Supplemental Material

Heavy Metal Lead Exposure, Osteoporotic-like Phenotype in an Animal Model, and Depression of Wnt Signaling

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Supplemental details of Raman spectroscopy analysis: Raman spectroscopy is capable of determining the biochemical composition of bone and has been applied to study both normal and osteoporotic tissue (Akkus et al. 2004; Boivin and Meunier 2003; Carden and Morris 2000). Differences in mineral and protein content between the rat femurs were characterized by metrics related to bone biochemistry. The mineral-to-matrix ratio (MTMR; PO_4^{3-} / CH_2 peak area ratio) describes the degree of phosphate mineralization. The carbonate-to-phosphate ratio (CTPR; CO_3^{2-} / PO_4^{3-} peak area ratio) describes the amount of carbonate substitution in the hydroxyapatite crystal lattice. Collagen maturity (1660 cm^{-1} / 1690 cm^{-1} peak intensity ratio) describes the ratio of mature (pyridinoline) to immature (dehydro-dihydroxylysinonorleucine) collagen cross-links. Finally, crystallinity (inverse of the PO_4^{3-} peak width at half-max intensity) is a measurement of mineral maturity, crystallite size, and the amount of substitution into the apatitic lattice (Faibish et al. 2006; Paschalis et al. 2001). Metrics were normalized by the average value calculated among rats in the control group.

Supplemental References:

Akkus O, Adar F, Schaffler MB. 2004. Age-related changes in physicochemical properties of mineral crystals are related to impaired mechanical function of cortical bone. Bone 34(3):443-453.

Boivin G, Meunier PJ. 2003. The mineralization of bone tissue: a forgotten dimension in osteoporosis research. Osteoporos Int 14 Suppl 3:S19-24.

Carden A, Morris MD. 2000. Application of vibrational spectroscopy to the study of mineralized tissues (review). J Biomed Opt 5(3):259-268.

Faibish D, Ott SM, Boskey AL. 2006. Mineral changes in osteoporosis: A review. Clin Orthop Relat Res 446:28-38.

Paschalis EP, Verdelis K, Doty SB, Boskey AL, Mendelsohn, Yamauchi M. 2001. Spectroscopic characterization of collagen cross-links in bone. J Bone Miner Res 16(10):1821–1828.

Supplemental Table S1

Primer Sequences used for Real-Time Polymerase Chain Reaction analyses.

Gene	Forward Primer	Reverse Primer
Adipsin (cfd)	CGGATGACGACTCTGTGCAG	CATCGCTTGTAGGGTTCAGGG
Alkaline phosphatase	TCCTGACCAAAAACCTCAAAGG	TCGTTCATGCAGAGCCTGC
aP2	TGGGGACCTGGAAACTCGT	TCTCTGACCGGATGACGAC
β-actin	TGTTACCAACTGGGACGACA	CTGGGTCATCTTTTCCAGGT
β-catenin (mouse)	ATGGAGCCGGACAGAAAAGC	GAATCCAAGTAAGACTGCTGCT
β-catenin (rat)	GCTGACCTGATGGAGTTGGA	TCTTCTTCCTCAGGATTGCC
C/EBP α	ATAAGAACAGCAACGAGTACC	GCGGTCATTGTCACTGGTC
C/EBP δ	CCACGACCCCTGCCATGTAT	TGTGATTGCTGTTGAAGAGGTC
Osteocalcin	AGGGAGGATCAAGTCCCG	GAACAGACTCCGGCGCTA
Osterix	ACTGGCTAGGTGGTGGTCAG	GGTAGGGAGCTGGGTTAAGG
PPAR-γ	TATGGGTGAAACTCTGGGA	TGGCATCTCTGTGTCACCAT
Runx-2	GCCGGGAATGATGAGAACTA	GGACCGTCCACTGTCACTTT
Type 1 collagen	GCATGGCCAAGAAGACATCC	CCTCGGGTTTCCACGTCTC

Supplemental Table S2

Biomechanical strength of lumbar vertebrae and long bones are decreased in Pb-treated rats.

	Stiffness	Max Load	Energy to Failure	Yield Force
Compression[a]	**(N/mm)**	**(N)**	**(mJ)**	**(N)**
Control	700.99 ± 61.91	280.69 ± 30.93	86.50 ± 10.53	158.00 ± 33.94
Pb-exposed	507.03 ± 20.12*	181.61 ± 21.05*	68.25 ± 6.07	123.43 ± 36.52
4-point Bending[b]				
Control	637.73 ± 36.91	264.42 ± 10.04	73.53 ± 7.39	242.99 ± 6.33
Pb-exposed	590.45 ± 79.40	204.58 ± 17.45**	50.52 ± 5.83*	178.73 ± 16.37**

Data represent mean ± SEM for 6 rats/group for compression and 9 rats/group for bending.

