Effects of Maternal Exercise on Embryonic Bone Development

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Introduction: Mechanical Stimulation is Essential for Fetal Development

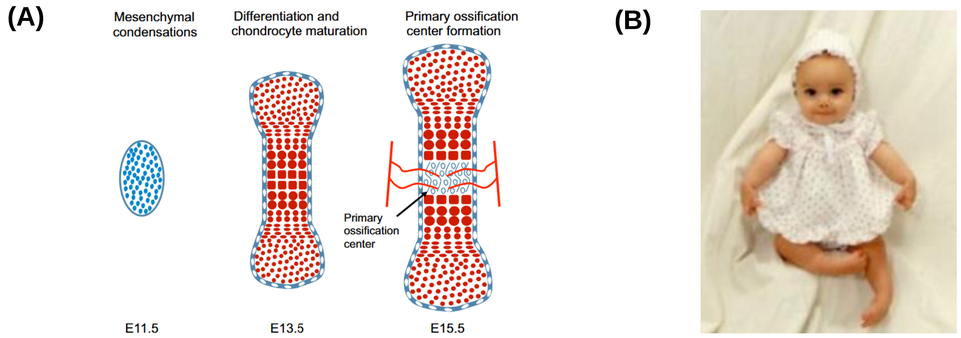

Fig. 1. Endochondral ossification is a critical process for fetal long bone development. Fetuses that lack mechanical stimulation, termed fetal akinesia, have impaired bone growth and skeletal deformities. **(A)** Process of endochondral ossification in embryonic mice [1]. **(B)** Child born with amyoplasia, associated with a lack of muscular development, caused by fetal akinesia [2].

<u>Objective</u> Determine the effects of supraphysiological mechanical stimulation by maternal exercise on embryonic skeletal development

Methods: Maternal exercise to provide extra-embryonic mechanical stimulation

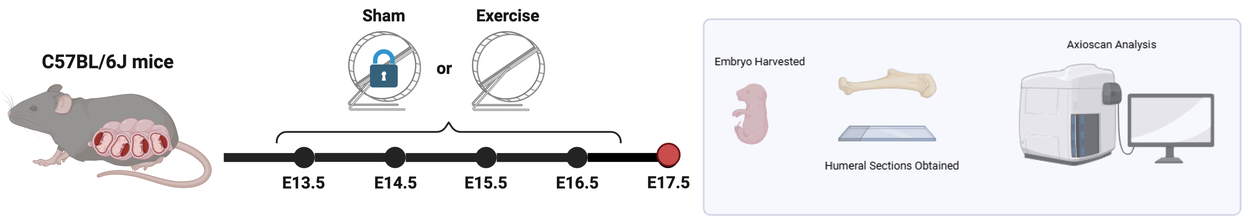

Fig. 2. Experimental design schematic. Female C57BL/6J mice were given at least 2 weeks to acclimate to home cage running wheels before being mated and then housed without wheels until embryonic day (E) 13.5, the initiation of cartilage template formation. Pregnant mice received *in utero* mechanical stimulation through encouraged wheel running exercise. Embryos were harvested at E17.5 for histological analysis.

- **Encouraged Wheel Running:** Pregnant mice received in utero mechanical stimulation through encouraged wheel running exercise for one hour daily from E13.5 to E16.5 (Exercise). Control mice (Sham) were subjected to a locked wheel for the same time period.
- **Histology:** Forelimb samples were collected from the E17.5 embryos and fixed for cryohistology. Sections were stained for collagen 10, phalloidin, and alkaline phosphatase.
- **Statistical analysis:** Student's t-tests were used to find significant differences between groups, and the Kolmogorov Smirnov test was used to find significant differences between distributions (α = 0.05).

Results: Effects of maternal exercise on length of hypertrophic zone

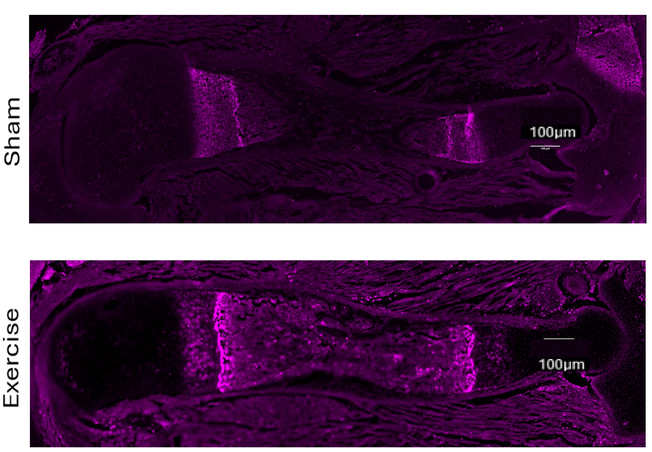

Fig. 3. Exercise results in decreased length of the hypertrophic zone. *Staining for COL10A1 is shown for Sham and Exercise groups. Quantification of distal hypertrophic zone length shows significantly lowered lengths for the Exercise group. This is most likely an indicator of more advanced bone growth in the exercise group. N = 7 embryos per group*

