Summer Opportunities in Anatomy Research

Virtual Poster Session
July 16, 2021

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12:05 PM	**Craniofacial Bone Mineral Density at Muscle Attachment Sites in Mice with Osteogenesis Imperfecta (OI)** Lauren Murabito University of Arkansas
12:15 PM	**An *In Silico* Method for Modeling the Nasal Cycle in 3D** Baonhu Tran University of Texas at Arlington
12:25 PM	**Student Attitudes Surrounding Active Learning in an Online Anatomy Class** Lauren Mitchell Kennesaw State University
12:35 PM	**Musculoskeletal Differences Between Amputated and Non-Amputated Lower Limbs** Caitlyn Finnerty The College of New Jersey
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Craniofacial Bone Mineral Density at Muscle Attachment Sites in Mice with Osteogenesis Imperfecta

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Osteogenesis imperfecta (OI) is a genetic disease commonly caused by an acquired or spontaneous mutation in the COL1A1 or COL1A2 gene. This mutation affects the production of type I collagen, a major component of bone that determines the toughness of bone, or its resistance to fracture. Thus, patients with OI present with brittle bones and higher rates of skeletal fractures. Additionally, defects in the collagen framework of developing bones impair their biomineralization. While the relationships among collagen defects, bone mineral density (BMD), and biomechanical loading have been investigated in long bones, little is known about these relationships in the skull. Previous work in our lab has investigated BMD at cranial locations which experience compressive forces during feeding, such as the jaw joint and alveolar bone adjacent to teeth. Muscles also contribute to cranial growth through the tensile forces they exert on bone during contraction, but BMD at muscle attachment sites in OI has not yet been investigated. The aim of this study is to quantify the BMD in a mouse model of OI, with a focus on skeletal sites associated with cranial musculature. We hypothesize that the mice with OI will have lower BMDs in skeletal areas associated with feeding muscles compared to their unaffected littermates.

To test our hypothesis, we used the homozygous recessive OI murine (OIM) model. This mouse has a COL1A2 mutation and exhibits a severe phenotype similar to type III OI. Adult (16 weeks old) OIM mice and wildtype (WT) littermates were CT scanned, and BMD was measured using the Bruker CTAnalyzer software. BMD was measured in 7 regions of interest encompassing muscle attachment sites on the mandible, zygomatic arch, and parietal bone. For each ROI, BMD values were compared between genotypes by a Kruskal-Wallis test.

We found that the OI mice had significantly lower BMD at the coronoid process ($p=0.007$), parietal bone ($p=0.031$), and posterior mylohyoid line ($p=0.007$) than WT mice. No significant differences were found in BMD at the gonial angle or along the zygomatic arch. This suggests a disproportionate weakness of the temporalis and mylohyoid muscles in mice with OI, but not in the masseter muscle. While type I collagen defects impact the structural organization of skeletal muscle, it is possible that masseter muscle function is preserved in OIM mice because of its key roles in rodent feeding and postural control of the jaw. The imbalance between temporalis and masseter muscles may contribute to the prevalence of dental malocclusions in type III OI.

By studying the interactions between muscle and bone in the skull, we will better understand the biomechanical forces that influence both normal and abnormal skull growth. This knowledge can be used to inform oro-motor therapies to improve feeding function, muscle strength, and facial bone quality for patients with OI. Future work will focus on longitudinal changes in muscle attachment sites in the mouse model of OI, as well as investigating structural and functional changes to the muscles themselves.

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An *In Silico* Method for Modeling the Nasal Cycle in 3D

