A

Mineralization

Non

Diff.

B Alkaline phosphatase

C

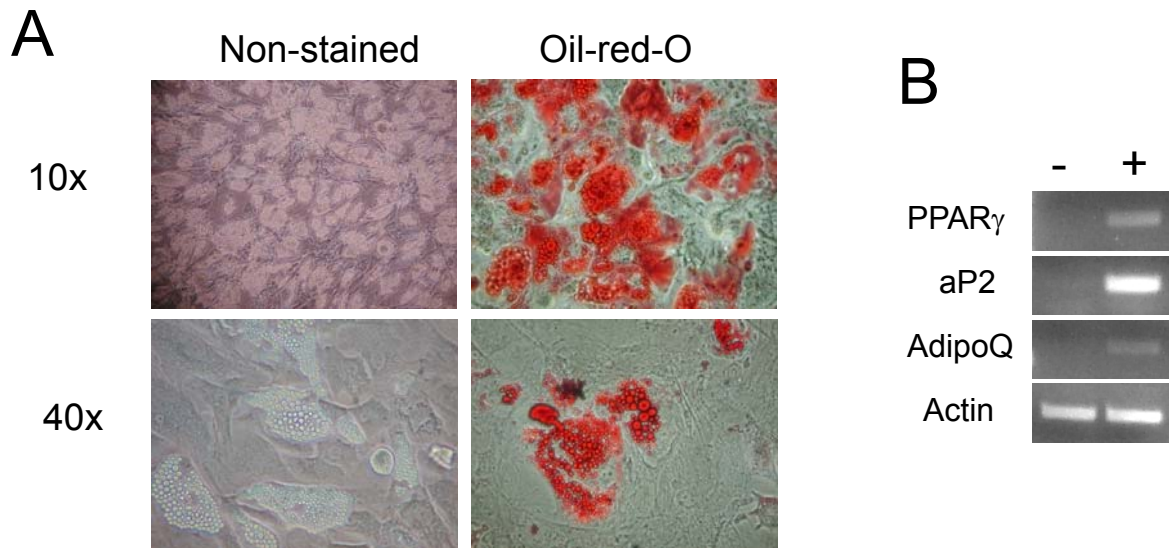|	-	+	Diff.
OSX			
ALP			
Actin			

Supplemental Figure 1: Differentiation of hBMSCs to osteoblasts. **(A)** hBMSCs were treated with osteoblast differentiation medium or αMEM (non-diff.) for 14 days and stained for mineralization with Alizarin red S (10x). **(B)** hBMSCs were differentiated for 7 days and stained for alkaline phosphatase. **(C)** RNA was isolated from control and differentiated cells and analyzed by RT-PCR for Osterix (OSX), alkaline phosphatase (ALP), and Actin (loading control).

