The role of iron in development and activity of osteoclasts

INTRODUCTION: Iron is an essential trace element. Membrane iron transporters are critical to maintain systemic/ cellular iron homeostasis to prevent metabolic disorders such as anemia and hemochromatosis. Recently, we described an increase in transcripts encoding Divalent Metal Transporter (DMT1), a membrane transporter for iron uptake, in osteoclastogenesis. To understand the role of iron and of DMT1 in osteoclasts (OC), we developed an *in vitro* iron uptake assay and generated osteoclast-specific DMT1-ko mice using the Cre/lox system, with Cre expressed under TRAP promoter.

METHODS: Osteoclast progenitor cells (OPC) were cultured with MCSF, RANKL ± iron chelator, DFO. After day 5, OC were harvested and incubated for up to 120 min with ^{55}Fe-Transferrin. Bone mass and structure of DMT1(OC)$^{fl/fl}$Cre+ lumbar vertebrae (L3-L5) were evaluated by microCT. OPC from DMT1(OC)$^{fl/fl}$Cre+ were cultured with MCSF and RANKL to assess the effects of DMT1 deficiency on OC proliferation and development. Levels of DMT1 transcripts were determined in mixed and homogeneous OC cultures by RT-PCR.

RESULTS: After 5 days, levels of transcripts encoding TfR1 and DMT1 were significantly increased in OC/DFO ($P < 0.0001$). Iron uptake in OC/DFO was significantly increased compared to OC w/o DFO ($P \leq 0.05$). Trabecular thickness in L5 vertebral bodies in male DMT1(OC)$^{fl/fl}$Cre+ mice was significantly decreased when compared to the Cre- control ($P \leq 0.05$). Levels of transcripts encoding DMT1 were decreased by 70% and 30% in OC derived from male and female DMT1(OC)$^{fl/fl}$Cre+ mice, respectively. Development of osteoclasts, as assessed by XTT and TRAP, was not affected by the loss in DMT1.

CONCLUSION: Iron deficiency *in vitro* induces a significant upregulation of both TfR1 and DMT1 transcripts in OC. Osteoclast-specific DMT1-ko efficiency is sex-dependent. In male DMT1(OC)$^{fl/fl}$Cre+ mice, the decrease in DMT1 transcripts leads to a decrease in trabecular thickness. However, no changes in cell viability and osteoclastogenesis were detected *in vitro*.