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The nasal cycle refers to the reciprocal pattern of alternating mucosal congestion and decongestion between the left and right nasal passages. As the nasal mucosa plays a major role in respiratory air-conditioning (heat and moisture exchange), it has been hypothesized that the nasal cycle provides a physiological mechanism that permits the congested side of the nose to temporarily recuperate while the opposite, decongested side meets functional demands for heat and moisture transfer. Yet, the inability to anatomically model varying levels of mucosal congestion has greatly limited testing of this hypothesis. Accordingly, the goal of this study was to develop an *in silico* method for accurately modeling the three-dimensional (3D) anatomy of the nasal airways with variable levels of mucosal congestion. A computed tomography (CT) scan of one male human head was selected for model generation. At the time of the CT scan, this individual displayed marked asymmetry between the left (L) and right (R) passages, with the left passage approximately 90% congested compared to only 10% on the right side (i.e., L/R = 90/10) based on our visual estimations. Using the Amira-Avizo software package, a protocol was then developed to permit controlled congestion and decongestion of the nasal mucosa. Three anatomical models were then rendered: a baseline model reflecting the asymmetrical (L/R = 90/10) airway occlusion at the time of CT; a decongested model completely lacking congestion bilaterally (L/R = 0/0); and a mid-cycle model in which airway occlusion was modeled at 50% on both sides (L/R = 50/50). Surface areas and volumes were collected for the left and right nasal airways of each model to calculate the surface area-to-volume ratios (SA/V), which serves as a measure for airway occlusion. Following theoretical expectations, the decongested model exhibited the lowest SA/V ratio (0.57) reflecting the large amount of airway volume relative to the mucosal surface area. In contrast, the SA/V ratios of the mid-cycle (0.72) and baseline (0.74) models were found to be comparably higher, reflecting a reduction in the overall airway volume of the nasal passages due to increased mucosal congestion. While these latter two models were found to have similar overall SA/V ratios, separate analyses of the left and right passages predictably demonstrated considerable differences, with the highly congested left nasal passage of the baseline model demonstrating a much higher SA/V (1.06) compared to the same passage in the mid-cycle model (0.84). These results conform to predications that, despite morphological asymmetry during the nasal cycle, the overall air-conditioning capacity of the nose remains relatively stable through the cycling process. The general concordance of our results with theoretical expectations suggests that the methodology developed for this project permits reliable *in silico* modeling of the nasal cycle. Accordingly, our study provides a valuable methodological contribution, allowing future studies to account for variation in nasal congestion while investigating the role of nasal morphology on respiratory airflow (using computational fluid dynamics analysis, etc.).

Support or Funding Information

Funding was provided by the American Association for Anatomy Innovation Program.

Student Attitudes Surrounding Active Learning in an Online Anatomy Class

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Anatomy classes can be challenging for students due in part to the nature of anatomy course material, in that students are required to learn a large amount of information in a short amount of time. Students who have not identified their study style or have yet to take classes that are materially-intensive may perform poorly. Additionally, when anatomy courses are presented online, students' learning can be impacted further due to the lack of access to typical in-class learning experiences such as peer interaction and dissection. To address these challenges, this study introduces an active learning technique to supplement peer-to-peer learning in an online, graduate anatomy course. We introduced an active learning technique called Jigsaw into an online anatomy course in the Spring of 2021. A previous study had evaluated Jigsaw within an in-person classroom environment with positive outcomes. The present study included 72 students who were split into 3 expert groups with 24 students per group. Two students from each expert group were assigned to one of 12 teaching groups, 6 students per group with 2 from each expert group A, B, and C. During Jigsaw, students were split up into expert groups in which they were expected to master a specific topic assigned to them. Students from the expert groups were then put into teaching groups, where each student would teach the other students about the material mastered in their expert group material. Students' attitudes surrounding Jigsaw were then evaluated using post-unit surveys and a final post-course survey. Results from the post-course survey indicated that 77.3% of students were either somewhat satisfied or very satisfied when asked about their satisfaction with Jigsaw. Understanding students' attitudes surrounding active learning techniques in the classroom can inform future anatomy educators as well as other instructors on assisting students in their learning process, especially in material intensive courses such as anatomy and as online learning becomes more commonplace.

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Musculoskeletal Differences Between Amputated and Non-Amputated Lower Limbs

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People with lower-limb amputations frequently experience greater risks of falls and overuse injuries. The forces acting on the human body during walking help to develop and maintain the shape, volume, and strength of musculoskeletal tissues. Conversely, altered walking patterns following limb loss may lead to tissue atrophy or degradation. Reductions in joint spaces are indicative of excess stress placed on the limb, which may lead to osteoarthritis. Bone loss in high stress regions like the femoral neck and shaft can reduce the bone's ability to resist compressive or rotational movements, making the bone more susceptible to fracture. The aim of this study was to measure gross and cross-sectional musculoskeletal differences between an individual's residual limb and intact limb to identify structures vulnerable to injury. We hypothesized that the residual limb, compared to the intact limb, would show reduced hip and knee joint spaces, cross-sectional bone properties, moment of inertia, and muscle and fat tissue area.

