Skeletal response to insulin in the naturally occurring type 1 diabetes mellitus mouse model

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Supplements;

Supplemental table 1: Antibodies

Antibody	Company	Catalog no	Dilution used
Cathepsin K antibody	Abcam	ab19027	1:200
pAKT(phospho Ser473) antibody	Genetex	89304-112	1:50
Glut1 antibody	Thermofisher scientific	PA1-46152	1:200
Glut4 antibody	Biogenesis	4670-1704	1:500
PDK4 antibody	Thermofisher scientific	12949-1-AP	1:50
Sclerostin antibody	R & D systems	AF1589	1:12
FGF23 antibody	R & D systems	MAB26291	1:100
Donkey Anti-Goat IgG H&L (HRP)	Abcam	ab97110	1:200
VECTASTAIN® ABC Kit (Rabbit IgG)	Vector labs	PK-4001	NA

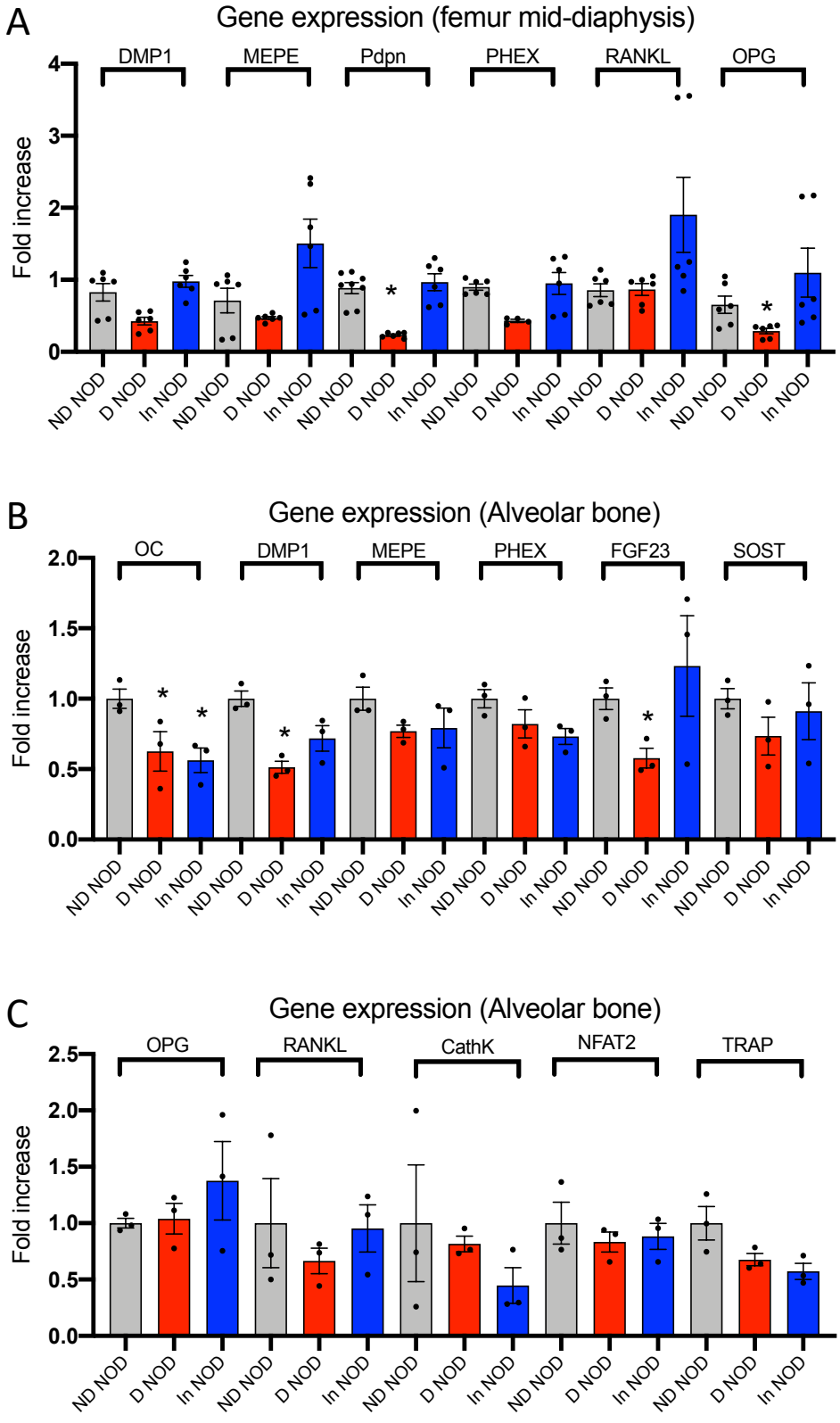
Supplemental table 2: qPCR primer sequences