Results: Effects of maternal exercise on Phalloidin intensity

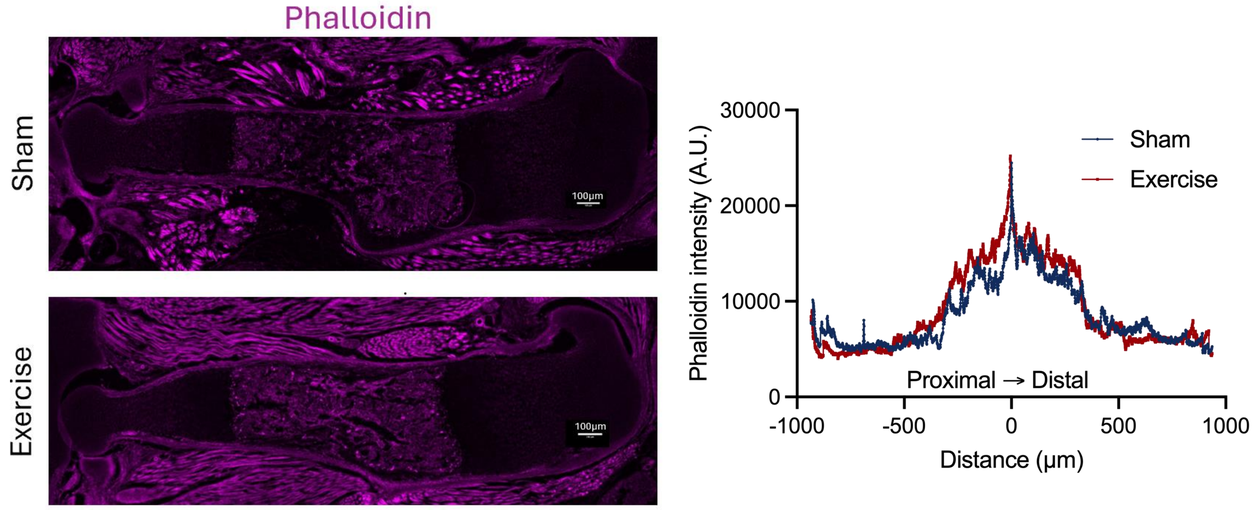

Fig. 4. Supraphysiological mechanical stimulation results in elevated Phalloidin levels. *Phalloidin intensity graph along the humerus with representative images. There is significantly heightened Phalloidin intensity in the Exercise group which indicates higher F-Actin levels and thus promoted osteogenesis. N = 8-9 embryos per group*

Results: Effects of maternal exercise on Alkaline Phosphatase Intensity

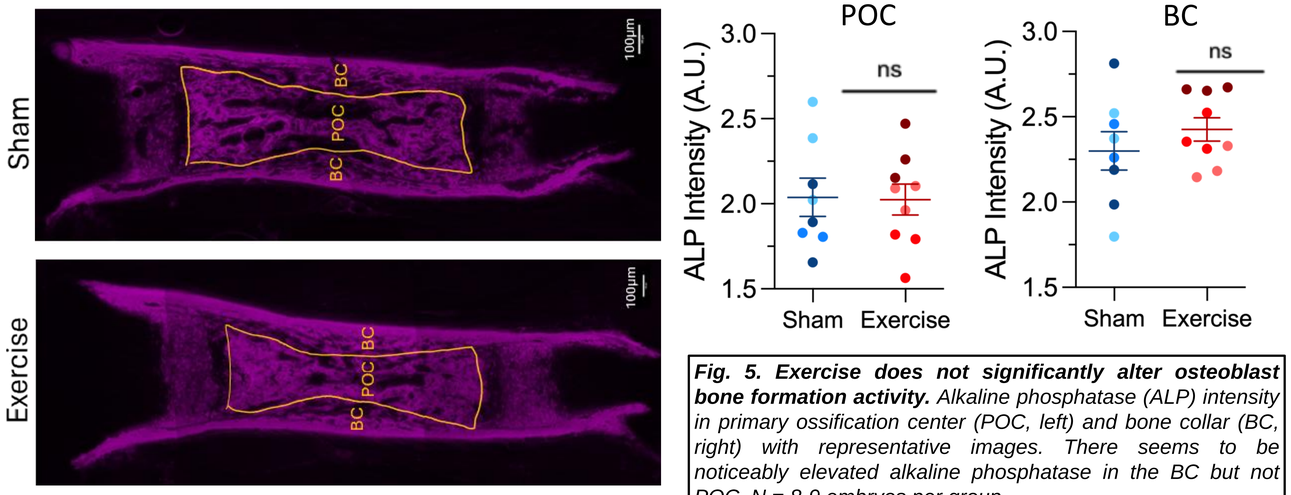

Fig. 5. Exercise does not significantly alter osteoblast bone formation activity. *Alkaline phosphatase (ALP) intensity in primary ossification center (POC, left) and bone collar (BC, right) with representative images. There seems to be noticeably elevated alkaline phosphatase in the BC but not POC. N = 8-9 embryos per group*

Discussion and Conclusion

- Further analysis with endomucin may provide additional insight into the POC since alkaline phosphatase intensity may be confounded by enhanced blood vessel infiltration, which would indicate POC maturity.
- Postnatal treatments for conditions caused by fetal akinesia are limited due to reduced morphogenic plasticity in the mature skeleton [3].
- Future studies will conduct similar histological analyses on embryos from muscular dysgenesis and Pax3$^{Spd/Spd}$ mice, which are models of fetal akinesia, to assess the therapeutic capacity of maternal exercise on fetal akinesia.

Maternal exercise enhances embryonic bone formation; thus, it could serve as a potential platform to study therapeutic effects of *in utero* mechanical stimuli during critical periods of bone formation.

References

[1] Kozhemyakina+ Development 2015; **[2]** Nowlan+ *Eur Cell Mater* 2015; **[3]** Hall+ Am J Med Genet 2021

Acknowledgements

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