

Project Description

1) **Title:** Skeletal Muscle Development in the *Sus* Feeding & Locomotor Systems

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LOCATION: NEOMED,

2) **Abstract:** This study utilizes a well-established precocial animal model it is possible to investigate aspects of newborn physiology, specifically changes in the musculoskeletal system from birth to adulthood. Currently, changes occurring at the tissue level of locomotor and feeding system skeletal muscle during ontogeny are not fully understood. This includes aspects of developmental plasticity and the extent to which genetic and environmental factors determine muscle fiber type. To increase our understanding of the changes which occur in different systems of skeletal muscle this study established the muscle fiber-type proportions of both the feeding and locomotor systems at three points of development (birth, pre-weaning, post-weaning) in a precocial species (pigs). This will be done through standard histological staining of muscle tissues (NADH-TR enzyme staining), and immunohistochemistry to isolate and count Type-I, Type-IIa, and Type-IIb muscle fibers. We hypothesize that the two systems will show similarities in fiber-type proportions at birth, but that the feeding system will show a unique and distinct maturation pattern, influenced by the changes which occur at weaning as the animals move from suckling to mastication. We expect that our results will add a critical understanding to the maturation of skeletal muscle, showing that as new ‘programs’ (mastication) are onboarded through development, muscle must subsequently respond to new functional requirements by adapting its fiber-type proportion to new demands.

3) **Significance of the Research:** Two muscle systems exist within the vertebrate body plan, which offer valuable comparisons and insights into the development of skeletal muscle: the feeding and locomotor systems. In precocial mammals these two systems are already functional at birth but continue to mature postnatally. Given their different origins and functions, comparison between feeding and locomotive skeletal muscle offers interesting and yet to be investigated insight into the way in which muscle develops during ontogeny. It also allows for investigation of how muscle growth in these two systems may be related to changing functional needs (i.e., weaning).

Muscles responsible for jaw movement, tongue mobility, and esophageal activity act together to facilitate chewing, swallowing, and food transport through the digestive tract, beginning with the initial intake of food. These structures must be developed sufficiently at birth to allow for suckling and swallowing, and they must undergo coordinated development for the subsequent shift to chewing and swallowing of solid foods. Non-nutritive sucking and swallowing begin *in utero* around 15 weeks of gestation with continuing refinement from this time. After birth, efficiency improves throughout neonatal life due to further development of the oral and pharyngeal structures and refinement of suckling and oral coordination. The masticatory muscles must also sufficiently mature in size and sensorimotor coordination to meet new functional demands exerted by weaning for the shift to textured, solid foods. However, the specific changes happening at the tissue level of these muscles to facilitate these refinements is unclear.

The locomotor system consists of skeletal muscles which enable movement, weight-bearing, stability, and coordination. Like feeding, this system must already be functional at birth in precocial mammals. As the individual develops, refinements occur to ensure successful locomotion over a range of different conditions, whilst maintaining consistent equilibrium with proper balance and postural control.

This study will bridge gaps in our understanding of how changes in a systems' needs, in this case weaning and the shift to mastication of solid foods, affect the underlying architecture of the systems components. By comparing fiber-type proportions it is our intention to show how refinement versus establishment of new biological programs are represented at an architectural level. The study age groups are aimed to be representative of time points covering a major milestone in the feeding system, acting as a lens through which to see how new programming is adopted by skeletal muscle.

Our hope is that this study will further the understanding of how musculoskeletal systems develop and change through ontogeny, when it is imperative that the organism's tissues must be able to grow whilst maintaining existing, and onboarding new, functional properties.

4) Goals and Objectives of the Research: By comparing the skeletal muscle fiber-type proportions across muscles of the feeding and locomotor systems in a precocial species, our data will elucidate whether the feeding system must go through distinct fiber-type shifts as mastication comes online, becoming the primary mode of nutrient acquisition.

5) Research Methods Learned by the Summer Fellow: The researcher will be trained to assist in all phases of the research project, including specimen preparation and analysis. The student will gain experience in basic histology and immunohistochemistry methods, including tissue preparation, staining skeletal muscle, differentiating and counting muscle fiber types, and analyzing data.

6) Research Methods and Data Analysis: Skeletal muscle tissue from the feeding system (Masseter, Temporalis, Digastric, Sternohyoid, Thyrohyoid, Genioglossus, Geniohyoid, Mylohyoid, & Hyoglossus) and locomotor system (Long & Medial Heads of Triceps brachii, Gastrocnemius, & Soleus) are frozen in isopentane. Muscle tissue will be cut into 10 μ m sections using a cryostat, stained, and counted for specific fiber-type cells. NADH-TR stain will be used to stain all muscles, providing a generalized overview of Type-I and Type-II muscle fibers. Antibodies specific to Type-I, Type-IIa, and Type-IIb myosin's will stain the more specific Type-II fibers. These protocols are already established. Using bright field microscopy, images will be taken of the tissue sections, and we will count the number of each fiber-type cell. Comparisons will then be made of the fiber-type proportions between the two systems, as well as within the different muscles of the feeding system as they pertain to suckling, mastication, and swallowing.

7) Expected Outcomes: Several possible outcomes are anticipated for this study. Our null hypothesis is that fiber-type proportions will follow the same maturation pattern in both the feeding and locomotor systems. If the feeding system shows a different profile compared to the locomotor system, it is likely due to the onboarding of a new system program (mastication) at

weaning. Our findings, regardless of outcome, will lay the foundation for future work quantifying fiber-type proportions across the two systems throughout development.

Student Fellow Training/Mentoring Plan: Funding is requested to support one summer research fellow. PI Young is committed to fostering the researcher's development for the summer. This goal will be achieved through a structured mentoring program, as described below.

Research will be conducted in Young's lab using frozen muscle tissue which is ready for analysis. Protocols are already established, and all necessary laboratory equipment and disposables are already in use as this is an ongoing project.

Besides benefiting from working alongside the PI, the student will be required to attend and present once at the weekly MSRFA journal club. The Musculoskeletal Research Focus Area sponsors a weekly journal club on the general topic of "Evolutionary Morphology", where the fellow would have the opportunity to share and discuss ongoing research findings and pertinent scientific publications. Finally, the student will design and present a poster for the end-of-program poster symposium at NEOMED.