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  |               |
| 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        |
|---------|-----------------------|-----------------------|
| Phex    | GAAAGGGGACCAACCGAGG   | AACTTAGGAGACCTTGAATC   |
| SOST    | AGCCCTAGAGAATGATGCCAC | CTTTGCGTCTAGGGATGGT   |
| DMP-1   | TTCGCTAGAATTGACTCTT   | TTGGGATGCTTCCTTAC     |
| FGF-23  | ATGCTAGGACCTGACCTTTAGA | AGCCAAACCAAGTTGAGGAAGT |
| Pdpn    | ACCGATGCCAGTGTGTCTG   | AGCACCTGTGTTGTTTAATTTT |
| MEPE    | GTCTGTTGAGCTCTCTCTT   | CACCACCCCTCGAGTCACAAA |
| RANKL   | CAGCATCGCCTCTCCTGTTA  | CTGCCATTTTCAATGAGACTC |
| OPG     | ACCAGAAAATGCATCACAG   | CTGCCATACACACACACTCACT |
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.

| Sample size | NOR | Non-diabetic NOD (ND) | Diabetic NOD (D) | Insulin treated NOD (In) | Significance |
|-------------|-----|-----------------------|------------------|--------------------------|--------------|
|             | 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, ND vs D, ND vs In, D vs In |
| T.Ar, mm\(^2\) | 1.431±0.029 | 1.416±0.016 | 1.346±0.014 | 1.372±0.027 | NOR vs ND, ND vs In |
| B.Ar, mm\(^2\) | 0.716±0.018 | 0.811±0.010 | 0.715±0.016 | 0.771±0.016 | NOR vs ND |
| M.Ar, mm\(^2\) | 0.714±0.014 | 0.605±0.009 | 0.631±0.013 | 0.601±0.019 | NOR vs ND |
| Cs.Th, mm   | 0.183±0.003 | 0.212±0.002 | 0.189±0.004 | 0.205±0.004 | NOR vs ND |
| J0, 1/mm\(^4\) | 0.251±0.011 | 0.273±0.006 | 0.234±0.006 | 0.252±0.009 | 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 | ND vs In, D vs In, In vs D |
| Tb.Th, mm | 0.057±0.001 | 0.064±0.001 | 0.053±0.002 | 0.060±0.002 | ND vs In, D vs In, In vs D |
| Tb.Sp, mm | 0.452±0.016 | 0.589±0.030 | 0.549±0.024 | 0.634±0.031 | ND vs In, D vs In, In vs D |
| 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  | ND vs In, D vs In |
| BV/TV, % | 20.216±1.030 | 23.578±1.002 | 16.447±1.013 | 15.908±2.014 | ND vs In, D vs In, In vs D |
| Tb.Th, mm | 0.059±0.001 | 0.074±0.002 | 0.057±0.002 | 0.061±0.002 | ND vs In, D vs In, In vs D |
| Tb.Sp, mm | 0.259±0.009 | 0.275±0.009 | 0.276±0.007 | 0.315±0.017 | ND vs In, D vs In, In vs D |
| Tb.N, 1/mm | 3.390±0.140 | 3.182±0.076 | 2.881±0.071 | 2.569±0.207 | ND vs In, D vs In, In vs D |
| BMD, g/cc | 0.449±0.017 | 0.533±0.019 | 0.375±0.018 | 0.386±0.032 | ND vs In, D vs In, In vs D |

**Alveolar Bone (mandible, between 1\(^{st}\) and 2\(^{nd}\) Molars):**

| Sample size | 10  | 26  | 21  | 10  | ND vs In, D vs In |
| BV/TV, % | 52.397±2.387 | 55.115±3.067 | 39.636±5.035 | 62.433±3.101 | ND vs In, D vs In, In vs D |
| Tb.Th, mm | 0.095±0.004 | 0.110±0.005 | 0.086±0.008 | 0.119±0.004 | ND vs In, D vs In, In vs D |
| Tb.Sp, mm | 0.085±0.005 | 0.128±0.006 | 0.145±0.005 | 0.105±0.011 | ND vs In, D vs In |
| Tb.N, 1/mm | 5.493±0.149 | 4.991±0.235 | 4.482±0.235 | 5.237±0.108 | ND vs In, D vs In |
| BMD, g/cc | 1.260±0.013 | 1.419±0.027 | 1.294±0.043 | 1.299±0.022 | ND vs In, D vs In, In vs D |

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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.