Kansas City Musculoskeletal Diseases Consortium
7th Annual Symposium on Musculoskeletal and Neuromuscular Diseases
UMKC – Pierson Auditorium, 5000 Holmes, Kansas City, MO
Friday, December 2, 2022
10:00 a.m. – 2:00 p.m.

10:00 am	**WELCOME** **Edward R. O'Connor,** PhD, MBA, FACHE, Executive Director, KCMD Consortium, Provost and Executive Vice President for Academic, Research and Student Affairs, Kansas City University
10:05 am	**INTRODUCTION OF KEYNOTE SPEAKER** **Richard J. Barohn,** MD, Executive Vice Chancellor for Health Affairs, University of Missouri – Columbia
10:10 am	**KEYNOTE SPEAKER** **W. David Arnold**, MD, Executive Director, NextGen Precision Health Initiative, University of Missouri – Columbia Sarcopenia has a lot of nerve! Neurobiological Mechanisms of Age-related Loss of Physical Function
11:00 am	**PREVIOUS KCMD AWARD WINNER PRESENTATIONS** Moderated by **Dr. O'Connor**
11:05 am	Erin Bumann, UMKC and Pamela Tran, KUMC, *"Genetic interaction between two ciliary paralogs regulates postnatal skeletal bone growth"*
11:20 am	Mazen Dimachkie, KUMC, *"Inclusion Body Myositis Treatment with Celution Processed Adipose Derived Regenerative Cells"*
11:35 am	Charlotte Phillips and Brittany Lafaver, MU, "*Characterization of Heart Abnormalities in Pre-translational Models of Osteogenesis Imperfecta"*
11:50 am	Hillary McGraw, UMKC, "*Foxg1a regulates cranial facial development in the zebrafish*" (Poster Presentation)
12:05 pm	**Working Lunch**
12:20 pm	Wen Liu, KUMC, "*Time trajectory of joint pain and compliance to an interval or continuous walking exercise in people with Knee Osteoarthritis*" (Virtual)

RRNMF Neuromuscular Journal 2023;4(1):42-73

12:35 pm Heather Wilkins, KUMC, "*Functional Biomarkers for ALS*"

12:50 pm Sarah Dallas, UMKC, "*Live Cell and Intravital Imaging of Bone Resorption Dynamics and Osteocyte Fate During Osteoclastic Bone Resorption*"

1:05 pm **Group Q&A –** Dr. O'Connor, Moderator

1:20 pm **POSTER PRESENTATIONS**

Ryan Anderson, UMKC, "*Maternal dietary vitamin A levels as a determinant of penetrance and severity of cleft lip/palate in a Wnt9b model*"

Kennedy Davis, KCU and Lisa Stehno-Bittel, KUMC, "*Joint Lubricants as Delivery Methods for Multipotent Stromal Cells*"

Andrew Heim, KUMC, "*Trial of Oxaloacetate in ALS (TOALS)*"

Claire Houchen, UMKC, *"A Novel PCR-based Strategy for Investigating Sexual Dimorphism in Avian Embryos: An Example in Bone"*

Stefan Lohfeld and Roland Klar, UMKC, "*3D printed multi-gradient microsphere scaffolds for guided osteochondral tissue engineering*"

Nuria Lara, UMKC, "*Role of Estrogen Receptor α in Bone-Muscle Crosstalk*"

Loretta Laughrey, UMKC, "*Multiscale, 3D finite element analysis using Micro-CT and confocal multiplexed images for correlation of osteocyte B-catenin signal pathway activation with predicted lacunar wall strain*"

Soumya Rao, UMKC, "*New insights into the genetic basis of the Oculo-Auriculo-Vertebral Spectrum (OAVS)"*

Maria Spletter, UMKC, "*Bruno1 Is Required Throughout Drosophila Indirect Flight Muscle Development to Regulate Dynamics Of Sarcomere Assembly And Growth*"

Jacob Thomas, MU, "*Preliminary Results from a Novel Movement-Based Concussion Screening Too*l"

Julian Vallejo, UMKC, "*Tibia Mechanical Loading Acutely Decreases Resting Heart Rate in Mice Likely via the Sympathetic Autonomic Nervous System*"

Sam Weiss, MU, "*Serial Subtraction Dual-Task Alters Lateral Step-Down Tibiofemoral Kinematics in Healthy Adults*"

Yixia Xie, UMKC, "*Confocal and Tissue Clearing/3D Imaging in Transgenic Mice Expressing a Membrane-GFP targeted to Mineralizing Cell Types*"

Padmini Giri, KUMC, "*An Interesting Case of Anti-Mi-2-Antibody Associated with Paraneoplastic Dermatomyositis in a Patient with Cervical Cancer*" (Virtual)

Richard Sherwood, MU, "*Maturation-based Prediction of Craniofacial Growth*" (Virtual)

Kun Wang, UMKC, "*Extracellular Vesicle-Mediated Communication Between Cells in Bone*"

2:00 pm **Closing Remarks: Dr. O'Connor**

The Executive Committee of the Kansas City Musculoskeletal Diseases Consortium:

- Edward R. O'Connor, PhD, MBA, FACHE, Provost and Executive Vice President for Academic, Research, and Student Affairs, Kansas City University, and KCMD Executive Director
- Richard J. Barohn, MD, Executive Vice Chancellor for Health Affairs, University of Missouri – Columbia
- Mazen M. Dimachkie, MD, FAAN, FANA, Vice Chairman for Research Programs & Executive Vice Chairman, Department of Neurology, University of Kansas Medical Center
- Yusheng (Chris) Liu, PhD, Vice Chancellor for Research, Office of Research and Economic Development, UMKC
- Christian L. Lorson, PhD, Associate Dean for Research and Graduate Studies, University of Missouri – Columbia
- Jeffrey M. Statland, MD, Professor, Department of Neurology, University of Kansas Medical Center

Previous KCMD Award Winner Presentation Speakers:

Kansas City University (KCU)

Kennedy Davis, Research Graduate Student, KCU College of Biosciences

University of Kansas Medical Center (KUMC)

Mazen M. Dimachkie, MD, Vice Chairman for Research Programs & Executive Vice Chairman, Department of Neurology

Wen Li, PhD, Associate Professor, Director of Neuromuscular Research Laboratory, Department of Physical Therapy & Rehabilitation Science

Lisa Stehno-Bittel, BSPT, PhD, Clinical Professor, Department of Physical Therapy, Rehabilitation Science, and Athletic Training

Pamela V. Tran, PhD, Associate Professor, Anatomy and Cell Biology, Department of Cell Biology & Physiology

Heather Wilkins, PhD, Assistant Professor, Department of Neurology; Assistant Director, Biomarker Core, University of Kansas Alzheimer's Disease Center

University of Missouri – Columbia (MU)

Charlotte L. Phillips, PhD, Professor, Director of Graduate Studies, Department of Biochemistry

University of Missouri – Kansas City (UMKC)

Erin E. Bumann, DDS, PhD, MS, Assistant Professor, Department of Oral and Craniofacial Sciences

Sarah L. Dallas, PhD, UM Curator's Distinguished Professor, Lee M. and William Lefkowitz Endowed Professor, School of Dentistry, Department of Oral and Craniofacial Sciences

Poster Presenters:

Kansas City University (KCU)

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Genetic interaction between two ciliary paralogs regulates postnatal skeletal bone growth

Erin E. Bumann[1] and Pamela V. Tran[2]

[1] Department of Oral and Craniofacial Sciences, School of Dentistry, University of Missouri-Kansas City
[2] Department of Cell Biology and Physiology, Jared Grantham Kidney Institute, University of Kansas Medical Center

Primary cilia are signaling organelles that receive mechanical and chemical cues in the extracellular environment, and are potent modifiers of skeletal growth. Mutation of ciliary genes causes syndromic disorders termed ciliopathies, which can manifest osteochondrodysplasias. We have generated a new mouse model of osteochondrodysplasia, generated by combinatorial deletion of two ciliary paralogs, *Thm1* (also known as *Ttc21b*) and *Thm2* (*Ttc21a*). While *THM1* mutations have been identified in patients with the ciliary osteochondrodysplasia, Jeune Syndrome, *THM2* is less characterized. We have found that in mice, $Thm2^{-/-};Thm1^{+/-}$ triple allele deletion causes small stature and micrognathia, and disrupts chondrocyte and osteoblast differentiation. Genetic downregulation of Hedgehog (Hh) signaling exacerbates the skeletal phenotype in mice, while Hh agonist, SAG, rescues the osteoblast differentiation defect *in vitro*. To determine the etiology of the bone defects and the clinical significance, future studies will investigate the osteoblast differentiation defect and bone remodeling in mouse, and co-occurrence of *THM2* and *THM1* rare variants in children with skeletal anomalies.

