to reduce the infection rate among the veterinary community, we must first understand the rate of exposure within the population. An enzyme linked immunoassay testing for the Toxoplasma gondii antibody was performed. The result demonstrated a low seroprevalence of the antibody in the population tested. The low seroprevalence observed could be due to factors such age, education level, and geographical places of residence throughout a lifetime. **Purpose of the Research or Description of the Problem:** Investigation into Toxoplasma gondii exposure; a “Neglected Parasitic Infection” listed by the Center for Disease Control and Prevention. This study quantifies the exposure to Toxoplasma gondii in a population of veterinary students at Western University of Health Sciences. This study will allow for the future analysis of risk factors and development of preventative measures. **Study Methodology:** Enzyme Linked Immunoassay. **Results:** Low Toxoplasma gondii antibody seroprevalence observed.

![Fig 1](image-url)
Conclusions: The low seroprevalence observed could be due to the age of the subjects. Risk for exposure to Toxoplasma gondii increases with age (Berger 2009). Veterinary students at the beginning of their career and still have decades of exposure opportunities ahead of them.

Education level is another factor lowering the likelihood of exposure. There is greater risk for exposure among those with lower education (Varella 2003). Veterinary medical students have a higher than average amount of secondary education.

Lastly, geographically western cities have been shown to have a lower seropositivity than other regions of the country (Jones 2001). Although not all the students in the College of Veterinary Medicine at Western University of Health Sciences are from the Southern California region, all the students have been exposed to animals from this region during their education.

References:
Ling, V.J. et. al. (2011) Toxoplasma gondii Seropositivity and Suicide rates in Women. The Journal of Nervous and Mental Diseases, 199(7).
Schluter, D. et. al. (2014) Animals are the key to human toxoplasmosis. International Journal of Medical Microbiology, 304 (917-929).
Background: The California Sea Lion (*Zalophus californianus*) is subjected to variable temperatures due to their dual aquatic and terrestrial lifestyle. Thermoregulatory mechanisms are crucial in maintaining homeostasis in the pregnant and reproductively active female California Sea Lion (Sentiel, 1995; Colgrove, 2009). This study is undertaken to explore the histomorphology of the ovarian plexus and ovarian bursa in the California Sea Lion. Understanding the anatomical relationship within these plexuses could expose a potential mechanism of thermoregulation and hormonal exchange in the genital tract of the female sea lion. A preliminary study described a venous plexus surrounding the ovary and ovarian arteries, which we will investigate, along with the reproductive tract as a whole, to test its contribution to thermoregulation and hormonal exchange in female pinniped reproduction.

Study Methodology: Five reproductive tracts from adult female sea lions (*Zalophus californianus*) were obtained from the Marine Mammal Care Center in San Pedro, California and placed in 10% neutral buffered formalin (NBF) for fixation. Two adult uteri were dehydrated, embedded in paraffin wax, and sectioned at 5 micrometers thickness. Sectioning included samples from the ovary, ovarian plexus, and ovarian bursal wall. These sections were mounted on glass slides. Tissues were stained with hematoxylin and eosin, trichrome, elastic, and toluidine blue stains (Bancroft 2008). Under light microscopy, the ovarian plexus vessels were photographed and measured arteries and veins for lumen diameter, wall thickness, and distance from neighboring vessels within the plexus.

Results: Preliminary results showed three possible vessel morphologies that could allow thermoregulation and/or hormonal exchange. First, there were various instances where an apposing vein wall (to a nearby artery) was thinner than the opposite side of the same vein wall. Arteries were found like this but the correlation graph did not prove to be significant due to incidence of sectioning. Further statistics comparing the proportion of distal wall to proximal wall need to be made for better results. Secondly, large number of venules and arterioles could be seen surrounding one artery (Fig.1) and this may facilitate hormonal exchange mentioned in Krzymowski (2012) article. Many of the venules collapsed because of post mortem. If the venules are dilated with cooler blood than the artery, it could be enough of a temperature gradient to bring down the temperature of blood within the artery. Lastly, engorged veins were observed throughout the ovarian plexus and within the ovarian bursal wall. If these vessels carry cooled blood and they surround the artery in this fashion, then temperature exchange would seem inevitable due to the thin walls of the veins and the amount of blood surrounding one artery.

