Fatty Acid Excess Increases Lipid Droplet Size and Modifies Gene Expression in Osteocytes

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The relationship between obesity and bone remains controversial, although recent studies suggest that obesity can confer an increased risk of osteoporosis resulting in bone fractures in skeletal sites such as the wrist. Osteocytes are the most numerous bone cells functioning as key regulators of bone formation, resorption, and renal phosphate homeostasis; therefore, they likely play an important role in obesity-induced skeletal pathology. Cells exposed to excess free fatty acids (FA) in obesity strive to detoxify the cytotoxic free FA by taking up and storing free FA into lipid droplets (LD). It is unknown how osteocytes respond to FA and how this would affect their functions.

In this study, we assessed the effects of FA overload on osteocytes using two cell lines, MLO-Y4 and IDG-SW3. We hypothesized that normal osteocyte energy metabolism is disrupted by excess FA, resulting in increased lipid storage and abnormal osteocyte function. To study this, MLO-Y4 cells were treated with palmitate to induce LD accumulation, and then stained to quantify LD. To determine palmitate's effects on osteocyte function, we performed qPCR analysis for lipid accumulation genes and osteocyte markers. To examine LD formation in IDG-SW3 mature osteocytes, LipidSpot fluorescent staining was performed; preliminary observations show LD accumulation.

Using this *in vitro* system, MLO-Y4 cells under fatty acid overload demonstrated a greater percent area and size of LDs. RT-qPCR analysis showed an increase in lipid storage genes, *Dgat2* (1.5-fold, $p<0.001$) and *Cidec* (4-fold, $p<0.05$). *Opg*, an inhibitor of osteoclast differentiation, was decreased (0.75-fold, $p<0.01$), whereas there was no change in *Rankl*, an inducer of osteoclast differentiation. *Dmp1*, an early osteocyte marker which plays a role in matrix mineralization, was increased (2-fold, $p<0.05$). This indicates that FA most likely disrupts osteocyte metabolism through increased lipid storage, which may alter osteocyte function to promote osteoclast activity leading to bone loss.