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Regional Patterns of Vascularity within the Limb Bones of Small and Large-bodied Bats

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Honors Project Proposal for Logan Usher

Introduction

Bats are the only mammalian animal to achieve powered active flight by flapping. This mode of locomotion has resulted in the development of unique adaptations, such as the modification of the hand to long, relatively flexible wing bones (Swartz et al, 1992, Swartz et al, 2007). Bats also have humeri and femora that are poorly vascularized or avascular compared to birds, with only larger bats typically displaying vascular canals (Pratt, 2018). Vascular canals provide passageways for blood vessels and nerves, which help maintain bone homeostasis, and support bones by resisting torsional stresses placed on them. The presence of a greater number of canals in large bats suggests that they may have a larger need for the support of nutrient and waste exchange than their dense lacunar-canalicular system can provide on its own. For example, Pratt (2016) examined bone microstructure of rat and hawk bones using synchrotron-based micro-Computed Tomography (SR micro-CT) and determined that this imaging modality can be used to non-destructively analyze bone (Pratt, 2016). Pratt (2018) further assessed vascular canals in birds and bats and found that bird bone architecture is significantly more laminar than bats, and that bats are often poorly vascularized. Cooper and Ball (2016) additionally examined the role of osteoprogenitor cells in bat bones, studying their skeletal support systems (Ball, et al., 2016).

Goals and Objectives

Previous studies, including those mentioned above, have documented greater vascularity in large bodied bats, but no in-depth investigation has been performed on vascular canal regionalization as it has been difficult to reconstruct the 3D structure of vascular canals. As such, the focus of the current project will be to analyze SR micro-CT data from various bat humeri and femora obtained by Pratt in order to examine differences between the patterns of vascularity within bones of small and large bodied bats. The potential benefit of this project is an increased understanding of the vascular networks of bats and how it may support bone homeostasis. The primary research objective is to determine whether small bodied bats and large bodied bats have similar regional patterns of vascularity in their humeri and/or femora. It is hypothesized that: 1) large bodied bats will possess greater vascularity than small bodied bats, and 2) humeri will be more highly vascularized than femora.

Methodology

The data being analyzed were obtained by Dr. Isaac V. Pratt, using phase-contrast enhanced synchrotron micro-Computed Tomography (SR micro-CT) to image bird and bat bone specimens (Pratt, 2018). SR micro-CT imaging was performed at the BioMedical Imaging and Therapy beamlines (BMIT) which are part of the Canadian Light Source synchrotron facility (Pratt, 2018). Both bend magnet and insertion device beamlines were used depending on availability of the beamlines (Pratt, 2018). Scans were reconstructed using the software program NRecon (Bruker microCT, Kontich, Belgium; Pratt, 2016). Image analyses will be performed using CTAnalyser version 1.18.4.0+ (CTAn; Bruker microCT) to assess several parameters

regarding bone vascularity. The parameters to be analyzed include: tissue volume (μm^3), canal volume (μm^3), canal surface area (μm^2), cortical porosity (%), canal surface to tissue volume ($1/\mu m$), canal diameter (μm), canal separation (μm), and canal number (#). Once the raw data have been obtained, statistical analyses will be conducted using JMP Pro version 14.1.0 (SAS Institute Inc., Cary, NC, 1989-2019). ANOVA tests will compare the averages of the above parameters from megabats and microbats as well as their femora and humeri to determine if there are any significant differences between each parameter, or a combination of them.

Tentative Timeline

Projected Completion Date	Description of Tasks
8/30/19	Compile data and analyze using CTAn
9/6/19	Finish initial analysis of data
9/13/19	Statistically analyze data using an ANOVA in JMP
9/20/19	Write initial results
9/27/19	Write introduction
10/4/19	Write methods and materials
10/11/19	Edit results, introduction, methods and materials
10/18/19	Continue edits of paper
10/25/19	Write discussion
11/1/19	Edit discussion
11/8/19	Begin writing poster, continue edits of paper

11/15/19	Finish poster and paper edits
11/22/19	Edit poster and send paper and poster to readers for review
11/29/19	Finalize paper and poster
12/6/19	Final paper submitted / poster presentation finished

I will further be assisting with additional duties in the Andronowski Lab including bone specimen cleaning and preparation for imaging, and cadaver dissections for the Human Anatomy for Biology Majors course.

Outcomes

The projected outcome of this project will be to provide further information regarding the vascular system and bone structure of bats. This information will build upon current knowledge of bone remodeling and bat physiology. If a greater number of canals are found in large bats compared to small bats, it suggests a need to support canaliculi exchanging waste and nutrients. If there are more canals found in humeri than femora, it suggests that the bone is responding to the torsional loads of flight. This knowledge may be further used to compare the bone microarchitectural support structures between bats and birds. Future comparisons between bat and bird bones may allow for a better understanding of the varying forces and strains powered flight has on bones and how various animals have adapted to them.

Academic Impact / Objectives

The objective of this project is to write a scientific journal article, as well as presenting a poster for the Honors college. Continuing work in the Andronowski Lab under Dr. Andronowski's supervision will focus on assisting the cadaver dissections for the Human Anatomy for Biology Majors class. The objectives are to assist with dissection and ensure the cadaveric structures are prepared for presentation to the class, as well as recording what work has been done. This aspect is motivated by my previous experiences with the class and will provide me with an even deeper understanding of the spatial relationships of structures within the body, and knowledge of anatomy.

The Honors Research Project will primarily build upon my knowledge in the field of biological research as well as my knowledge in human anatomy. This project will further develop skills associated with laboratory techniques and procedures, from computer image processing and analysis, to data analysis and statistics. The opportunity to assist in writing and publishing an article in a scientific journal, most likely the Journal of Anatomy, adds greatly to my undergraduate experience as scientific publication is not something many undergraduates get a chance to work with, as well as building upon other research opportunities I have had through the Tiered Mentoring program. The cadaver component adds to my experience with human tissue and anatomy, as well as prepares me for any further work with cadavers or live humans in research and/or clinical settings. Very few undergraduates get to have experience learning from human cadavers, and this project will further the incredible opportunity of working with a cadaver. Both experiences envelop my experience in the Biology program, as they both rely on information I have gained from many different classes and papers read and written for class or research. Both further rely on information gained to properly analyze, write and perform the

tasks at hand and understand the impact and influence of the knowledge gained. From the curriculum and outcomes, it is expected I will learn and apply information on bone anatomy and physiology, anatomy and comparative methods between similar groups.

References

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