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Dlk1/Pref-1 (delta like1/preadipocte factor-1) is an imprinted gene encoding a transmembrane protein that belongs to EGF-like repeats protein family. We have recently identified Dlk1/Pref-1 as negative regulator for differentiation of human mesenchymal stem cells into osteoblasts and adipocytes (Abdallah BM, et. al., JBMR, May,19(5):841-852, 2004). To further investigate the in vivo effect of Dlk1/Pref-1 on bone, we generated mice expressing high serum levels of FA1 (biological soluble form of Dlk1) using the hydrodynamic-based gene transfer procedure. Full length of mouse Pref-1 cDNA was subcloned under human ubiquitin promoter and rapidly injected via tail vein into BALB/cA male mice (16 weeks old, n =15) every 2 weeks over a period of 2 months. DNA, mRNA analysis, immunohistology and ELISA for FA1 were assayed to identify the expression of the transgene. Bone mass and structure were determined by PIXImus (Lunar^R) and micro-CT (Scanco^R) respectively. We could only localize the plasmid in the liver and no complications were detected due to transgene expression. Serum levels of FA1 in Dlk1 injected mice (Dlk1$^+$mice) was elevated by more than 15 folds compared to control saline injected mice (control) (198.0 ± 74.3 ng/ml vs 13.4 ± 1.1 ng/ml, $p<0.001$, respectively). Dlk1$^+$mice displayed lowered body weight and reduced total fat mass (13.5%, $p< 0.05$ and 17.8%, $p< 0.05$ respectively). Interestingly, Dlk1$^+$mice displayed (16.6 %, $p<0.005$) lowered total bone mineral density (BMD) compared to controls and BMD was negatively correlated with the circulating levels of FA1. Micro-CT analysis revealed significantly decreased micro-architectural parameters of trabecular bone in the distal femur and proximal tibia of the Dlk1$^+$mice compared to controls (see table).
Naked DNA delivery by hydrodynamic injection is a simple and safe procedure for evaluating the effect of genes on bone phenotype in vivo. Our data suggest that Dlk1/Pref-1 is a novel regulator of bone mass.

Groups (n=8)/ Measurments	BV/TV	TbTh (µm)	TbSp (µm)	TbN (1/mm)	CD (1/mm^3)
Distal femur	0.34 ±0.11	0.07±0.01	0.19±0.03	5.80±1.10	155.1±37.4
Control group					
Dlk+ group	0.19 ±0.06	0.06±0.00	0.24±0.04	4.60±0.84	113.3±37.8
Proximal Tibia	0.20±0.07	0.07 ±0.01	0.24±0.04	4.60±0.57	98.6±21.27
Control group					
Dlk+ group	0.14±0.03	0.06 ±0.01	0.25±0.02	4.26±0.42	75.5±25.7

P<0.05 for all tested parameters
TbTh , Sp ,N=trabecular thickness, space, number, CD=connectivity