Inclusion Body Myositis Treatment with Celution Processed Adipose Derived Regenerative Cells

Heim, AJ; Soder, R; Bhavsar, D; Agbas, A; Kosa, E; Ciersdorff, A; Pasnoor, M; Jawdat, O; Jabari, D; Farmakidis, C; Chandrashekhar, S; Dimachkie, MM

Abstract

Background: Inclusion Body Myositis (IBM) is a progressive, debilitating disease causing both proximal and distal muscle weakness, characteristically most prominent in the quadriceps and finger flexors. Over time it can lead to severe disability, including loss of hand function, falls due to quadriceps muscle weakness and foot drop, dysphagia, and eventually respiratory muscle weakness. The Celution System is a closed, automated system intended to digest adipose tissue in order to further extract, wash, and concentrate stromal stem cells and other associated progenitor cells intended for autologous reimplantation in a real-time bedside manner and has been tested in vascular disease and wound healing.

Objectives: The primary objective of this study is to assess the safety in IBM of an autologous graft consisting of adipose-derived regenerative cells (ADRCs) derived from the Celution. Secondary efficacy measures are included. This study is conducted under IDE 25043.

Methods: ADRCs were isolated and purified from human abdominal subcutaneous fat tissue using the Celution 800/CRS. ADRCs were processed aseptically, had a mean viability of 90% and were suspended in lactated Ringer's solution for infusion. Enrollment is staggered into 3 groups. Nine IBM subjects are randomized 2:1 in groups of 3 to late (Part 1) versus early (Part 2) ADRC autologous graft injections. Stem cell injections will occur unilaterally at 2 sites in the flexor digitorum profundus muscle and 6 sites in the quadriceps group of muscles. Subjects are injected on the side of the body that muscle strength is graded between 6 and 9 for finger flexion and knee extension using the Kandell 0-10 scale at the screening visit. A total of 30 million cells is injected into the subject between the 8 injection sites. Subjects are followed up every 3-6 months for two years after the stem cell injections for safety and efficacy measures.

Results: All 9 IBM subjects have been enrolled in the trial. In totality 3 subjects have received stem cell injections. On October 26, 2022, the third group participant received the ADRC autologous graft injections via EMG-guidance. The remaining 6 subjects will receive injections in 2023 with study completion estimated for 2025. In collaboration with Dr. Abdulbaki Agbas, we are collecting whole blood samples for assessing the profile of biomarker protein TDP-43 at various visits in Part 1 and Part 2.

Discussion: While study results are not yet available, the stem cell injections have been so far well-tolerated. The study-related adverse events have been limited to expected transient effects of the liposuction procedure. TDP-43 assessment data is being collected and will be analyzed by capillary electrophoresis Immunoassay (CEI).

Acknowledgements: This study received funding from the Kansas City Musculoskeletal Consortium and the Vice Chancellor of Research at the University of Kansas Medical Center.

Characterization of Heart Abnormalities in Pre-translational Models of Osteogenesis Imperfecta - KCMD Research Progress Update

Charlotte L. Phillips PhD[1,2], Brittany Lafaver[1]*, Departments of Biochemistry[1], and Child Health[2], University of Missouri, Columbia MO 65211 (*poster presenter)

Lixin Ma PhD[3,4], Li Lee PhD[3,4], Departments of Radiology[3], University of Missouri, Columbia MO 65211, VA-Biomolecular Imaging Center[4], Truman VA Hospital, Columbia, MO 65201

Maike Krenz MD[5,6], Departments of Medical Pharmacology and Physiology[5] University of Missouri, Dalton Cardiovascular Research Center[6], Columbia, MO 65211

Michael Wacker PhD[7], Krish Sardesai[7], Julian Vallejo MS[7], Evan Pierce[7], Department of Biomedical Sciences[7], School of Medicine, University of Missouri-Kansas City, Kansas City, MO 64108

Osteogenesis Imperfecta (OI) is a heritable connective tissue disease that affects 1:10,000 births. OI patients often present with brittle bones and scoliosis. Recent investigations have begun to elucidate the presence of inherent muscle weakness and cardiopulmonary complications. Previously cardiopulmonary complications were attributed to thoracic spinal deformities, however recent evidence demonstrates that patients with low grade or no scoliosis also exhibit cardiopulmonary manifestations. Echocardiographic clinical studies suggest that diastolic dysfunction in the OI patient population is common, often accompanied by valvular regurgitation. Of the extracellular matrix in the normal heart myocardium, approximately 85% of total myocardial collagen is type I, which is important in maintaining the structural integrity of the myocardium. Whether the presence of reduced or abnormal collagen levels alters cardiovascular health in OI patients remains to be fully elucidated, as cardiovascular complications are thought to be the second leading cause of death in OI.

The osteogenesis imperfecta murine (*oim*) mouse models severe type III human OI in its homozygous (*oim/oim*) form. In the present study 4-month-old wildtype (Wt) and *oim/oim* littermates underwent functional studies, followed by sacrifice, where the hearts were weighed and flash frozen. Analyses of the wet weights of male and female *oim/oim* and Wt hearts demonstrated increased cardiac mass in *oim/oim* compared to age and sex matched Wt hearts. Preliminary cardiac MRI analyses demonstrated that *oim/oim* hearts exhibited increased left ventricular volume (end-diastole and end-systole) and decreased ejection fraction relative to Wt hearts. Introductory echocardiography studies show that 2 out of 5 *oim/oim* males and 2 out of 5 *oim/oim* females have significantly increased peak blood flow velocities in the ascending aorta. This indicates that a subset of *oim/oim* mice developed aortic valve stenosis. Initial electrocardiography studies suggest the conduction pathway in *oim/oim* hearts is not altered. Cardiac tissue evaluated by RT-qPCR showed elevated Brain Natriuretic Peptide (BNP) expression in the female *oim/oim* heart, as well as a myosin heavy chain (MHC) isoform switch, represented by lowered α-MHC and raised β-MHC in males. Overall, these investigations suggest *oim/oim* mice demonstrate altered cardiac function as well as gene expression of BNP, indicating potential cardiac stress. Further investigations are needed to elucidate the mechanisms in the pathogenesis of the cardiac manifestations in the *oim/oim* heart and its implications to cardiovascular health in patients with OI.

Funding Sources:

- Kansas City Consortium on Musculoskeletal Diseases (Collaborative Research for neuromuscular/ Musculoskeletal Disorders)
- NIH/NIAMS – R21 (1R21AR077813-01)
- NIH T32 Training Grant Fellowship, University of Missouri- B.L. was supported by T32 GM008396

Foxg1a regulates cranial facial development in the zebrafish

Nusaybah Ibrahim, Laylah Liwaru and Hillary McGraw
University of Missouri-Kansas City, School of Science and Engineering

Foxg1 is a forkhead transcription factor with a critical role in neural development. Foxg1 regulates many cellular behaviors including proliferation, differentiation and mitochondrial function. In humans, Foxg1 mutations are linked to Foxg1 syndrome, which is defined by defects in neural development, intellectual disability, language deficits, movement disorders, disrupted circadian rhythm, and social withdrawal. Additionally, Foxg1 mutations are linked to cranial facial defects in some human patients, thought a specific role during development has not been described. Our work focuses on a zebrafish *foxg1a*^{a266} mutant line, which was generated using CRISPR-Cas9 genome editing (Thyme et al. 2019). Preliminary analysis of the *foxg1a*^{a266} mutants reveals defects in cartilage elements of the developing jaw. The mutants have abnormal joint development, narrow heads, and early lethality. Understanding these connections of cranial facial defects and the *foxg1a* mutation will help in uncovering how the mutation affects the development of other vertebrates and aspects of Foxg1 syndrome.

