

## **Cx43 Deletion in Osteocytes Results in Cortical Osteocyte Apoptosis, Periosteal Expansion and Reduced Bone Material Stiffness**

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Connexin (Cx) 43 is the most abundant gap junction protein in bone. Deletion of Cx43 exclusively from osteoblasts and osteocytes (Cx43<sup>fl<sup>ox</sup>/-</sup>;OCN-Cre mice) results in increased cortical osteocyte apoptosis (1). In the femur, this is accompanied by increased osteoclast recruitment on the endocortical side of the midshaft and the consequent enlargement of the marrow cavity area. In addition, Cx43 deletion results in a decrease in bone material stiffness (Young's modulus), a measure of the intrinsic rigidity of the material, in the femoral diaphysis (2).

To single out the contribution of Cx43 removal from osteocytes to the observed phenotype, we generated mice in which Cx43 was deleted exclusively from osteocytes. Towards this end, we mated mice producing Cre recombinase under the control of an 8kb fragment of the DMP1 promoter, that confers expression in osteocytes only, with Cx43<sup>fl<sup>ox</sup>/fl<sup>ox</sup></sup> mice, to generate Cx43<sup>fl<sup>ox</sup>/fl<sup>ox</sup></sup>;DMP1-Cre mice (named Cx43<sup>ΔOT</sup>). Cre mRNA (assessed by qPCR) and the deleted form of Cx43 (identified by PCR) were amplified in bone and not in heart or kidney from Cx43<sup>ΔOT</sup> mice, or in tissues from control Cx43<sup>fl<sup>fl</sup></sup> mice, demonstrating that Cx43<sup>ΔOT</sup> mice exhibit Cre activity only in bone. In addition, osteocyte-specific Cre expression was demonstrated by mating 8kbDMP1-Cre with 8kbDMP1-GFP mice to obtain calvaria cells containing green fluorescent protein (GFP)-expressing osteocytes. After sorting by GFP expression, Cre mRNA was detected only in GFP+ osteocytes and not GFP- osteoblasts.

In femoral mid-diaphysis, osteocyte apoptosis was remarkably higher in Cx43<sup>ΔOT</sup> mice (12.6±7.4%) compared to controls (1.1±1.4%); the percentage of empty lacunae, an additional sign of osteocyte death, was also elevated in Cx43<sup>ΔOT</sup> mice (13.1±6.9%) compared to controls (0.8±0.5%). This was associated with a 26% higher femoral marrow cavity area and 20% increase in total cross-sectional area (p < 0.05), likely the result of increased endocortical resorption and periosteal apposition, respectively.

As in Cx43<sup>fl<sup>ox</sup>/-</sup>;OCN-Cre mice, 3-point bending assays revealed a 22% lower bone material stiffness in Cx43<sup>ΔOT</sup> mice femora. In addition, Cx43<sup>ΔOT</sup> mice exhibit significantly higher circulating osteocalcin levels, whereas C-telopeptide levels, although higher, did not reach statistical significance, suggesting increased remodeling in mice lacking Cx43 in osteocytes.

In summary, Cx43 deletion exclusively from osteocytes is sufficient to induce osteocyte apoptosis, alter bone geometry and change bone material stiffness. We conclude that Cx43 expression in osteocytes is required to maintain osteocyte viability and adequate levels of bone formation and resorption. Our study also supports the notion that accumulation of apoptotic osteocytes is followed by resorption (3-5). We hypothesize that Cx43 is required in osteocytes to sense physiological survival stimuli and to orchestrate both arms of the remodeling process.

## References

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