Fig. 1. Elastic stain showing internal and external elastic lamina of medium size muscular artery surrounded by large number of arterioles, venules and capillaries, which may play a role in thermoregulation. Line scale=200 µm.

Fig 2. Notice the dilated large vein with two different wall thicknesses with the side facing the artery much thinner. 10x magnification
Conclusions: In conclusion, the morphologies observed were thought to be unique and conducive to heat and hormonal exchange. To confirm this hypothesis more work needs to be done. Three adult females still remain to be sectioned and processed which is something planned for the nearby future. Two samples were taken for TEM and will be measured and photographed. Lastly, a latex injected specimen needs to be dissected, photographed, and a schematic to be drawn of all blood vessels in the region.

References:

(Gorby) Divvyjot Singh, Student's College: College of Pharmacy, Graduation Year: 2018, Advisor's Name: Dr. Nazarian, Advisor's College: College of Pharmacy, Source of Funding: WesternU Pharmaceutical Sciences, Pain Evoked Neuronal Activation In the Amygdala and the Bed Nucleus of Striata Terminalis of Formalin Treated Rats, Author List: (Gorby) Divvyjot Singh BS, Arbi Nazarian PhD, Alex Armendariz BS

The Purpose of this study was a follow up to the established foundation of morphine’s effect on attenuating pain response dealing with the affective (emotional) component formalin evoked pain in male and female rats. As shown previously, the affective component in brain signaling is not very well understood; hence a better understanding can provide better validity to analgesic medications and clinical therapy (Harton, et al). The aim of the project was to examine possible changes in neuronal activation in brain regions known to be involved with the affective component of pain. To that end, rats were pretreated with varying doses of morphine and then administered diluted formalin into the hindpaw to induce pain. Ninety minutes after formalin administration, rats were fixed and the brain and spinal cords were harvested. Brains were sectioned using a sliding microtome (50um in thickness). Immunohistochemistry was performed on the brain sections to identify the immediate early gene c-fos in the regions of interest (Amygdala and the Bed Nucleus of the Striata Terminalis, BNST). The study is currently ongoing, although, significant amount of troubleshooting has taken place thus far to optimize the histological assay and related steps. Cell bodies of the immunofluorescent c-fos gene in the Amygdala and BNST region will be quantified and reported as results. Purpose of the Research or Description of the Problem: Determining pain signaling, induced by formalin injections, by staining for c-fos gene in the Amygdala and the Bed Nucleus Striata Terminalis (BNST) and a correlation to affective component of rat brain. Study Methodology: Microtome Sectioning: Microtome was kept
cold using dry ice (-20°C). Sections of the brain were initially stored in Paraformaldehyde (PAR) and then transferred 4% Paraformaldehyde in sucrose solution. A sectioning stage was built using the 4% PAR in sucrose. Rat brain was first divided into areas of interest and then sliced at 50 microns in thickness. Each area of interest sections were placed in anti-freeze wells and stored in the -20°C freezer. C-Fos Staining (Immunohistochemistry): Brain sections were removed from the freezer (in anti-freeze solution), and placed in rinsing wells. The sections were rinsed with 0.01 PBS (phosphate buffer saline) four times, ten minutes each (4 X 10 min). Next, Primary Antibody (Ab) was prepared using a vehicle, of 80% PBS (0.01M), 10% Normal Goat Serum (NSG), 10% of Triton X-100 (3%). The Ab (c-fos; Santa Cruz) was diluted 1:20,000. These sections were incubated in the Ab overnight at 4°C in fridge on a shaker. The next day, the sections were again rinsed with 0.01 PBS (4X10min). A secondary Ab was prepared with similar vehicle but the concentration of dilution was 1:1000. The sections were incubated with the secondary Ab for 90 minutes at room temperature. The sections were then rinsed with 0.01 PBS (4X10min) and then mounted on slides. *The protocol is specific to Dr. Nazarian’s Lab.

Mounting of sections on slides: Stained sections were stored anti-freeze. The sections were transferred to PBS solution and were next mounted on slides (25 X 75 X 1.0mm; Fisher brand). The slides were allowed to dry overnight and were cover slipped. The slides were analyzed using Fluorescent microscope. Fig 1 Currently, being processed. Will vary to the former study. Image below is only a representation of the regions (Amygdala, and BNST), and not the representation of c-fos staining for this project.