Time trajectory of joint pain and compliance to an interval or continuous walking exercise in people with knee osteoarthritis

Wen Liu, Ph.D. [1]
Batool Alkhamis PT, Ph.D.[1,2]
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Abstract

Introduction People with knee osteoarthritis (KOA) often complain of worsening joint pain post-exercise and fail to meet the recommended physical exercise. A past study showed that one bout of interval walking (IW) may reduce pain level while one bout of continuous walking (CW) may raise pain level in people with KOA, but it is unknown how the joint pain will respond to IW or CW in a long-term. **Purpose** In this pilot randomized clinical trial, we examined the changes of joint pain after each session of IW or CW exercise over 6 weeks and changes of compliance to the IW or CW exercise in people with KOA. **Methods** Twenty-two participants with KOA were enrolled and randomly assigned to either an IW or CW group. The intervention involved 30 minutes of walking exercise, 3 times/week over 6 weeks. The IW was a 30-minute walking in 2 bouts (15 minutes each) with 30-40 minutes of a resting interval, while the CW was a 30-minute walking in one continuous bout. The joint pain level of each participant was assessed using a visual analogue scale (VAS) prior to and immediately after each walking session. The data of compliance to walking exercise was collected using an exercise diary from each participant. **Results** Nine participants in each group completed the intervention. The joint pain pre and post walking exercise showed a similar trend of decreasing over time in both IW and CW groups. However, after each walking session, the joint pain decreased in the IW group but increased in the CW group. The difference in changes of joint pain after each walking session was significant between the two groups. The compliance to walking exercise was consistently high in the IW group throughout the study but declined significantly in the CW group after the first four weeks due to worsening of the joint pain. **Conclusion** The results of this study indicated that the regulation of the joint pain at resting status or during exercise might involve different systems, specifically local versus central system. The IW may be a better form of a walking exercise than the CW for people with KOA.

Functional Biomarkers for ALS

Whited K[1], Lillig K[1], Gerringer C[1], Higgs K[1], Colgan S[1], Saavedra L[1], Roath K[1], Walker M[1], Clay R[1], Taylor N[2], Bright A[2], Kosa E[3], Swerdlow RH[1], Jawdat MD[1], Jabari D[1], Agbas A[3], Statland JM[1], Barohn RJ[2], Wilkins HM[1]
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Background: Amyotrophic lateral sclerosis (ALS) pathological features include mitochondrial dysfunction and loss of proteostasis (protein aggregation). Mitochondrial dysfunction and proteostasis are intricately linked biological modalities which are key targets for upcoming clinical trials in ALS. These clinical trials will benefit from functional blood-based biomarkers. Our overall goal is to clinically validate blood based mitochondrial and proteostasis biomarker protocols for use as indices of target engagement in future clinical trials.
Objectives: We are comparing mitochondrial and proteostasis biomarkers within 150 ALS and 50 control subjects over eight months.
Methods: We have currently enrolled 20 ALS subjects from KUMC and MU-Columbia neuromuscular clinic. Each subject will undergo four blood draws at 2–4-month intervals. We measure lymphocyte mitochondrial biomarkers, including mitochondrial membrane potential (TMRE), mitochondrial superoxide (MitoSox), mitochondrial mass (MitoTracker), and apoptosis (Annexin V). Using these biomarkers, we employ an algorithm to determine a mitochondrial health index (MHI). We also measure plasma neurofilament light (NfL) values and phosphorylated TDP43 in plasma and platelets. Data are compared with clinical assessments including forced vital capacity (FVC) and the ALS functional rating scale (ALSFRS).
Results: Current data suggest that lymphocyte MHI values decrease at each visit, while NfL levels are relatively stable but elevated. Lymphocyte MHI does not correlated with NfL. However, individual biomarkers do correlate with clinical assessment. Mitochondrial superoxide in lymphocytes correlates with FVC. Lymphocyte mitochondrial membrane potential correlates with plasma NfL levels.
Discussion: Overall, our data show that lymphocyte mitochondrial function could be a biomarker of ALS progression.

Live Cell and Intravital Imaging of Bone Resorption Dynamics and Osteocyte Fate During Osteoclastic Bone Resorption

Sarah L. Dallas[1], Valerie Kirtley[1], Anthony Meljanac[1], Yixia Xie, LeAnn Tiede-Lewis[1], Eleanor Ray[1], David S. Moore[1]

[1]University of Missouri-Kansas City

Abstract

Live cell imaging allows us to gain novel insight, from a dynamic perspective, into the fundamental processes controlling bone formation and resorption during bone development, maturation, repair and aging. Using transgenic mice with fluorescently tagged osteoclasts, osteocytes and collagen, we have performed timelapse live cell and intravital imaging to visualize the dynamic and resorptive behavior of osteoclasts in their natural bone environment and determine the fate of osteocytes after bone resorption.

Transgenic lines, including mice with red fluorescently tagged osteoclasts (LysM-Cre/tdTomato), green or red fluorescently tagged osteocytes (Dmp1-AcGFPmem) or (Dmp1-Cre/tdTomato) or green fluorescently tagged type I collagen (COL1A2-GFP*tpz*), were crossed in various 2-color combinations. Intravital multiphoton imaging was performed in 4-6wk old mice and confocal or widefield epifluorescence live cell imaging was performed using calvarial explants from 10-14 day old mice in the presence or absence of stimulators and inhibitors of osteoclastic resorption. Live imaging in bone explants provided resolution sufficient to visualize osteoclast dynamics as well as the dynamics of their sealed zones (actin rings). Dual imaging of osteoclasts with GFP-collagen has enabled us to correlate osteoclast cell dynamics with collagen resorption kinetics. The osteoclasts had a multi-lobed morphology and actively resorbing osteoclasts were morphologically distinct from inactive ones, exhibiting a more compact, less branched morphology. Over long-term timelapse imaging (up to 96h), osteoclast morphology was continuously changing, with amoeboid-like streaming of the cell, repeated extension and retraction of cell processes and formation of varying numbers of transient sealed zones rings (up to 3-4 per osteoclast) that correlated with bone resorption activity. Osteoclasts showed complex cell dynamics including fission, fusion and recycling. In control bones osteoclast maximum velocities were up to 52.6±5.7μm/h, with an average of 13.5±1.4μm/h. Treatment with PAM3CSK4, an inflammatory stimulator of bone resorption, decreased the maximum and mean velocities to 38.3±2.1 and 8.8±0.4μm/h but increased the directionality of osteoclast motion. PAM3CSK4 also increased the mean number of actin rings per osteoclast from 0.27±0.11 to 1.4±0.25 and decreased osteoclast fusion, while increasing osteoclast survival. Dual imaging of osteoclasts/osteocytes showed that the majority (>90%) of osteocytes in areas of bone that were resorbed underwent cell death/apoptosis, a small fraction (~5%) were internalized within vacuoles and only in rare cases did osteocytes appear to survive osteoclastic resorption by migrating out of their lacunae. These data provide new insight into the dynamic behavior of osteoclasts in their natural bone environment, how these dynamics are altered in inflammatory bone resorption, and can be used to enhance our understanding of the mechanisms of action of bone therapeutics.

Maternal dietary vitamin A levels as a determinant of penetrance and severity of cleft lip/palate in a Wnt9b model.

Ryan Anderson[1], Akiko Suzuki[1], Zaib Malik[1], Liza Cox[1], & Timothy Cox[1,2]

Departments of [1]Oral & Craniofacial Sciences and [2]Pediatrics, School of Dentistry, University of Missouri-Kansas City, Kansas City, MO 64108, USA

Vitamin A (VA) and its derivatives (retinoids) play essential roles during embryogenesis and particularly facial development, with both severe VA deficiency and vast excess of VA derivatives having been linked to CL/P risk. Previous studies have shown that knockout of *Retinol binding protein 4* (*Rbp4)* severely limits the mobilization of liver retinol stores and that elevated dietary intake is required to ensure sufficient retinol supply to peripheral tissues. This dietary-dependence underpins the *Rbp4* KO line as a sensitive and tunable model to study the effects of VA on development and disease.

