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## Feeding After Overnight Fast Enhances Bone's Response To Mechanical Loading In Mice

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Concentrations of gastro-entero-pancreatic hormones change in fasting and fed states and many have potent regulatory effects on bone. We hypothesise that effect of exercise on bones depends on concentrations of these hormones and therefore both timing of eating and nature of ingested food.

To test this hypothesis we performed experiments to determine osteogenic effects of mechanical loading that mimics exercise after different periods of withholding food or feeding, where we expect altered concentrations of gut-derived hormones, using a mouse model.

Groups of 17-week-old C57BL/6 male mice (n=7) were either fasted overnight (16hrs) or allowed free access to food. Fasted mice were then given access to food for 1hr, 2hrs or 3hrs. All mice then underwent axial loading of the right tibiae with a peak force of 13N, 3 times weekly for 2 weeks (40 cycles of loading with 9sec rest periods between each). Left tibiae were non-loaded controls in each animal. Fluorochrome labels were injected at the start and end of experiments. Response of bones to loading was determined by micro-computed tomography and dynamic histomorphometry.

Loading caused significant adaptive responses in loaded right tibiae of all mice. However the change in cortical thickness induced by loading was 36% greater in animals that were fasted then fed for 2hrs than in loaded bones of animals fed ad-lib before loading (fasted fed loaded:  $0.049 \pm 0.007$ mm vs ad-lib fed loaded:  $0.036 \pm 0.004$ mm,  $p < 0.05$ ). Preliminary analysis of total BFR/BS and MAR of the tibiae of fasted then 2hrs fed mice was consistent with the microCT analysis (BFR/BS fasted fed loaded:  $3.59 \pm 1.22 \mu\text{m}^3/\mu\text{m}^2/\text{d}$  vs non-loaded control:  $0.02 \pm 0.03 \mu\text{m}^3/\mu\text{m}^2/\text{d}$ ,  $p < 0.01$ ; MAR  $4.90 \pm 1.79 \mu\text{m}/\text{d}$  vs  $0.07 \pm 0.06 \mu\text{m}/\text{d}$ ,  $p < 0.001$ ) compared with tibiae of ad-lib fed mice (BFR/BS loaded:  $1.04 \pm 0.14 \mu\text{m}^3/\mu\text{m}^2/\text{d}$  vs control:  $0.12 \pm 0.16 \mu\text{m}^3/\mu\text{m}^2/\text{d}$ ,  $p < 0.001$ ; MAR  $2.00 \pm 0.46 \mu\text{m}/\text{d}$  vs  $0.33 \pm 0.15 \mu\text{m}/\text{d}$ ,  $p < 0.001$ ). Loading after 1hr or 3hrs following overnight fast increased cortical bone thickness, but not differently from ad-lib fed mice.

Food ingestion for 2hrs after overnight fasting potentiated bone's osteogenic response to mechanical loading in mice, indicating interactions between the timing of food ingestion and bone adaptation. These findings have potential for translation into benefits for people by providing information on when to exercise in relation to meals, which may help to build and maintain healthy musculoskeletal system throughout lifecourse including in older age.

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