References:
Harton, et al. Morphine on Formalin-Induced Somatic and Affective Components of Nociception in Male and Female Rat. Department of Pharmaceutical Sciences, Western University of Health Sciences.

Acknowledgements: I am grateful for all the support Dr. Nazarian and Alex have contributed in my growth in their research lab. Special thank you to Western University for giving me the opportunity to participate in this research.
Charcot neuroarthropathy begins in an acute phase in which the affected limb is severely inflamed. This is exacerbated by patients with peripheral neuropathy who continue to ambulate the limb which then causes cascade of bone degradative processes occur leading to bone fractures which can in turn stimulate further inflammation and the whole process can repeat in a cyclical fashion. The inflammation eventually subsides and osseous structures affected re-ossify malpositioned leading to dysfunctional deformities such as rocker bottom foot which often require reconstructive surgery or amputation below the knee.

Current treatment for the acute phase of charcot neuroarthropathy involves immediate off-loading with a total contact cast. After the acute phase concludes, the patient is transitioned to a weight-bearing Charcot restraint orthotic walker.

Denosumab (Prolia) is a potent RANKL inhibitor which reduces the rate of bone turnover and potentially reduce degradative osseous effects of acute Charcot neuroarthropathy. It has been shown to reduce fracture risk in patients with osteoporosis (3-6). Because of the ability of denosumab to inhibit a key cytokine in the inflammatory processes, it may have potential beneficial uses in the treatment of acute Charcot neuroarthropathy.

**Study Methodology:** This will be an open-label phase 1 trial with a duration of 1 year. A total 6 subjects will receive treatment of Prolia (60mg injection) along with the standard offloading with a total contact cast. Patients will follow up weekly for 2 weeks, biweekly up until 6 months, and then the final two visits will be at 9th and 12th month. At each visit, foot skin temperature readings will be recorded using an infrared thermometer. The primary endpoint of this study the reduction of skin temperature of the treatment limb compared to a baseline established upon the initial visit. Additionally, a 100-point Visual Analog Scale measure of pain will be given at each visit to determine improvement of symptoms associated with Acute Charcot Neuroarthropathy. Radiographic imaging and basic laboratory tests including markers for bone turnover (calcium and bone specific alkaline phosphatase) will be obtained at each visit and assessed for any changes throughout the duration of the study.

**Results:** The project is in the process of IRB approval and funding. There are no results to report at this time. We have been working closely with the IRB and funding sources this summer to get approval. Multiple IRB revisions and company requested revisions to study protocol and informed consent have been made to try to get this study off the ground. In addition we have also begun to draft a budget, accounting for all the medications, equipment, manpower and other costs necessary to complete this phase 1 trial.

**References:**
Dana Lin, College of Podiatric Medicine, Graduation Year: DPM 2018, Advisor’s Name: David Shofler, DPM, Advisor’s College: College of Podiatric Medicine, Source of Funding: Western University of Health Sciences Summer Research Fellowship, The combine effects of social history, culture, and social support in diabetic foot care management among Latinos, Author List: Dana Lin, BS, David Shofler, DPM

**Background:** The Latino population is one of the fastest growing minorities within the United States. However, there has not been an overall improvement in health outcomes of the Latino community. Poorer health outcomes as well as higher morbidity rates of chronic diseases among Latinos such as diabetes, cardiovascular disease, and obesity can have implications within the lower extremity. For example, foot complications can arise consisting of lack of nerve sensation from diabetes, poor circulation to the feet from peripheral arterial disease, and foot pain from obesity. These various foot problems can greatly affect one’s quality of life in terms of not being able to walk comfortably which also reduces the ability to do physical activity. Furthermore, many common life-threatening illnesses, from cardiovascular diseases to increase risk of some cancers, are associated with a sedentary lifestyle. Because of this, there is a need to closely examine the health disparities among Latinos that have resulted in poorer health outcomes and its impact on foot-related conditions. Examining the combined effect of social and cultural factors is significant in tailoring an effective foot care treatment and management plan for Latino patients and thus can reduce the risk of amputations. In addition, feet has such a strong relationship with diabetes that it is essential that a podiatrist be involved as part of the diabetes care and management team. Evaluating the health disparities of Latinos requires the consideration of social history in combination of the cultural aspect that play a role in their reported foot health complications; all of which may further the cultural competence and betterment of self-care and foot care management.

