Mechanosensitive ion channel MSL8 is required for pulsatile growth and normal pectin deposition in *A. thaliana* pollen tubes

Joshua H. Coomey[1], Kari Miller[1], Elizabeth Haswell[1]
[1]Biology Department, Washington University in St. Louis, St. Louis, MO, USA.
coomey@wustl.edu

Abstract:
In flowering plants, pollen is the male gametophyte that not only performs the critical role of fertilization, but also represents a unique and accessible system for interrogating plant cell mechanics. Pollen endures rapid rehydration upon contact with the stigma, and germinates the fastest growing cell in the plant kingdom. A key component in this robust mechanical system is MscS-Like 8 (MSL8), a mechanosensitive ion channel. Our previous work has suggested that MSL8 serves as an "osmotic safety valve", regulating pressure in the germinating pollen tube by releasing anions when the plasma membrane experiences tension and preventing pollen tube rupture. However, we have recently identified defects in the cell walls of *msl8* mutant pollen, suggesting a role independent of osmoregulation. MSL8 channel function appears to be required for pulsatile growth dynamics and typical pectin deposition, suggesting a mechanism where ion release into the apoplast regulates wall dynamics in a mechanosensitive manner.

Mechanical loading effects on hypertrophic cardiomyopathy pathophysiology using iPSC derive micro heart muscle

Jingxuan Guo[1], David Schuftan[2], Huanzhu Jiang[2], Druv Bhagavan[2], Lavanya Aryan[2], Jonathan Silva[2], **Nathaniel Huebsch[2-4]***

1. Department of Mechanical Engineering and Material Science
2. Department of Biomedical Engineering
3. NSF Science and Technology Center for Engineering Mechanobiology
4. Center for Cardiovascular Research, Center for Regenerative Medicine, Center for Investigation of Membrane Excitability Diseases, Washington University in Saint Louis

Advances in genetics have associated mutations in the contractile sarcomere apparatus of cardiomyocytes with hypertrophic cardiomyopathy, the most frequent cause of sudden cardiac death in the young. It is challenging to predict genotype-phenotype relationships in hypertrophic cardiomyopathy due to incomplete penetrance. For example, different patients from the same family with identical genomic variants develop different symptoms. Non-genetic, environmental factors such as blood pressure are critical in heart function and disease. Therefore, understanding how mechanical factors contribute to phenotypes in diseases like hypertrophic cardiomyopathy is critical for developing effective therapeutics. However, limited studies have been performed to investigate the mechanical effects on engineered heart tissue physiology.

Here, a tissue engineered in vitro micro-heart muscle model was developed using cardiomyocytes derived from human induced pluripotent stem cells to investigate how mechanical resistance in combination with genetic mutations trigger the hypertrophic cardiomyopathy phenotype. Mechanical loading induced by material stiffness trigger early structure defects in micro-heart muscle derived from human induced pluripotent stem cells with a hypertrophic cardiomyopathy mutation. This led to micro-scale sarcomere structural defects and contractile dysfunction, causing impaired energetics, cellular hypertrophy and profound dysregulation of calcium handling. These studies illustrate the importance of physiologically relevant engineered tissue models to study inherited disease mechanisms with induced pluripotent stem cell technology.

A Mechanical Model for Analysis of Glenohumeral Stability

Shicheng Li[1], Iden Kurtaliaj[2], Jennifer Kunes[2], Lynn Ann Forrester[2], Michael Rowley[3,4], Mikhail Golman[2], Sharon Swartz[3], Stavros Thomopoulos[2], Guy Genin[1]

[1]Washington University, St. Louis, MO; [2]Columbia University, New York, NY; [3]Brown University, Providence, RI; [4]California State University, Hayward, IN

Research on shoulders of bats provides insight into the mechanisms of shoulder instability. Past research, although probing into the mechanical behaviors of the shoulders, does not provide quantitative analysis regarding the contribution of fibers to the stability of the shoulder. We developed a theoretical analysis of the interaction between the glenoid, humerus, and tendons, using MATLAB to build a mechanical system. Different tests with various parameters input show that the relationship between an external, constantly oriented force and the displacement of the bottom end of the humerus follows a non-monotonic change. With other geometric parameters held unchanged, different combinations of glenoid depth and tendon angles affect the energy which the system can absorb before reaching instability. Future research should aim to optimize the model by adding viscoelasticity, improving the geometry of the model, and exploring the effects of other parameters on the stability of the system.

Bone's Response to Mechanical Loading from Female and Male Mice Vary Across Eight Inbred Strains

Nicole Migotsky[1], Surabhi Kumar[1], John T. Shuster[1], Matthew J. Silva[1]
[1]Washington University in St Louis, St. Louis, MO
n.migotsky@wustl.edu

INTRODUCTION: In the United States, more than 54 million people over the age of 50 suffer from osteoporosis or low bone mass, which contributes to an increase in fracture risk, pain, and loss of independence. I addition to losing bone mass with age, bone's anabolic response to mechanical stimulus diminishes indicating a loss of mechano-sensation or -transduction. Both osteoporosis and low bone mass are moderately heritable and recent studies have shown that bone's response to mechanical unloading is also heritable. However, little is known about the influence of genetics on cortical bone's response to mechanical loading. In this study we utilized males and females from eight genetically diverse mouse strains from three subspecies of the common mouse (M. m. musculus, M. m. castaneus, M. m. domesticus). We loaded these mice to an equal mechanical strain and measured the induced bone formation. We hypothesize that loading induced bone formation will vary with mouse strain and sex and be moderately heritable.