To study the interaction between maternal dietary VA and specific cleft susceptibilities, we have crossed the *Wnt9b* KO – the best-characterized single gene CL/P model - on to the *Rbp4* KO background. Pregnant dams were switched, post conception, from a sufficient diet (23IU/g) to either a low VA (4IU/g) or high VA (40IU/g) diet. Embryos were then imaged using optical projection tomography for qualitative and quantitative facial phenotyping. Our data reveal a window of optimal maternal dietary VA, where the incidence of CL/P was reduced to <20%. Notably, doubling maternal VA levels increased both CL/P severity and incidence (to ~65%), whereas vitamin A insufficiency led to a switch from bilateral clefting to midline facial clefts and repatterning of the frontonasal tissue. These findings suggest that optimization of maternal dietary VA during the early stages of pregnancy may reduce the chances of a child being born with CL/P. Ongoing work is determining whether this holds true for other CL/P risk alleles.

Funding: UMKC Work Study program and an Endowment for Dental and Musculoskeletal Research.

Joint Lubricants as Delivery Methods for Multipotent Stromal Cells

Kennedy Davis, Megan Hamilton, Douglas Bittel, Michael Filla,
Lisa Stehno-Bittel, PhD
Kansas City University, University of Kansas Bioengineering Program, and Likarda, LLC, Kansas City, Missouri, USA

Multipotent Stromal Cells (MSC) are utilized as therapeutic agents for addressing tissue regeneration for musculoskeletal conditions, including knee osteoarthritis (OA). Currently, cell therapies lack FDA-approval for injections to alleviate joint OA. To overcome this barrier, some clinicians are utilizing autologous stem cell transplants that are not regulated. Yet, the results are mixed with a majority of patients indicating little or no relief. Major challenges in the clinical application include poor MSC viability after isolation, extreme shear stress of the injections, maintenance of the cells in the joint capsule, and the harsh inflammatory environment of knee OA. As hyaluronic acid (HA) is an innate polymer of synovial joints that maintains cartilage viscoelastic integrity, HA-based cell delivery systems are of interest. While MSCs could be delivered in these uncrosslinked gels, it is hypothesized that they will not trap the cells in the knee joint long enough to have an effect. The aims of this study were: 1) to determine whether a commercial HA joint lubricant (Monovisc) could maintain MSC viability under different conditions, and 2) to determine the ability of cells delivered in Monovisc to reverse knee joint degeneration in an OA rat model.

Trial of Oxaloacetate in ALS (TOALS)

Heim, AJ; Lillig, K; Statland, J; Jabari, D; Wilkins, H; Agbas, A; Choi, I;
Lee, P; Zhang, N; Gerringer, C; Dimachkie, MM; Swerdlow, R; Barohn, RJ; Jawdat, O

Abstract

Background: Amyotrophic Lateral Sclerosis (ALS) is a progressive and fatal neurodegenerative disease. ALS affects nerve cells in the brain and spinal cord which control muscle movement. Death of the motor neurons leads to denervation of skeletal muscles resulting in muscle atrophy, weakness, fatigue, fasciculations, respiratory failure, and death. The majority of patients with ALS die within three to five years from symptom onset. The exact cause of motor neurodegeneration remains uncertain; however, mitochondrial dysfunction has been implicated having a role in motor neuron death in ALS. Oxaloacetate (OAA) is a Krebs cycle and gluconeogenesis intermediate, which enhance glycolysis flux, supports oxidative phosphorylation, and modifies bioenergetics-related infrastructures as supported by both human and animal model studies.

Objectives: The primary objective of this study is to determine safety of OAA administration and if reducing mitochondrial stress is a viable treatment strategy for ALS. The study design includes a dose escalation which will determine the maximal tolerated dose of OAA and whether OAA improved biomarkers of mitochondrial stress.

Methods: Enrollment is staggered into a 3+3 dose escalation of oral oxaloacetate for 28 days. First patient's cohort, consisting of three subjects, has received 1000mg OAA twice daily. If no dose limiting toxicity observed, second patient cohort has received 1500 mg OAA twice daily. Finally, third cohort has received maximum 2500 mg OAA twice daily. Subjects have three onsite visits and weekly phone call visits throughout the duration of the study to evaluate safety and efficacy. Subjects undergo an MR spectroscopy of brain glutathione at the Hoglund Biomedical Imaging Center at Screening and Day 28. In collaboration with Dr. Abdulbaki Agbas and Dr. Heather Wilkins, platelet TDP-43 and pTDP-43, and mitochondrial biomarker blood sample data were collected at screening, baseline, and day 28 for all cohorts.

Results: To date, 13 subjects have enrolled and completed the 28-day dosing period. The final cohort evaluating the 2500 mg twice daily dose is currently enrolling 5 more subjects. Related adverse events have been limited to gastrointestinal upset, which is an expected side effect of OAA.

Discussion: OAA has been well tolerated and enrollment has continued into the final cohort of 2500 mg twice daily. Reported adverse events have been limited and expected with ALS progression. Safety and biomarker blood samples are being collected for analyzing platelet TDP-43 and pTDP-43 by employing capillary electrophoresis Immunoassay (CEI).

Acknowledgements: This study received funding from the Kansas City Musculoskeletal Consortium

A Novel PCR-based Strategy for Investigating Sexual Dimorphism in Avian Embryos: An Example in Bone

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Sex is known to influence human skeletal development, making it critical to consider sex as a biological variable in bone development research. We use embryonic duck (*Anas platyrhynchos*) and quail (*Coturnix japonica*) to answer questions about the development of bones, such as the lower jaw. There is known sexual dimorphism in adult duck skeletons, but not in quail. However, the impact of sex on embryonic lower jaw bone gene expression is unknown in both our avian models and in humans. Such studies are challenging because sex is morphologically unidentifiable in embryos of most avian species, therefore molecular techniques are required for sex identification. Molecular sex identification of embryonic birds can be difficult, particularly in species outside of the widely-studied chick (*Gallus gallus*). To improve upon a polymerase chain reaction (PCR) technique in chick that identifies histidine triad nucleotide binding protein W (*HINTW)*, which is located on the female-specific W chromosome, we developed a novel quantitative PCR (qPCR)-based technique to identify the sex of avian embryos using *HINTW* in multiple species, such as chick, duck, and quail. Messenger RNA was extracted from embryonic quail and duck lower jaw tissue at two developmental timepoints: one just before bone calcification begins in the facial complex and one when it is largely calcified. Expression of eight genes related to bone development were analyzed ($n=3$-4/stage/sex, $p<0.05$). Statistically significant sex differences were noted only in duck matrix extracellular phosphoglycoprotein (*MEPE)* with expression 4-fold higher in males, and duck receptor activator of nuclear factor κ B (*RANK)* with expression 2-fold higher in males. None of the other genes analyzed demonstrated sexual dimorphism, including those with large fold changes between stages (matrix metalloproteinase-9 (*MMP9)*, osteoprotegrin (*OPN),* and *MMP13 (*in quail)), those with mild fold changes (collagen type I alpha 1 chain (*COL1A1*), fibroblast growth factor-23 (*FGF23), MEPE* (in quail), and *MMP13* (in duck)), and those with biologically minor changes (runt-related transcription factor 2 (*RUNX2)*, and *RANK* (in quail)). None of the genes analyzed in quail demonstrated sexual dimorphism, which aligns with the lack of sexual dimorphism in adult quail skeletons. In contrast, our data suggest possible sexual dimorphism in the mRNA expression of some bone-related genes in embryonic duck, whose adult skeletons are dimorphic. This study demonstrates our novel *HINTW* qPCR technique to identify the sex of avian embryos could be a useful tool for including sex as a biological variable in analysis of a variety of tissues and cells used in developmental biology research.

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3D printed multi-gradient microsphere scaffolds for guided osteochondral tissue engineering

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Osteochondral joint regeneration is one of the most challenging tissue engineering endeavors. It involves the recapitulation of two distinct and yet similar biological processes, which are joint chondrogenesis versus subchondral osteogenesis, requiring proper temporal and spatial signaling coupled with guiding mechanical stimulation and correct geometrical configurations. These factors have made it difficult for tissue engineers to design biomaterials that can replicate nature's designs. A promising solution to this issue are synthetically derived microspheres, which can be used to encapsulate therapeutic substances to provide a temporally controlled release mechanism. 3D printing techniques that could be used to create gradient scaffolds to control the spatial release of signaling biomolecules from load-bearing scaffold applications as needed for joints, currently cannot distribute microspheres in the desirable fashion.