The purpose of this study is to investigate further into health disparities among Latinos, and the impact of whether their social history and culture correlates with their foot complications especially in diabetic patients. Cultural factors may play a role in this together with social history in that Hispanic culture traditional remedies may be preferred as well as the strong cultural family aspect in which medical treatments may be delayed due to putting the family needs before medial needs. Much of the existing studies focus predominately on specific diseases that Latinos have shown in proportionate numbers such as diabetes, heart disease, and obesity. In regards to health disparities, previous lower extremity studies have also hinted at similarities in terms of social history and foot health correlation, but none have taken account Latinos’ cultural factors in regards to all the culturally related remedies and strong family aspect that are everyday variables in their lives. Evaluating the health disparities of Latinos requires the consideration of their culture and social histories such as socioeconomic and health issues (lack of affordable medical care, obesity, chronic disease) that play a role in one’s reported foot health complications and health status.

Past lower extremity published research have also shown that Hispanics are disproportionately affected in having higher incidence rates of lower limb amputation related to diabetes. In this study, the hypothesis was that the higher incidence rate could be due to the higher prevalence of vascular disease, neuropathy, and history of lower-extremity complications; however, this hypothesis was not supported by the data and suggest that other variables are involved which requires further investigation. These variables attributed to the higher amputation risk could be due to the differences in access to care, patient education, patient compliance, self-care management, and cultural issues. One of the hypotheses in this study is that social history in the life of Latinos as well as cultural factors plays a greater role in their reported foot care and diabetes management. The need to explore cultural as well as social history risk factors in health among Latinos is imperative and may provide some potentially effective management strategies to improve not only foot health but also overall health outcomes for the Latino community. For instance, since one’s social history is implicated in one’s own health, the care of chronic illnesses that greatly affect the foot such as those of diabetes, obesity, and heart disease could focus more equally on managing stress, coping with depression, and getting family social support along with managing of glucose levels, following a low-fat diet, and maintaining healthy weight through physical activity, all of which can be even more of an effective form of chronic illness and foot self-care management. Furthermore, allowing evident disparities in education and other socioeconomic factors to continue among the Latinos will only persist and worsen the health disparities that greatly impact many Latinos’ health outcomes today. This research will contribute to a better understanding in the features of what needs to be incorporated into one’s self-care and foot care management in order to improve the health disparities and in the social determinants of foot health conditions within the Latino population.

**Study Methodology:** A systematic literature review was performed to identify previous research topics that have been conducted to use as a systematic review to develop the survey instrument. Current preparations are being made for IRB submission so that the next phase will be the recruitment of participants to be surveyed, obtain informed consent
forms, collect data, and begin data analysis. The survey participants will be adult diabetics who identify as having a Hispanic/Latino background. The sample size of about eighty or more Latino participants will be used to gather data from them. This research study will implement surveys to measure the effect of social history and culture on foot health outcomes among the diabetic Latino community. The survey guide will include questions of social history, diabetic health assessment questions, familial social support, and general demographic questions. The surveys will also be translated into Spanish for non-English speaking respondents. Surveys will consist of a majority of close-ended questions.

The screening process involved in patient recruitment will be to review the patient medical history/ laboratory data such as the last glucose and HbA1C results. The majority of potential participants will have had lower extremity physical examination as well as communication with the primary attending podiatrist to confirm any foot ailment diagnosis. The staff podiatrist will examine each potential participant to identify any lower limb complications and risk factors related to diabetes such as past history of lower extremity pathology in ulcers, neuropathy, peripheral vascular disease, and foot pathologies/deformity. Neuropathy will most likely be examined with a Semmes-Weinstein monofilament and vascular disease will be evaluated with a non-palpable or low foot pulse (from the dorsalis pedis and posterior tibial arterial pulses).

**Results:** The current status of this research consists of the near completion in the development of the survey questionnaire instrument and the preparation of the IRB application. The last phase of the research has yet to be concluded which will be to gather the data and complete data analysis.