METHODS: **Mice**. Female and male mice (n = 12/strain/sex) were received from Jackson Laboratory (Jax) at 7-10 wks and aged until skeletally mature (22 wks) before mechanical loading. Eight inbred mouse strains, including five common lab strains (A/J, C57BL/6J, 129S1/SvImJ, NOD/ShiLtJ, NZO/HlLtJ) and three wild-derived strains (CAST/EiJ, PWK/PhJ, WSB/EiJ), were evaluated. **microCT**. One or two days prior to the start of loading, the right and left tibia was microCT scanned *in vivo* (pre-scan) while the mouse was anesthetized. Three weeks later, each tibia was re-scanned *in situ* after sacrifice (post-scan). Bones were quantified for cortical bone morphology and values were compared before and after loading. Pre- and post-scans were registered per limb to quantify formed and resorbed volumes on the periosteal and endocortical surfaces. Loading induced changes were quantified as changes in loaded limb (RT) – changes in non-loaded limb (LT). **Loading**. While mice were anesthetized, the right tibia was placed in a custom loading fixture with the knee and ankle secured. Each mouse was loaded for 60 cycles at 4Hz with a Haversine waveform to a pre-determined peak force to engender 1500με. After loading, mice received analgesic to alleviate any pain. Mice were loaded 5 days per week for 2 weeks starting on Day 0. Calcein green and alizarin red were administered intra peritoneal (IP) on Day 4 and Day 11, respectively. On Day 18, mice were sacrificed with carbon dioxide inhalation. **Dynamic Histomorphometry**. After the microCT post-scans, the left and right tibias were dissected clean of all muscle, stored in 70% ethanol, dehydrated, and processed for MMA embedding. Transverse sections were cut from the location of peak bone formation (5 mm proximal to the TFJ). Sections were imaged on a Leica confocal with a 10x objective and analyzed using Bioquant for mineralizing surface, mineral apposition rate, and bone formation rate (lamellar and woven bone). Relative values were calculated as loaded limb (RT) – non-loaded limb (LT). **Statistics**. Results were analyzed in GraphPad using ANOVAs to investigate (mouse) strain, sex, and loading effects; significance was defined as p<0.05.

RESULTS: Preliminary data has been collected for 81-136 mice (n = 136 uCT, n = 81 DH) spanning the eight mouse strains. For bone area, total area, pMOI, and cortical thickness, the change over time has a significant strain-loading interaction for both males and females, indicating the amount of formed bone (loading effect) depends on mouse strain. Additionally, the loading-induced change in all morphology parameters is significantly different between the strains via two-factor ANOVA. Specifically, 129S1 show the largest loading induced changes for all 5 morphology parameters. Tissue mineral density is not affected by loading. Since all mice are skeletally mature, little change is expected in the non-loaded limb over time. However, the non-loaded, left tibia of WSB mice all have increases in bone size leading to an insignificant loading response. Time-lapse microCT shows significant strain differences for formed and resorbed bone volumes on the periosteal and endocortical surfaces. Overall, loading induces bone formation and reduces bone resorption. 129S1 mice have the most newly formed bone on both the periosteal and endocortical surfaces. CAST males have the most dramatic reduction of bone resorption on the periosteal surface. On the endocortical surface, NZO female mice have increased bone resorption in response to loading, indicating an increase in bone remodeling. The relative bone formation rate (rBFR) is significantly different between mouse strains in a sex dependent manner. 129S1 have the highest rBFR on both bone surfaces, matching the results from time-lapse microCT.

DISCUSSION: We mechanically loaded mice from eight genetically diverse strains to assess how loading induced bone formation is genetically regulated. The response to loading was investigated using bulk microCT, image-registered microCT, and dynamic histomorphometry, allowing the quantification of gross geometric changes, formed and resorbed volumes, and osteoblast number and activity, respectively. Loading induces bone formation and reduces bone resorption in a mouse strain dependent manner. 129S1 mice add the most bone due to robust formation on both the endocortical and periosteal surfaces. A/J mice, although similar in size to B6 and 129S1, do not anabolically respond to a loading stimulus of 1500με. The wild-derived mice respond moderately with PWK females having the most robust response.

SIGNIFICANCE/CLINICAL RELEVANCE: Knowing the response to mechanical stimulation is influenced by genetics motivates future studies to uncover what variation in the genome, and therefore specific genes, are responsible for modulating the anabolic response to loading. This could provide targets for therapeutics to increase bone formation in low bone mass diseases such as osteoporosis or provide genetic screening markers to predict how well a patient will respond to exercise intended to increase bone mass.