Computerized tomography (CT) scans of 10 males (42-79 years) were obtained from the New Mexico Descendant Image Database (NMDID). 3D Slicer software was used to measure gross skeletal properties, hip and knee joint dimensions, and cross-sectional muscle and fat tissue areas. BoneJ software was used to quantify cortical bone thickness and moment of inertia (a proxy for resistance to torsion and deformation) at the proximal, middle, and distal femoral shaft. A Wilcoxon Signed-Rank test was used to assess the differences between residual and intact limbs. The significance level was set at $\alpha \leq 0.10$ due to a small sample size.

Femoral neck width ($p = 0.077$) and muscle tissue area ($p = 0.010$) were significantly lower in the residual limb compared to the intact limb. Maximum cortical bone thickness ($p = 0.046$) and standard deviation cortical bone thickness ($p = 0.022$) were significantly lower in the residual limb in the distal femoral shaft compared to intact limb.

Loading inequalities between the residual and intact limb are likely to contribute to the observed skeletal and muscular differences. This suggests that following amputations, residual limbs are at risk of fracture in both the femoral neck and distal diaphysis regions. Also, muscle tissue area was significantly reduced in the residual limb, indicating muscle atrophy. There were no significant differences in hip or knee joint spaces between the residual and intact limbs, possibly due to our limited sample size.

A better understanding of the musculoskeletal properties associated with fall risks and overuse injuries could reduce the likelihood of experiencing these risks in populations with lower-limb loss. Additionally, both bone health and risk of lower-limb amputation are associated with metabolic disorders such as type II diabetes. Further research investigating musculoskeletal properties in individuals with and without diabetes will provide insight into the interactions between diabetes and musculoskeletal health subsequent to limb loss.

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Osteogenesis Imperfecta and The Middle Ear Ossicles

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Introduction: Osteogenesis Imperfecta (OI) is a disease that affects 20,000 people in the US. It is caused by a genetic mutation in one of four genes encoding the three alpha-helices that makeup collagen.
Many of the human body structures and systems involve collagen and bones in some way. In thehead alone, research can be focused on the teeth/dentine, nasal, and skull—the premise of my research focuses on the ears. Current literature on OI and its effects on the ear is scarce, but of the research at present, patients with OI are found to have varying forms of hearing loss, from conducive to sensorineural to mixed, all worsening with age. Furthermore, current research indicates structural abnormalities in the ear of OI patients, including thin, fragile ossicular chain,fixation of a thickened or obliterated footplate, and/or thickened hyper vascularized mucosa. As aforementioned, a gap in knowledge on the topic exists yet current OI research on hearing suggests that there are effects in humans. However, what these effects are have not been investigated in detail or in a large sample size. My study is looking at these affects in a large, neonatal sample of mice bred to have OI in order to document these affects. I hypothesize that there are significant differences between the ear bones in adult mice that are wild type (normal pathology) and those with OI, which would entail impaired hearing with OI.

Method Used: The skulls of 25 adult mice were scanned using high resolution CT technology, 12 with OI, and 13 wild-type mice. The imaging software Fiji was used to isolate only the skull scans that include the middle ear and crop down the skull only to the middle ear. The program 3D Slicer was used to extract 3D surfaces of the middle ear ossicles from the CT scans, post-modificationvia Fiji. Differences between the OI mice and wild-type mice were then observed. The 3D models, and subsequent observations, were limited to the resolution of the scans.

Results: Preliminary observations show that OI mice have more ossification and distortion of structure as evident by spike outgrowths on the leg and head of the malleus as well as the leg of the incus.
These differences are not profound but found on multiple scans between OI and wild-type mice.In both the OI and wild-type mice, the stapes and stapedial footplate could not be located.

Conclusions: The hypothesis is tentatively supported; however, the resolution of scans needed to be higher to really examine the morphology. Current research literature indicates that the stapedial footplateof the stapes fuses with the oval window causing conductive hearing loss. Higher resolution scans will allow future research to investigate the stapes differences between OI and wild-type mice. Future studies should also include analysis and measurement tools to be implemented to elucidate the difference between wild-type and OI mice to either support the hypothesis or alternatively weaken it.

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