**Conclusions:** The last phase of the research has yet to be completed in the data collection and analysis. The results will be from this data analysis to which the findings and importance in the findings can then be discussed.

**References:**

Michael Czulinski, College of Osteopathic Medicine, Graduation Year: 2018, Advisor’s Name: Dr. Glen Kisby, Advisor’s College: College of Osteopathic Medicine, Source of Funding: COMP Start-up Funds, Effect of environmental chemicals on tau and synuclein expression in human neural stem cells, Author List: Michael E. Czulinski, Morgan Florek

Environmental chemicals may perturb neurodevelopment by inducing epigenetic changes during key periods of early brain development. By treating human induced pluripotent stem cell (iPSC)-derived neuroprogenitors/neural stem cells with chemicals that both perturb epigenetic mechanisms and stably affect brain development it is possible to characterize the effects of 5-deoxyazacytidine, trichostatin A, valproic acid and Bisphenol A, methylazoxymethanol (MAM), in hNPCs. Initial experiments developed a protocol for seeding a 96-well plate in addition to optimizing the cell density for later toxin characterization. Optimal cell density was determined on both Sure-Bond® and Sure-Bond Plus® coated wells using light microscopy and immunohistochemistry. Current analyses are focused on markers of neuronal and glial cell differentiation to identify epigenetic changes to tau and synuclein expression following chemical exposures.

Human fetal brain development can generally be described as having three phases of development: early, middle and late gestational periods. The early phase of neural development is characterized by a highly regulated proliferative phase. The subsequent middle and late phases are periods of neural cell differentiation. In the latter stages, neural cells form complex neural circuits containing specialized differentiated cells (e.g., neurons, astrocytes, oligodendroglia). Neural proliferation and differentiation are two highly regulated time-dependent phases of development which are tightly regulated by the epigenome. These phases are particularly vulnerable to epigenetic changes, which are believed to be the underlying cause of several neurodevelopmental (e.g., schizophrenia, autism) and neurodegenerative disorders (e.g., dementia progressive supranuclear palsy). Early life exposure to environmental chemicals is considered an important trigger of these diseases, but the mechanism is poorly understood. Thus, developmental abnormalities may result from the action of environmental chemicals on epigenetic processes that regulate normal neurodevelopment. By treating human neural stem cells (i.e. induced pluripotent stem cell (iPSC)-derived neuroprogenitors, hNPCs) with chemical agents that are known to interfere with epigenetic mechanisms, their effects on the development of hNPCs at key stages of neuronal differentiation can be described. Our first goal is to establish a protocol to optimize the seeding of hNPCs on a 96-well plate on both Sure-Bond® and Sure-Bond Plus® substrates and the characterization of the hNPCs. The next set of studies will establish the time-points that recapitulate normal human brain development as hNPCs.
differentiate into various cell subtypes. This work will be performed with iPS-derived hNPCs undergoing proliferation and differentiation in 96-well plates. Fluorescent-labeled antibodies will be used to distinguish the different cell subtypes that arise at each time-point tested and their relative proportions among the total cell population. Once the differentiation model is established, we will test the effects of five chemicals with known and/or suspected ability to perturb normal differentiation by an epigenetic mechanism. The chemicals tested will include two well-characterized epigenetic inhibitors, 5-deoxyazacytidine and trichostatin A, which inhibit DNA methylation and histone deacetylation, respectively. The other chemicals of interest include Bisphenol A, methylazoxymethanol (MAM) and valproic acid. MAM is an established neurodevelopmental toxin. Utilizing human neuroprogenitor cells, an in vitro “brain in a dish” model of neurodevelopment, will provide a better understanding of the role of environmental chemicals in neurodevelopmental disorders. More specifically, our studies will attempt to identify discrete time points and cell-specific markers for aberrant changes in neuronal differentiation following exposure to environmental chemicals with epigenetic activities.