A

	Non-stained	Oil-red-O
10x		
40x		

B

Supplemental Figure 2: Differentiation of hBMSCs to adipocytes. (A) hBMSCs at confluency were treated with differentiation medium for 7 days and photographed under light microscopy. Lipid accumulation is clearly visible. (10x and 40X). Bottom panels; Cells were then stained with Oil-red-O to confirm lipid accumulation (10x and 40X). **(B)** Adipocytes were generated as in (A) and gene expression analyzed by RT-PCR for PPARγ, aP2, adiponectin (*AdipoQ*) and actin. (Rep. of 3 patients).
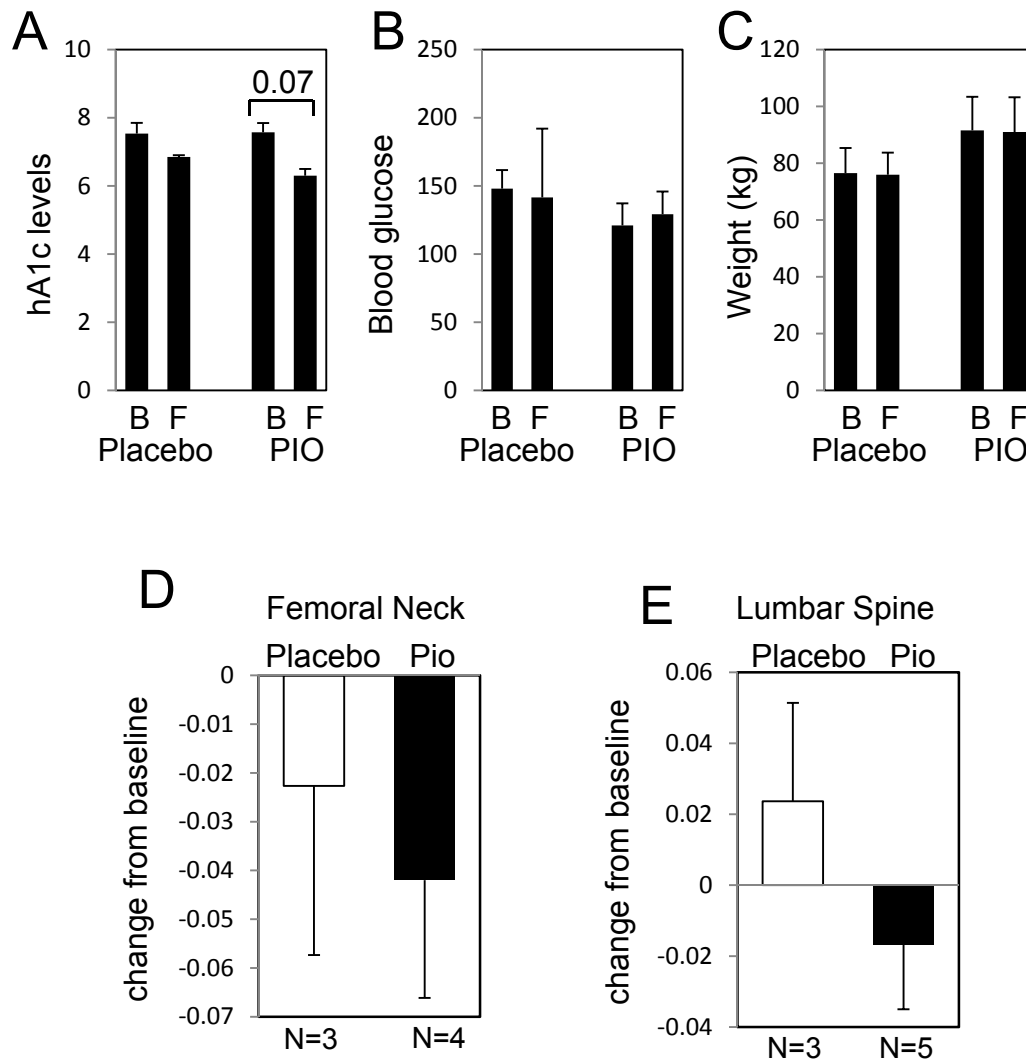
Supplemental Figure 3: Baseline and final response of study subjects treated with Pioglitazone or placebo. Pre-diabetic volunteers had (B:baseline) blood, weight, and BMD measurements taken (by DeXA) and the same parameters measured (F:final) after 26 weeks of placebo (N=3) or PIO (N=5). Blood hA1C (**A**) and glucose (**B**) were measured as well as weight (**C**). BMD was measured by DEXA at Femoral Neck (**D**) and Lumbar Spine (L1-4) (**E**) and results are expressed as the mean +/- SEM change from baseline for each patient. Changes were not statistically different.

Supplemental Table 1: Bone metabolism markers

Plasma	N	Osc	+/-	CTX	+/-	Adiponectin	+/-
Placebo	3	-5.2	5.8	-0.9	12.4	-0.8	13.9
Actos	4	-1.0	11.7	-8.4	15.7	48.3	42.5

Values are calculated as change from baseline for each patient and are expressed as mean +/-SEM for each group.

Supplemental Table 2: Primers used for qRT-PCR

Gene	Forward	Reverse
18s	5'-CAGCCACCCGAGATTGAGCA-3'	5'-TAGTAGCGACGGGCGGTGTG-3'
PPARγ2	5'-CCAGAAAGCGATTCCTTCAC-3'	5'-CGGAGCTGATCCCAAAGTT-3'
aP2/Fabp4	5'-TGGAAACTTGTCTCCAGTGAA-3'	5'-TGATGATCATGTTAGGTTTGGC-3'
AdipoQ	5'-CCATCTCCTCCTCACTTCCA-3'	5'-TAGAACAGCTCCCAGCAACA-3'
RUNX2/Cbfa1	5'-CAGTAGATGGACCTCGGGAA-3'	5'-CCTAAATCACTGAGGCGGTC-3'
Osc	5'-TGAGAGCCCTCACACTCCTC-3'	5'-CCTCCTGCTTGGACACAAAG-3'