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The Role of Leptin in the Local Control of Fracture Healing

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Purpose: Leptin is a hormone with pleiotropic actions regulating bone formation and angiogenesis. Osteoblasts are under dual leptin-dependant regulation: central anti-osteogenic negative regulation acting via the hypothalamus and local pro-osteogenic positive regulation. We hypothesized that leptin is expressed in fracture healing and the deficiency of leptin leads to impaired healing that can be rescued by local application of either recombinant leptin or vascular endothelial-derived growth factor (VEGF).

Methods: Ten week old wild type C57BL/6 control mice and leptin knockout (*ob/ob*) mice were used. **Arm 1:** Leptin expression in normal fracture healing was determined by creating closed mid-diaphyseal femur fractures stabilized with intramedullary fixation in 56 wild type mice. Fracture callus tissue was harvested at 1, 3, 5, 7, 10, 14 and 21 days and RT-PCR analysis was performed. **Arm 2:** The effect of leptin deficiency on fracture healing was determined by an open model of stabilized mid-diaphyseal femur fracture healing. A total of 84 mice underwent fractures: 42 wild type controls and 42 *ob/ob* mice and tissues were harvested at 14, 21 and 42 days. At each time point radiographic, histologic and qCT analysis was performed. **Arm 3:** Recombinant leptin in a collagen carrier was drip applied at the fracture site in two separate groups of 42 *ob/ob* mice each: Group I used 10 µg leptin dose and Group II used 100 µg leptin dose. Tissues were harvested at 14, 21 and 42 days and radiographic, histologic and qCT analysis was performed. **Arm 4:** 2 µg of recombinant VEGF was drip applied at the fracture site in 12 *ob/ob* mice and tissue harvested for radiographic and histologic analysis at 14 and 21 days. 2-factor ANOVA and Students t-test was used for statistical analysis.

Results: Leptin mRNA was detected in callus tissue at multiple time points. Delay in callus maturation was demonstrated radiographically in the *ob/ob* mice compared to the controls. Histologically, *ob/ob* fractures demonstrated delay in callus organization with significantly greater hypertrophic chondrocytes ($p < 0.05$) compared with controls at 14 and 21 days. *ob/ob* fractures demonstrated increase in total callus height and total callus volume by qCT ($p < 0.05$). Application of local leptin at both doses reversed the delay seen in the *ob/ob* fractures radiographically and histologically at 14 and 21 days. VEGF also reversed the delay in *ob/ob* fractures.

Conclusions: Leptin is expressed locally during fracture healing. Leptin deficiency leads to a delay in fracture healing that can be reversed by local application of leptin or VEGF. We describe a hitherto unrecognized role of leptin as a potential angiogenic factor in the program of fracture healing.