Muscle Fiber-typing of the *tibialis anterior* in Offspring of Maternal Overnutrition Mice

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Introduction

The *in utero* environment has a significant impact on the growing fetus' physiology. For example, mice of maternal undernutrition demonstrate low birth weight and are predisposed to metabolic syndrome later in life[1]. Additionally, the offspring of maternal undernutrition exhibit differences in their muscle fiber-type composition, but only when they are food restricted[1].

We have previously shown that mitochondrial dysfunction links undernutrition *in-utero* with metabolic disease in adulthood. We are now evaluating the effect of maternal overnutrition on disease in the offspring. We are using a model which has been previously characterized by Samuelsson *et al.* where the mothers are fed a high fat, high sucrose (HFHS) diet[2]. One aspect of this project is to investigate muscle fiber-type in the offspring to determine if there are underlying differences in skeletal muscle respiration. This fiber-typing may potentially lead to the discovery of the physiological mechanism causing this altered mitochondrial respiration.

Methodology

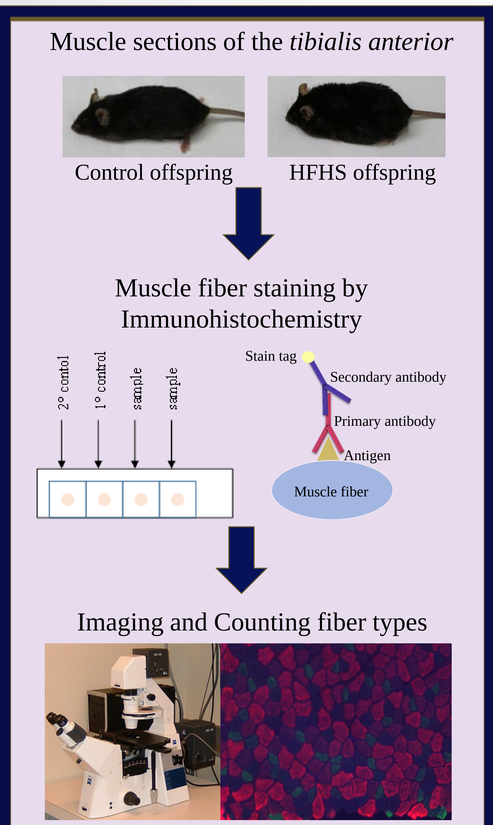

Figure 1. *Tibialis anterior* staining and analysis. Sections of muscle of both control and HFHS offspring were taken and stained by immunohistochemistry on slides. Antibodies specific to each fiber type were used. Images were taken on Zeiss Axio Observer microscope and analyzed with ImageJ.

Results

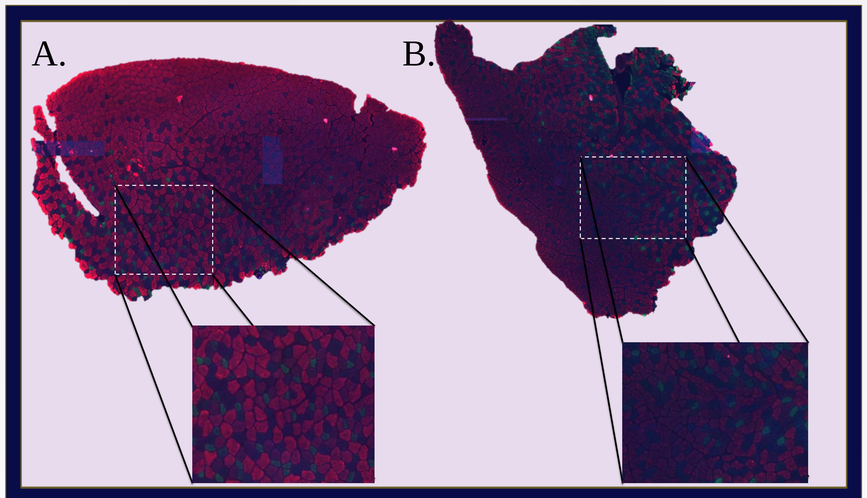

Figure 2. Stained *tibialis anterior* cross-sections. Blue stain=type I fiber; green=type IIa fiber; red=type IIb fiber; unstained=type IIx fiber. **A)** *tibialis anterior* cross section of control group offspring. **B)** *tibialis anterior* cross section of maternal high fat/high sucrose overnutrition (HFHS) group offspring.