Gene	Forward primer	Reverse primer
Osteocyte markers		
Phex	GAAAGGGGACCAACCGAGG	AACTTAGGAGACCTTGACTCACT
SOST	AGCCTTCAGGAATGATGCCAC	CTTTGGCGTCATAGGGATGGT
DMP-1	TTCGCTGAGGTTTTGACCTT	TTGGGATGCGATTCCTCTAC
FGF-23	ATGCTAGGGACCTGCCTTAGA	AGCCAAGCAATGGGGAAGTG
Pdpn	ACCGTGCCAGTGTTGTTCTG	AGCACCTGTGGTTGTTATTTTGT
MEPE	GTCTGTTGGACTGCTCCTCTT	CACCGTGGGATCAGGATACA
RANKL	CAGCATCGCTCTGTTCCTGTA	CTGCGTTTTCATGGAGTCTCA
OPG	ACCCAGAAACTGGTCATCAGC	CTGCAATACACACACTCATCACT

Supplement Table 3: mCT analysis of the osseous system of NOR, non-diabetic (*ND*), diabetic (*D*) and insulin treated (*In*) NOD mice. Data presented as mean+/-SD, one-way ANOVA with $p<0.05$ was considered significant. Comparisons that were insignificant are labeled *NS*.

	NOR	Non-diabetic NOD (ND)	Diabetic NOD (D)	Insulin treated NOD (In)	Significance
Sample size	10	26	19	14	Presented p<0.05
Femur mid-diaphysis, cortical bone parameters:					
BV/TV, %	50.039±0.544	57.268±0.409	53.064±0.921	56.237±0.907	NOR vs ND NOR vs D NOR vs In ND vs D
T.Ar, mm^2	1.431±0.029	1.416±0.016	1.346±0.014	1.372±0.027	NOR vs D ND vs D
B.Ar, mm^2	0.716±0.018	0.811±0.010	0.715±0.016	0.771±0.016	NOR vs ND ND vs D
M.Ar, mm^2	0.714±0.014	0.605±0.009	0.631±0.013	0.601±0.019	NOR vs ND NOR vs D NOR vs In
Cs.Th, mm	0.183±0.003	0.212±0.002	0.189±0.004	0.205±0.004	NOR vs ND NOR vs In ND vs D D vs In
J_0, $1/mm^4$	0.251±0.011	0.273±0.006	0.234±0.006	0.252±0.009	NOR vs ND ND vs D
Length (Le), mm	15.648±0.127	15.815±0.127	15.316±0.128	15.857±0.065	*NS*
Robustness (Le/T.Ar)	0.091±0.002	0.090±0.001	0.088±0.001	0.087±0.002	*NS*
BMD, g/cc	1.337±0.004	1.338±0.005	1.348±0.007	1.328±0.006	*NS*
Femur distal metaphysis, trabecular bone parameters:					
BV/TV, %	6.387±0.402	6.812±0.719	5.818±0.514	5.194±0.883	*NS*
Tb.Th, mm	0.057±0.001	0.064±0.001	0.053±0.002	0.060±0.002	NOR vs ND ND vs D D vs In
Tb.Sp, mm	0.452±0.016	0.589±0.030	0.549±0.024	0.634±0.031	NOR vs ND NOR vs In
Tb.N, 1/mm	1.122±0.061	1.053±0.104	1.097±0.094	0.843±0.123	*NS*
BMD, g/cc	0.123±0.005	0.112±0.009	0.112±0.007	0.097±0.013	*NS*
Lumbar Skeleton (L5):					
Sample size	7	6	6	6	
BV/TV, %	20.216±1.030	23.578±1.002	16.447±1.013	15.908±2.014	ND vs D ND vs In
Tb.Th, mm	0.059±0.001	0.074±0.002	0.057±0.002	0.061±0.002	NOR vs ND ND vs D ND vs In
Tb.Sp, mm	0.259±0.009	0.275±0.009	0.276±0.007	0.315±0.017	NOR vs In
Tb.N, 1/mm	3.390±0.140	3.182±0.076	2.881±0.071	2.569±0.207	*NS*
BMD, g/cc	0.449±0.017	0.533±0.019	0.375±0.018	0.386±0.032	ND vs D ND vs In
Alveolar Bone (mandible, between 1st and 2nd Molars)					
Sample size	10	26	21	10	
BV/TV, %	52.397±2.387	55.115±3.067	39.636±5.035	62.433±3.101	NOR vs ND NOR vs D NOR vs In ND vs D D vs In
Tb.Th, mm	0.095±0.004	0.110±0.005	0.086±0.008	0.119±0.004	NOR vs ND NOR vs In
Tb.Sp, mm	0.085±0.005	0.128±0.006	0.145±0.005	0.105±0.011	*NS*
Tb.N, 1/mm	5.493±0.149	4.991±0.235	4.482±0.235	5.237±0.108	NOR vs D
BMD, g/cc	1.260±0.013	1.419±0.027	1.294±0.043	1.299±0.022	NOR vs ND ND vs D ND vs In

Supplement Figure 1: Gene expression of osteogenic markers at the femur diaphysis, and alveolar bone of the mandible. (A) Expression of osteocyte markers at femur diaphysis. (B) Expression of mature osteoblast and osteocyte markers in alveolar bone. (C) Expression of osteoclast markers in alveolar bone.



Supplement Figure 2: Diabetic NOD mice show alterations in the expression of GLUT1, PDK4, and p-AKT and GLUT4. GLUT1 (A), PDK4 (B), (C) pAKT, and (D) GLUT4 positive osteocytes in cancellous bone of the L5 vertebra. Representative sections from ND-NOD, D-NOD and In-NOD groups, quantifications are provided in Figure 7.