**Methods:** A standardized optimal protocol for seeding a 96-well plate was established to consistently assess the effects of the chemicals of interest on hNPCs. Preliminary cell density studies included comparing the adherent substrates Sure-Bond® and Sure-Bond Plus® for the optimal seeding of hNPCs on a 96-well plate. The seeding density on Sure-Bond® ranged from 20,000-70,000 cells/cm² in increments of 10,000 cells/cm². The seeding density on Sure-Bond Plus® ranged from 5,000-30,000 cells/cm² in increments of 5,000 cells/cm². After plating, the hNPCs were monitored via light microscopy over a 21-day differentiation period. The cells were fixed with 4% buffered paraformaldehyde on day 21 for immunoprobing and staining. DAPI is a blue stain used to identify all cell nuclei. An antibody to Sox II (nuclear protein) and Nestin (cytoskeletal protein) was used to probe specifically for undifferentiated hNPCs.

**Results:** Our initial studies developed a standard protocol for hNPCs in a 96-well plate that we believe is optimal for future dose-response studies with the previously identified toxins. Optimal cell density is defined as being the lowest number of hNPCs that we could apply to each well of a 96-well plate to maintain cell viability and morphometric analysis of the growing cells (e.g., axon elongation, neurite number, branching, ...etc). The cell substrates Sure-Bond® and Sure-Bond Plus® identified an ideal plating density of 25,000-30,000 cells/cm² for both (Figure 1-3). This ideal plating range was established by evaluating the cell densities by both light microscopy and immunohistochemistry for those characteristics that will be used for future quantitative studies. In addition, preliminary toxin experiments have shown some success utilizing the optimal plating density of a 96-well plate in viability assays. Data from these dose-response studies has been collected and the results are being analyzed. The first toxins we tested were MAM, BMAA, and Streptozotocin. The latter two toxins are environmental toxins that are related to MAM.

**Sure-Bond® Cell Density Experiment**

<table>
<thead>
<tr>
<th>Cells/cm²</th>
<th>Day 1</th>
<th>Day 21</th>
</tr>
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**Figure 1:** Sure-Bond® was evaluated for optimal cell density over a 21-day period. All images are taken with a 10x objective on an EVOS microscope. On day 21, the 20,000 cells/cm² group displayed minimal survival (no observable cells) while hNPCs at 30,000 cells/cm² appeared healthy, proliferative, and developed axons. Higher plating densities became sequentially more overgrown with neurites making it particularly hard to identify neuronal structures (i.e., dendrites, axons).

**Sure-Bond Plus® Cell Density Experiment**
Figure 2: Sure-Bond Plus® was evaluated for optimal cell density over a 21-day time-period. All images were taken with a 10x objective on an EVOS microscope. On day 21 day, the 25,000 cells/cm² wells displayed minimal survival (no observable cells) while hNPCs at 30,000 cells/cm² appeared healthy, proliferative, and developed axons.

**Sure-Bond Plus®**: Immunofluorescence of hNPCs at near optimal plating density

Figure 3: Images of near optimal plating densities of hNPCs determined by fluorescence microscopy. Neuronal morphology (e.g., axon elongation, neurite number, branching, … etc) was visible at both cell densities an indication that these cell densities will be useful for future toxin experiments as well as the characterization of hNPCs. DAPI is a blue stain used to identify all cell nuclei. Antibodies to Sox II (red; nuclear protein) and Nestin (green; cytoskeletal protein) were used to specifically identify undifferentiated hNPCs in our cultures.

**Conclusion:** Cell density studies utilizing both light microscopy and immunohistochemistry identified the optimal cell densities (ranging from 25,000-30,000 cells/cm²) for seeding a 96-well plate. We demonstrated that these these plating densities are optimal for both Sure-Bond® and Sure-Bond Plus® coated 96-well plates. Light microscopy specifically supports that hNPCs at minimal survival plating density of 25,000 cells/cm². Immunofluorescent probing of the 96-well plates at the optimal seeding density allowed for better visibility of neuronal morphology and thus confirmed that at the same plating densities the morphology of toxin treated hNPCs could be identified. Together, these studies are critically important for evaluating the effect of toxins on the development of hNPCs, which has been successfully shown in preliminary analysis of MAM, BMAA, and Streptozotocin treated hNPCs. In parallel studies, analytical techniques are developed (i.e., Alamar-Blue, Calcein-AM, and PI) to quantitatively assess the effect of various toxins on cell viability and for identifying the LD₅₀ for each toxin discussed.

**References:**