We have recently overcome this issue and have generated a 3D printed scaffold comprised solely of PLA microspheres with varying porosities and geometrical alterations that better mimic the architectural foundation of bone. Not only are we now able to generate such a solely microsphere comprised scaffold but have successfully generated the first 3D printed microsphere scaffold with a 3D material gradient simulating the structure of an osteochondral joint matrix that would foster possible cellular migration, differentiation and tissue formation into cartilage and bone.

Our theory is that with the ability to precise control release of signaling biomolecules temporally and spatially within one scaffold, we can better study cell response on certain stimuli, e.g. the amount of growth factors released at any time, or cascading different factors in a certain order, while also providing preferred architectural environments for cell invasion and proliferation. This research will then enable us to create scaffolds that better mimic the natural process and hence create superior tissue-engineering based therapies to heal bone and cartilage defects.

Role of Estrogen Receptor α in Bone-Muscle Crosstalk

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Estrogen plays critical roles in bone and muscle health by signaling through its two main receptors, ERα and ERβ. To investigate the role of ERα in bone-muscle crosstalk, we deleted this receptor in skeletal muscle by crossing ERα$^{f/f}$ mice with the tamoxifen inducible HSA-MCM-Cre mouse to generate ERα$^{-/-sm}$ mice. Cre^{-} littermates were used as controls. Mice were injected with tamoxifen one month prior to sacrifice, at either 5 mo old, or 11 mo old (n=3-8/group). Extensor digitorum longus (EDL) and soleus muscles were used for *ex-vivo* contractility analysis. Femurs were used for μCT analysis and biomechanical analysis.

At 6 months of age, muscle specific deletion of ERα impacted several muscle properties. Male ERα$^{-/-sm}$ EDL weight was lower (16.2±2.9 vs 13.3±0.4 mg, $p<.05$), produced lower absolute force (267.8±35.8 vs 214.3±22.7 mN, $p<.05$), and had lower relaxation rate (3100±407 vs 2485±257 mN/s, $p<.05$) at submaximal frequencies, and had improved fatigue resistance ($p<.05$). Muscle deletion of ERα also resulted in altered trabecular bone parameters only in male mice. Femurs of ERα$^{-/-sm}$ males had higher Tb.BMD (0.39±0.06 vs 0.46±0.05 g.cm^{-3}, $p<.05$), Tb.BV/TV (13.8±2.6 vs 20.8±4.4%, $p<.01$) and an increase in Tb.N (4±1.1 vs 5.7±1.0 1/mm, $p<.05$). ERα$^{-/-sm}$ male tibias had an increase in Tb.Th (0.054±0.01 vs 0.062±0.01, $p<.05$). ERα$^{-/-sm}$ female mice had improved fatigue resistance in EDL muscle compared to control ($p<.05$), but bone parameters were not altered.

At 12 mo, there were no differences between ERα$^{-/-sm}$ and control male mice in muscle and bone properties. ERα$^{-/-sm}$ female EDL muscles produced lower absolute and specific force (54.1±2.8 vs 40.5±3.9 N/cm^2, $p<.01$) at high frequencies. However, there were no differences after a fatigue regime and exposure to caffeine, suggesting alterations in excitation contraction coupling after repetitive stimulation which restored force to control levels. There were no changes in bone properties in the femurs of ERα$^{-/-sm}$ female mice.

In summary, deletion of ERα after development reduced ex vivo contractility in EDL, while improving fatigue properties. ERα expression in skeletal muscle in 6-month-old male mice suppresses trabecular bone formation, but these changes were not present in 12-month-old mice. These data support an important role of ERα-mediated signaling in bone to muscle crosstalk at the biochemical level that changes with aging.

Multiscale, 3D finite element analysis using Micro-CT and confocal multiplexed images for correlation of osteocyte β-catenin signal pathway activation with predicted lacunar wall strain.

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Abstract:

It is well known that mechanical loading of bone activates β-catenin signaling pathways in osteocytes, initiating an osteogenic response. The mechanotransduction process involved is not fully understood, although it is hypothesized that strain in the lacunocanalicular walls surrounding osteocytes is correlated with osteocyte activation. We have previously shown that cyclic compression loading results in heterogeneous activation of β-catenin signaling in neighboring osteocytes which are thought to experience similar global (whole tissue) strains. Determining whether this heterogeneity is a result of heterogeneity in the local (lacunocanalicular) bone strains experienced by osteocytes will improve our understanding of the mechanisms involved in initiating the osteogenic response, possibly leading to better treatments or therapies for bone diseases like osteoporosis.

The right ulna of a TOPGAL β-galactosidase (β-Gal) LacZ reporter mouse was subjected to cyclic compression loading, and the left ulna was used as a non-loaded control. The β-Gal substrate DDAOG was used to stain activated osteocytes with the fluorescent product DDAO whose signal is related to the level of β-catenin pathway signaling activity in cells. Using the loaded bone, Micro-CT scans and 40x confocal images were analyzed to measure relative osteocyte activation levels and predict the bone strain distribution in 5 finite element models with similar FE characteristics. Strain values surrounding the lacunae were filtered to look at average strains, the average of strains greater than the overall average + 3 standard deviations, and the average of strains in the top 5% of all values. Linear regression was used to evaluate the correlation between strain and signal intensity.

DDAO signal was observed in all osteocytes in the non-loaded and loaded samples, indicating a basal level of β-catenin pathway signaling regardless of loading history. The average DDAO signal intensity in osteocytes in the non-loaded bone was 1.2×10^6 ranging from 1.2×10^4 to 2.3×10^6, while in loaded bone the average was 3.6×10^7 ranging from 3.4×10^6 to 9.1×10^7, demonstrating the anticipated increase in osteocyte activation due to loading, and heterogeneity of activation between neighboring osteocytes.

The average effective strain distribution in the 5 models was ~2500 $\mu\epsilon$, with a maximum strain of ~70,000 $\mu\epsilon$. Comparing DDAO signal intensity with average strain in FE elements near the lacunar walls (see Figure) indicated an increase of osteocyte activation with increasing strain overall. However, when strain data was filtered to include only elements with strains that were significantly higher than average, the linear regression was less likely to show a positive relationship between activation and strain. R^2 values were only significant in one

of the 5 models examined.

The results suggest that strain in the lacunar walls is not the most significant factor in determining osteocyte activation levels. A possible alternative factor that cannot be addressed by this study is the effect of fluid flow shear stress, which is also widely believed to influence osteocyte activation.

