In humans, high dose glucocorticoids lead to bone loss primarily by decreasing bone formation and increasing osteoblast apoptosis. Glucocorticoids produce apoptosis by down-regulating Bcl-2, a cell survival factor (1). We hypothesize that by targeted overexpression of Bcl-2 and thereby inhibiting osteoblast apoptosis, we would prevent glucocorticoid-induced decreased bone formation. To prove this hypothesis we used the Col2.3Bcl-2 transgenic mouse that overexpresses human Bcl-2 (hBcl-2) in mature osteoblasts via the 2.3 kb Col1a1 promoter fragment of Type I collagen driving 1.7 kb of human Bcl-2 (hBcl-2) cDNA. Three Col2.3Bcl-2 (CD-1 background) mouse founder lines were established and previously characterized (2). The Col2.3Bcl-2 mice and their nontransgenic littermates were treated with glucocorticoids. Bones were harvested, and analyzed by TUNEL assay for apoptosis and by histomorphometry for bone formation parameters.

Methods
Three-month old wild-type (WT) and Col2.3Bcl-2 mice were treated i.p. with 1 mg/kg body weight of dexamethasone or vehicle every other day for 21 days (8 mice/group) or 35 days (9 mice/group). To dynamically label forming bone, seven days before sacrifice, 100 mg/kg body weight of calcein was administered followed by 90 mg/kg of xylene orange 2 days before sacrifice. Femurs, vertebrae and calvaria were harvested, fixed, and embedded in methylmethacrylate or in paraffin for dynamic histomorphometry and static histomorphometry-immunocytochemistry, respectively. For histomorphometric analysis, 5-micron-thick serial sections were cut. Static and dynamic histomorphometric measurements were made in a blinded, nonbiased manner using the BioQuant computerized image analysis system (BIO-QUANT, R & M Biometrics, Nashville, TN) interfaced with a Nikon E400 microscope. The terminology and units used are those recommended by the Histomorphometry Nomenclature Committee of the American Society for Bone and Mineral Research (3). Immunocytochemical detection and quantification of apoptosis was determined by terminal deoxynuleotidyl transferase-mediated dUTP nick end labeling (TUNEL) using an In Situ Cell Death Detection Kit (Roche Diagnostics distributed by Boehringer Mannheim, Indianapolis, IN) on paraffin-embedded tissue.

Results
The percent osteoblast cells undergoing apoptosis by TUNEL was determined by counting TUNEL+ and TUNEL- osteoblasts in the trabeculae proximal to the epiphyseal growth plate and along the periosteal and endosteal surfaces of the cortical bone in 4-6 WT and Col2.3Bcl-2 mice. We observed a 77.1% decrease in apoptosis of trabecular osteoblasts, and a 52% and 55% decrease in apoptosis in the endosteal and periosteal osteoblasts, respectively, in vehicle-treated Col2.3Bcl-2 mice compared to vehicle-treated WT mice. In WT mice treated for 21 days with 1 mg/kg body weight of dexamethasone, a 12.1-fold increase in apoptotic cells was found compared to vehicle-treated WT mice. In the glucocorticoid-treated Col2.3Bcl-2 mice there was only a 4.0-fold increase in apoptotic osteoblasts compared to vehicle-treated Col2.3Bcl-2 mice. Histomorphometric analysis of femurs from WT mice treated with dexamethasone for 21d demonstrated a significant decrease in the mineralization apposition rate (MAR) (**=p<0.002) and bone formation rate (BFR) (**=p<0.01) (Table 1). We found similar results in mice treated with or without 1 mg/kg dexamethasone for 35 days. The decrease in the % trabecular bone area (BA/TTA%) was not significant with either 21 or 35 days of glucocorticoids. No decrease in MAR or BFR was found in the dexamethasone-treated Col2.3Bcl-2 mice. The expression of human Bcl-2 in the Col2.3Bcl-2 mice was able to block the glucocorticoid-induced inhibition of MAR and BFR.

Discussion: Bcl-2 appears to be an important regulator of apoptosis in osteoblasts and to affect osteoblast function and bone formation rates. Overexpression of Bcl-2 in the bones of the Col2.3Bcl-2 transgenic mice, was shown to inhibit glucocorticoid-induced apoptosis and to prevent the decrease in bone formation produced by glucocorticoids.

References