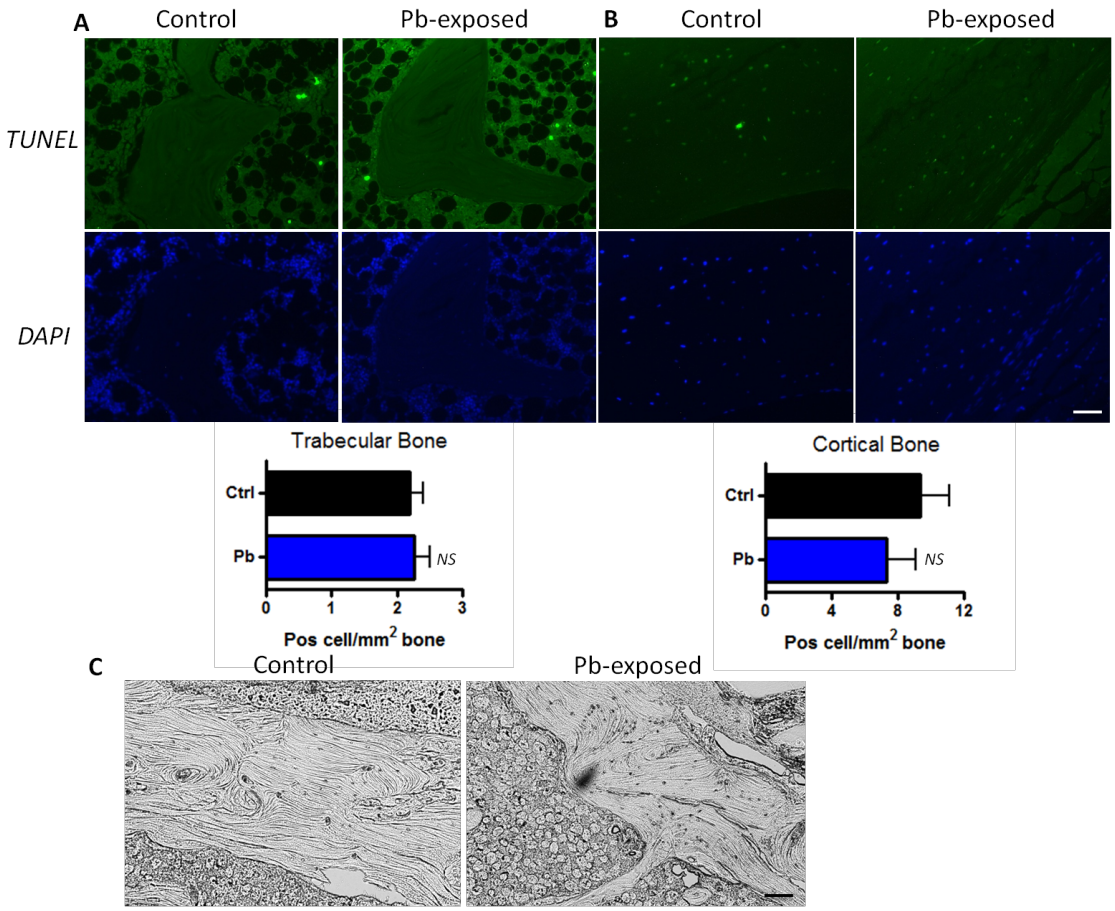
[a]Third lumbar vertebra were subjected to compression to failure testing. [b]Femurs were subjected to 4-point bend testing. *Significant at $p < 0.05$, **significant at $p < 0.005$.

Supplemental Table S3

Biomechanical strength of lumbar vertebrae and long bones are decreased in Pb-treated rats.

Raman Metric	Control	Pb-exposed
MTMR	1.00 ± 0.13	0.76 ± 0.09*
CTPR	1.00 ± 0.07	1.13 ± 0.08*
collagen maturity	1.00 ± 0.10	1.47 ± 0.19*
crystallinity	1.00 ± 0.002	0.992 ± 0.005*

Biochemical parameters of each group derived from the Raman spectra and normalized to control values. Data represent mean ± SEM for 4 rats/group, *$p < 0.05$



Supplemental Figure S1

Pb exposure had no effect on cell viability and bone structure. No change was seen in positive TUNEL staining between 0-Pb and 50-Pb treated rats in either trabecular (A) or cortical bone (B). Normal woven bone was observed in cortical bone (C). Data represent mean ± SEM for 3 samples. Scale bar: (A, B) 500 µm, (C) 100 µm, n=3.