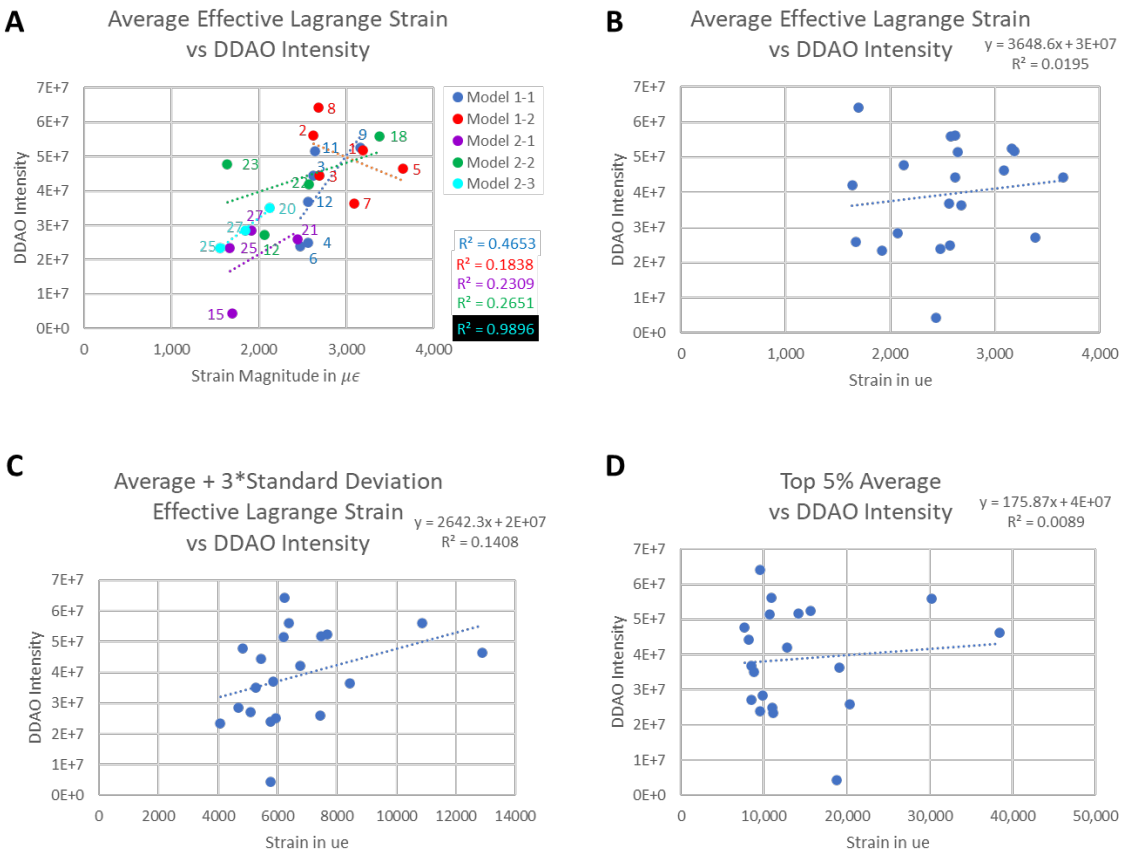

Figure: Linear regression of DDAO signal intensity (relative osteocyte activation) and effective Lagrange strain in loaded ulna. A) Average strain of elements surrounding each lacuna with each model depicted separately. Data labels indicate lacuna number. Note that overlapping models have similar results (lacunae 3, 25, and 27). Model 1-2 has a negative slope. Only model 2-3 has a significant R^2 value. B) Average strain of elements surrounding each lacuna for all 5 models. C) Filtered data representing only elements whose strain is higher than the average plus 3 standard deviations. D) Filtered data representing only elements whose strain is in the top 5% of values. Note in figures B, C, and D that the slope of the regression line lessens as elements with lower strain are eliminated from the data.

New insights into the genetic basis of the Oculo-Auriculo-Vertebral Spectrum (OAVS)

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Oculo-Auriculo-Vertebral Spectrum (OAVS) represents a wide range of symptoms involving three intimately related, rare disorders involving, but not limited to, eyes, ears and spine. It is recognized by its frequently asymmetric facial presentation that can variably include microtia, maxillary and mandibular hypoplasia, pre-auricular or lateral facial tags, and cervical vertebral defects. The phenotypic spectrum shares notable overlap with, but distinct from, the mandibulofacial and acrofacial dysostoses and other disorders of branchial arch development.

The prevailing view is that OAVS is genetically heterogeneous with significant environmental contributions: the most convincing being exposure to elevated levels of retinoids. Further support for disrupted retinoid signaling as a contributing factor has come from our characterization of a mouse mutant that displays an OAVS-like phenotype and carries an inversion/deletion mutation that results in elevated expression of two adjacent retinol dehydrogenase genes. In patient studies, three missense variants have been described in *MYT1* – which encodes a transcriptional suppressor of retinoid signaling - in a cohort of 225 patients. Array CGH studies have also implicated a number of loci including 14q22 (involving craniofacial genes *OTX2*, *SIX1* and *SIX6*) and 4p16 (involving *HMX1*).

In an effort to identify additional single gene and structural causes of OAVS, we have employed a combined exome- and whole genome sequencing approach on a sub-cohort of OAVS patients that were selected from a larger clinically-phenotyped cohort of 80 patients. The initial sub-cohort is comprised of 9 sporadic cases and 2 families exhibiting autosomal dominant inheritance. We have identified a number of candidate genes and will present findings using a zebrafish model of one of these candidates, which supports it as a bona fide OAVS gene.

Bruno1 Is Required Throughout Drosophila Indirect Flight Muscle Development to Regulate Dynamics Of Sarcomere Assembly And Growth

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Abstract:
The differential expression of structural protein isoforms influences cytoskeletal assembly and contractile properties. CELF family RNA binding proteins are important regulators of RNA processing, but we do not fully understand how misregulation of CELF proteins leads to defects in sarcomere assembly, growth and function. Bruno1 (Bru1, Arrest) encodes a CELF1/2 homolog in *Drosophila* that regulates flight muscle specific alternative splicing. Here we show that Bru1 is required throughout muscle development to regulate cytoskeletal assembly and growth dynamics. During early myofibril formation before 48h APF, using both temporally-restricted RNAi knockdown and overexpression, we show that misexpression of Bru1 leads to disorganization of the actin cytoskeleton, aberrant myofiber compaction and defects in pre-myofibril formation. Transcriptomic and proteomic analyses revealed misexpression and isoform switches in diverse structural proteins regulating sarcomere growth and actomyosin interactions. Live-imaging assays confirmed aberrant contractility of *bru1* mutant myofibers. By monitoring incorporation of fluorescent actin and myosin proteins during myofibril maturation after 56h APF in *bru1* mutant IFM, we show that lateral sarcomere growth is dramatically misregulated, leading to exacerbation of pre-existing defects, myofibril fusion and formation of hollow myofibrils. A progression in the severity of cellular and molecular phenotypes from 80h APF to adult distinguishes hypercontraction from earlier growth defects, and temporally restricted rescue can partially alleviate hypercontraction in late pupal and adult stages. Taken together, our data indicate that Bru1 regulates cytoskeletal growth and remodeling throughout myogenesis, including cytoskeletal rearrangement necessary for myofibril formation as well as the balance in length versus lateral growth of the sarcomere. Defective RNA processing due to misexpression of CELF proteins thus causes wide-reaching structural defects and progressive malfunction of affected muscles.

Preliminary Results from a Novel Movement-Based Concussion Screening Tool

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Background: Following concussion, individuals are 2-3 times more likely to sustain a subsequent lower extremity injury. Current methods used to screen for concussions are subjective and not based on movement tasks which simulate the demands of sport.
Research Question: The purpose of this study was to assess the ability of a novel movement-based concussion screening tool (MPASS) to quantify differences between individuals with and without concussion.
Methods: 17 individuals (22.59±1.66 yrs., females=12) participated in this study. Five participants suffered a concussion within 1-month before data collection. Twelve had not suffered a concussion within the past year. All participants completed the same battery of functional tasks: walking (control, serial subtraction by seven dual-task, and head-shaking), Romberg balance tests (firm-surface and foam-surface with eyes-open, eyes-closed, and eyes-closed head-shaking), and reaction time. During walking tasks, lower extremity spatiotemporal parameters were collected via Kinect depth-sensing camera. During balance tasks, a force plate recorded center of pressure and Kinect recorded center of mass. Reaction time was recorded using an Arduino-based reaction board while center of pressure was simultaneously recorded via force plate. This resulted in 133 unique variables for each participant. Principal component analysis (PCA) was used to reduce the dimensionality of the data. All statistical analyses were complete in RStudio (v4.2.0).
Results: Five principal components (PCs) were retained using Horn's Parallel Analysis for component retention. These 5 PCs explained 65.35% of dataset variance. Visual analysis of the first 3 PCs demonstrated clear separation between the two groups(concussion and control). Analysis of each variable's contribution to retained PCs revealed significant contributions from center of mass data during balance tasks, stride and step length during walking, and step length during head-shaking walking. This suggests results from these tasks are most vital in distinguishing individuals with concussion from those without concussion. However, further samples will be required before this can be determined.
Significance: Results demonstrate promising initial results for concussion screening using MPASS.
Acknowledgements: This study was funded in part by the University of Missouri Coulter Biomedical Accelerator.