Frozen sections of the muscle were prepared, and analysis of muscle fiber-type was conducted by immunohistochemistry. Stained tissue sections were imaged on a Zeiss Axio Observer microscope, and quantification of fiber-type carried out using ImageJ (Figure 1 & 2).

The staining revealed that the major muscle fiber type in the *tibialis anterior* was typeIIb, the fast glycolytic type, along with some typeIIa, the fast glycolytic fibers and typeIIx, the unstained fibers. the data was analyzed using Graphpad it was found that there was no difference between maternal over-nutrition offspring and the control offspring in the muscle fiber-type composition of the *tibialis anterior* (Figure 3).

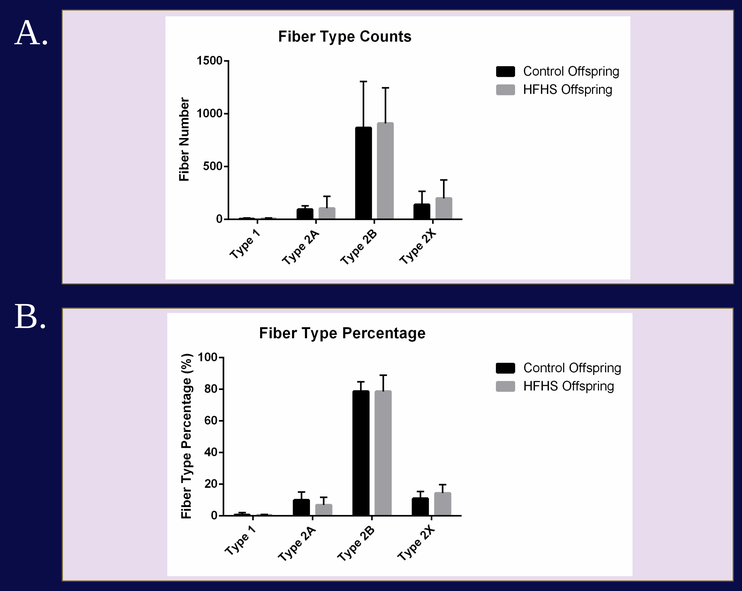

Figure 3. Analysis of counted stained cross sections of muscle. **A)** There is no significant difference in the individual fiber type counts between the control and HFHS offspring. **B)** There is no significant difference in the percentage of fiber types between the control and HFHS offspring.

Conclusion

We found no difference in composition of muscle fiber-types between the mice of maternal overnutrition and the control group. As we have only looked at one muscle (*tibialis anterior*), it remains a possibility that other muscles may have differences in fiber type composition. It is also possible that there is another more complex underlying physiological mechanism that could explain the maternal overnutrition offspring's altered mitochondrial respiration that has been observed.

We were able to demonstrate that the major muscle fiber-type in the *tibialis anterior* was type IIb, which is consistent with the literature[3].

References

1. Beauchamp B *et al*. (2015). Low birth weight is associated with adiposity, impaired skeletal muscle energetics, and weight loss resistance in mice. *International Journal of Obesity*, 39(4), 702-711.
2. Samuelsson A *et al*. (2007). Diet-Induced Obesity in Female Mice Leads to Offspring Hyperphagia, Adiposity, Hypertension, and Insulin Resistance: A Novel Murine Model of Developmental Programming. *Hypertension*, 51(2), 383-392.
3. Augusto V *et al*. (2004). Skeletal Muscle Fiber Types in C57BL6J Mice. *Brazilian Journal of Morphological Sciences*, 21(2), 89-94.

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