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Tibia Mechanical Loading Acutely Decreases Resting Heart Rate in Mice Likely via the Sympathetic Autonomic Nervous System

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Bone adapts to mechanical forces by altering its overall structure and mass. Sensation of applied load in bone occurs in osteocytes via fluid flow shear stress generated throughout the lacunocanalicular system during mechanical strain. This results in the release of intracellular signaling molecules including prostaglandin E2 which ultimately leads to the activation of bone formation and suppression of bone resorption. In addition, the response to bone loading is blunted during the setting of osteoporosis and aging. Previous studies from our group have demonstrated that osteocyte-secreted factors enhance muscle contractility and improve muscle cell proliferation and differentiation. Moreover, recent reports have suggested that bone may directly regulate heart function and the acute stress response. We therefore hypothesized that bone mechanical loading would elicit an acute increase in cardiac muscle functional output. To test this hypothesis, we performed acute *in vivo* mechanical loading (2 Hz, 300 cycles, 9.25 N) on the right leg tibias of anesthetized young adult (2-6 months old) CD-1 mice while simultaneously monitoring heart depolarization/repolarization parameters, heart rate and heart rate variability (HRV) using lead II electrocardiogram (ECG). In both male and female mice, tibia loading resulted in an immediate and transient reduction in resting heart rate (0.93 fold decrease from baseline, $n=6\text{-}7$, $p<0.01$) accompanied by an increase in HRV (1.23 fold increase from baseline, $n=6\text{-}7$, $p<0.01$) which returned to baseline levels upon completion of the loading process. ECG intervals including QRS and corrected QT (QTc) were not altered during tibia loading nor during a 30 minute period subsequent to loading ($p>0.05$). The speed at which the heart responded to loading suggested that this response could be mediated neuronally. We therefore injected lidocaine (2.5mg/kg) into the hindlimb near the tibia to inhibit local neuronal afferent activity prior to loading and found that the decrease in heart rate was significantly attenuated (vehicle: 0.90 vs. lidocaine: 0.97 fold change from baseline, $n=7\text{-}8$, $p<0.05$). To further delineate the specific arm of the nervous system which mediates the heart rate changes we observed during loading, we injected mice with muscarinic acetylcholine receptor antagonist atropine (2mg/kg) or $\beta1/\beta2$ receptor antagonist propranolol (10mg/kg) and found that propranolol (vehicle: 0.88 vs. propranolol: 0.98 fold change from baseline, $n=7\text{-}8$, $p<0.05$) but not atropine (vehicle: 0.89 vs. atropine: 0.94 fold change from baseline, $n\text{-}8\text{-}11$, $p>0.05$) significantly inhibited the heart rate decrease suggesting a likely role for changes in sympathetic autonomic tone on the heart during bone loading. In conclusion, our study has uncovered a novel bone-heart neural reflex likely involving afferent neurons in the hindlimb and changes to sympathetic tone. In the future, it will be important to determine how this bone-neural-heart reflex contributes to heart physiology during the settings of exercise, osteoporosis and aging.

Serial Subtraction Dual-Task Alters Lateral Step-Down Tibiofemoral Kinematics in Healthy Adults

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INTRODUCTION: Prior research has shown cognitive dual tasking can have negative impacts on gait[1]. Less is known regarding the effects of dual tasking on lower extremity patterns of movement. However, it is known cognitive dual tasking can lead to increased injury risk while performing dynamic limb movements[2]. Greater understanding of these injuries, and factors which may contribute to them, is necessary to improve treatment and prevention efforts. The purpose of this study was to use the Mizzou Knee Arthrometer Testing System (MKATS) to assess, one aspect of lower limb movement, differences in tibiofemoral motion related to dual tasking during activities.
METHODS: The MKATS uses an electromagnetic motion tracking system (Polhemus Patriot System, Polhemus, Colchester, VT) attached to a 3D printed housing which covers the femoral epicondyles with a separate clamp on the tibia. This configuration accurately captures knee motion across three planes of motion (frontal, sagittal, and transverse) after proper calibration determining the knee axis of rotation and anatomical axes. Following calibration, with every task, the participant performed 5 repetitions of the lateral step-down (LSD) on each leg. The first trial was performed without cognitive dual tasking. Second and third trials consisted of the Stroop test followed by Serial Sevens (SS) dual task.
RESULTS: 19 healthy individuals (22.05±1.61 yrs., 173.92±9.21 cm, 67.99±12.65 kg) participated in this study. At maximum knee flexion, significant kinematic differences were found between control and SS conditions, but not between control and Stroop. Knee abduction angle was significantly lower (right side: M = -1.35, SD = 3.54, left side: M = -2.14, SD = 2.88) during SS than during control (right: M = -1.04, SD = 3.61; left: M = -1.42, SD = 2.90). Transverse plane rotation of the left knee (M = -1.86, SD = 4.45) was externally rotated significantly higher during SS than control (M = -2.53, SD = 4.30). The transverse plane rotation of the right knee did not show that significance. No variables showed significant differences between control and Stroop conditions (all $p > 0.05$). 18/19 participants were right leg dominant.
DISCUSSION: The results indicate differences in tibiofemoral kinematics dependent on the type of cognitive dual task being done. Specifically, the SS dual task led to greater results regarding tibiofemoral kinematics while performing a LSD than the Stroop test. In addition, the nondominant left leg also yielded a greater number of significant results than the right dominant leg. This suggests an increased rate of cognitive dual tasking on nondominant limb kinematics during LSD compared to the dominant limb. More research should follow to explore more varied populations simulating other everyday dynamic movements.
ACKNOWLEDGEMENTS: Data collection possible with resources from the University of Missouri Motion Analysis Center and funding by the University of Missouri Coulter Biomedical Accelerator Program.
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Confocal and Tissue Clearing/3D Imaging in Transgenic Mice Expressing a Membrane-GFP targeted to Mineralizing Cell Types

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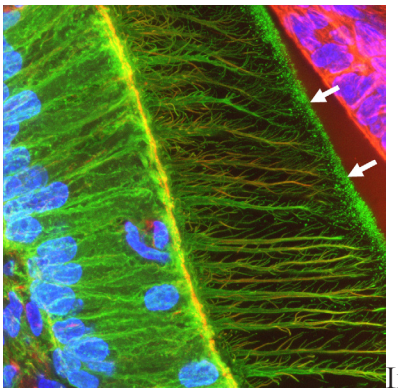
High resolution 3D imaging of cells in mineralized tissues is challenging due to the light scattering properties of mineral and extracellular matrix. Imaging fine detail of dendritic cells, such as osteocytes, cementocytes and odontoblasts requires cell-specific staining methods that target the membrane or substructures within cell processes. Using transgenic mice expressing a membrane-targeted GFP driven by the dentin matrix-1 promoter (Dmp1-mGFP mice), we have used 3D confocal imaging and tissue clearing/3D imaging to examine tissue expression of the mGFP reporter and fine cellular detail of mineralizing cells in the mandible and dentition of Dmp1-mGFP transgenic mice.

Mandibles were prepared from 7-10 day and 2 month old Dmp1-mGFP mice or transgenic mice co-expressing Dmp1-mGFP and a LysM-Cre/tdTomato reporter to target osteoclasts. Samples were decalcified and 50m cryosections were counterstained with alexa555-phalloidin to visualize F-actin and DAPI to visualize nuclei. Alternatively, samples were processed intact using the PEGASOS tissue clearing method [Jing et al: Cell Res. 28(8):803-818 2018], with and without decalcification. Specimens were imaged by confocal, multiphoton or lightsheet microscopy.



In 7 day mandibles the Dmp1-mGFP transgene was expressed in osteocytes, odontoblasts, some pulpal cells adjacent to odontoblasts and a subset of late osteoblasts on the bone surface. The membrane-targeted-GFP enabled visualization of fine detail and branching of odontoblast processes (fig.1) and osteocyte dendrites. In 2 month mice, Dmp1-mGFP expression was also observed in cementocytes. The Dmp1-mGFP transgene labeled abundant extracellular vesicle (EV)-like particles (~ 80-500nm), found at the distal tips of odontoblast processes at the dentin-enamel junction (Fig 1, arrowheads) and throughout the mineralized matrix surrounding osteocytes and cementocytes, which likely represent matrix vesicles associated with initiation of mineralization. Confocal imaging of intact mandibles without tissue clearing did not allow imaging deeper than the alveolar bone. In contrast, PEGASOS clearing allowed deep tissue imaging throughout the alveolar bone and teeth, with imaging depth limited primarily by the objective working distance. Lightsheet imaging enabled 3D reconstruction of Dmp1-mGFP and LysM-Cre/td tomato reporters in the entire mandible and teeth and virtual sectioning in any plane. This also revealed expression of Dmp1-GFP in a subset of cells within blood vessels supplying the cervical loop region of the incisor, consistent with vascular pericytes.

In summary, the Dmp1-mGFP mouse is a valuable model for studying differentiation and imaging the fine structure of mineralizing cell types in craniofacial tissues and is compatible with high resolution confocal imaging, tissue clearing/3D lightsheet imaging and live cell imaging.

An Interesting Case of Anti-Mi-2-Antibody Associated with Paraneoplastic Dermatomyositis in a Patient with Cervical Cancer

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Introduction: Dermatomyositis (DM) is a type of idiopathic inflammatory myositis (IIM). DM is often associated with malignancies. Myositis associated antibodies (MSAs), have been helpful to stratify patients with IIM and have been used as a marker for cancer associated myositis. MSAs such as anti-TIF1- γ and anti-NXP2 antibodies, are known to be associated with cancer more frequently than other MSAs. Anti-mi-2 antibodies have historically been less likely to be associated with malignancy. It is more commonly associated with classic cutaneous findings of dermatomyositis (1). Some cancers that are more commonly associated with dermatomyositis include breast, lung, rectum, and kidney (3). It is uncommon for cervical cancer to be associated with dermatomyositis. We present a case of paraneoplastic dermatomyositis with anti-mi-2 Antibodies, associated with inoperable cervical cancer.

Case: A 53-year-old female of Hispanic descent, with history of steatohepatitis and diabetes who presented to the outpatient clinic for dermatomyositis. She developed proximal muscle weakness 6 months prior which became progressive and further workup by her primary physician revealed an elevated Creatine kinase (CK) level over 5000 U/L, mildly elevated ESR 29 mm/hr and CRP 5.4 mg/dL. She was initially evaluated for rhabdomyolysis but there was no significant improvement in CK (improved to over 3000 in one month). Biopsy of the right biceps muscle active myopathic process with muscle fiber necrosis, elevated MHC-1 staining, and accentuated perifascicular atrophy, all suspicious for dermatomyositis. The patient endorsed symptoms of mechanics hands for two years prior to this presentation, for which her general practitioner reportedly was treating her with various ointments for dry/cracked skin but was unhelpful. The patient was placed on high dose, long term prednisone taper with improvement of skin findings and myopathy. Age-appropriate cancer screening was pursued, which revealed moderately differentiated cervical squamous cell carcinoma, which was deemed inoperable requiring radiation and chemotherapy. Myomarker panel demonstrated a weakly positive anti-mi-2 antibody only. Other serology was positive for ANA 1:320 with homogenous pattern. She is currently following with concerned specialties for treatment of dermatomyositis and cervical cancer.

Discussion: Dermatomyositis is an uncommon inflammatory myositis. It may present with cutaneous features and muscle inflammation. This condition is already distressing and can be debilitating if not treated promptly, but it also acts as warning signal for malignancy. Anti-mi-2-antibodies are commonly seen MSAs in DM. They are known to be associated with typical cutaneous findings and mild myositis (7). Dermatomyositis can precede neoplasm in 40% of cases; both conditions may occur together (26%) or cancer may occur first (34%). The incidence of carcinoma in association with DM varies from 15 to 34% (3). The majority of DM is idiopathic, but 15-30% may present as paraneoplastic syndromes (4). In our patient, she had features of Mechanic's Hands about 2-years prior to her overt clinical picture of DM and diagnosis of cervical cancer. Given that cervical cancer is known to be slow growing, can takes 3-5 years to progress, and generally easily treatable if caught early, it is likely that this patient had a paraneoplastic manifestation of DM a couple of years ago with the development of cervical cancer. The feature of Mechanic's Hands was not properly identified by the patient's general practitioner. It was thought to be a feature of dry skin. Her condition eventually progressed to an inoperable stage requiring treatment with Methotrexate and IVIG for DM as well as radiation and chemotherapy for cervical cancer. This case also highlights the uncommon anti-mi-2-antibody positivity in a DM patient and malignancy. There could even be a consideration for ethnicity. In a PubMed search for 'Dermatomyositis and Hispanic' turned out less

than 20 relevant results. One study (6) conducted specifically in Mexico, reported a high prevalence of anti-Mi-2 antibodies, in Mexican DM, however they related it to environmental factors, as they have also mentioned a lower prevalence of Mi-2-antibodies in Mexican Americans compared to patients in Mexico. There are large scale studies on IIM conducted in Europe and Asia, but not many in America.

Conclusion: There are very few studies that report anti-mi-2-antibodies associated with cancer. Paraneoplastic DM associated with cervical cancer is also rare. This case serves to underline the vast differences in presentation of patients with IIM and MSAs, although in some cases can be helpful for predictive outcome, can vary in different patient populations. Given that our country is fortunate to have a melting pot of ethnicities, more studies analyzing MSAs in IIM and associated development of malignancy should be pursued. More research on this topic in our own country will lead to better cancer surveillance guidelines and improved patient care in this patient population.

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Maturation-based Prediction of Craniofacial Growth

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Objectives: To examine differences in timing of ontogenetic parameters for craniofacial measures in untreated pediatric subjects, age 3 to 25 years, using chronological age and skeletal age.
Methods: Double logistic models of craniofacial growth were fit to 158 male and 145 female participants of the Fels Longitudinal Study (Yellow Springs, OH) with longitudinal lateral cephalograms (minimum 5 time points/participant) and matching skeletal maturity assessments from hand/wrist radiographs. Separate models were fit based on chronological age and skeletal age. Age at peak growth velocity (aPGV) was calculated for these individuals based on their individual growth velocity curves (first derivative of the predicted trait measure).
Results: Comparison of the two models clearly demonstrate how prediction of the timing of aPGV is improved by the use of skeletal age rather than chronological age. When chronological age is used, aPGV has a 95% CI of 8.9-13.1 years (females) and 11.1-15.1 years (male). When skeletal age is used aPGV has a 95% CI of 10.05-13.3 years (females) and 13.41-14.2 years (male). These maturity estimates effectively shift the "age" axis for each individual allowing it better match with the patient's actual pace of growth. The consideration of skeletal age in place of chronological age when evaluating growth could have a distinct clinical advantage resulting in optimized treatment timing and reduced patient (and physician) burden.
Conclusions: The discovery of measurable differences in growth trajectories in children demonstrating different patterns of skeletal maturity continues our long-term aim of providing individualized predictions of craniofacial growth, ultimately improving decisions in treatment timing.

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Extracellular Vesicle-Mediated Communication Between Cells in Bone

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Abstract
Cell shedding of exosomes/extracellular vesicles (EV) provides an important mechanism for cell-cell communication. These EV deliver their cargo of proteins, mRNAs and miRNAs to target cells, thereby altering their differentiation and function. Using transgenic mice expressing a membrane targeted GFP in osteocytes, we have previously shown that osteocytes and other mineralizing cell types deposit EV in the bone matrix and that GFP-positive EV can be found in the circulation. EV from osteocyte-enriched IDG-SW3 cells or primary calvarial cells promoted osteoblast-to-osteocyte differentiation and their cargo was altered by treatment with the bone regulatory hormone, PTH. To further characterize the cargo of osteocyte EV and their function in bone, multiple approaches were used. Western blotting, combined with proteomic analysis of EVs from osteocyte-enriched IDG-SW3 cells by LC/MS/MS using Tandem Mass Tag labeling technology revealed a proteome of > 3000 proteins that was enriched for exosome markers CD81, ALIX, and RAB5. The osteocyte EV contained all of the top 20 and 93 of the top 100 exosome markers listed in the Exocarta database. Osteocyte markers PHEX, MEPE, E11, RANKL and sclerostin were present in the EV as well as proteins playing a role in biomineralization, membrane fusion/exocytosis, motility/neurite outgrowth, extracellular matrix assembly, and Wnt/β-catenin, FGF and TGFβ signaling. Treatment of undifferentiated IDG-SW3 cells with EV from osteocyte enriched IDG-SW3 cell cultures induced expression of early osteocyte marker genes and induced mineralization, suggesting that they promote osteoblast to osteocyte transition and have overlapping functions with matrix vesicles, known to play a role in bone mineralization. EV from PTH-treated IDG-SW3 cells also induced osteoclast formation in whole marrow cell cultures. Live cell and intravital imaging showed that osteocytes shed EV from their cell membrane and dendrite tips and that osteocytes adjacent to blood vessels may release EV into the circulation. Together, our data suggest that EV shed by osteocytes may play an important role in regulation of both osteoblast and osteoclast function in bone and may potentially communicate with distant target cells via the circulation to regulate their function.