Article

Adipose tissue denervation blunted the decrease in bone formation promoted by obesity in rats

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**SUPPLEMENTARY FIGURES**



**Figure S1.** Static histomorphometric parameters in Sham or denervated (Dnx) groups under standard diet (SD) or high fat diet (HFD). (a) trabecular thickness – Tb.Th, (b) osteoid thickness – O.Th. Open bars (Sham groups) and closed bars (Dnx) represent median and interquartile range. Statistical analyses were performed by two-way ANOVA with Bonferroni post-hoc test. P<0.05 was considered statistically significant.



**Figure S2.** HFD did not alter calcium metabolism. Biochemical parameters evaluated at the end of protocol did not differ among groups: (a) serum calcium; (b) urinary fractional excretion of calcium – FeCa (%); (c) serum 25-hydroxyvitamin D3. Bars represent median and interquartile range. Statistical analyses were performed by two-way ANOVA with Bonferroni post-hoc test. P<0.05 was considered statistically significant.



**Figure S3.** Denervation surgery confirmation. Denervation of retroperitoneal white adipose tissue (rWAT) was confirmed by western blot quantification of tyrosine hydroxylase protein, which was significantly lower in SD+Dnx and HFD+Dnx groups when compared to non-denervated groups (Sham SD and HFD). (a) representative western blots of relative protein levels and (b) relative quantification of tyrosine hydroxylase protein. Bars represent median and interquartile range. Statistical analyses were performed by two-way ANOVA with Bonferroni post-hoc test. P<0.05 was considered statistically significant.

**SUPPLEMENTARY TABLES**

**Table S1.** Composition of the diets used in the study.

|  |  |  |
| --- | --- | --- |
| **Ingredients (g/kg of diet)** | **SD** | **HFD** |
| Cornstarch | 720.7 | 248.7 |
| Sucrose | - | 100.0 |
| Casein | 140.0 | 200.0 |
| Soybean oil | 40.0 | 40.0 |
| Cellulose | 50.0 | 50.0 |
| Lard | - | 312.0 |
| AIN-93M Vitamin mixture | 10.0 | 10.0 |
| AIN-93M Mineral mixture | 35.0 | 35.0 |
| L-cystine | 1.8 | 1.8 |
| Choline bitartrate | 2.5 | 2.5 |
| Butyl hydroquinone | 0.008 | 0.008 |

SD: standard diet; HFD: high-fat diet

**Table S2.** List of antibodies used for Western Blot.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Protein/Epitope** | **Source and Catalog number** | **Host** | **Application** | **Dilution** | **Application specific details** |
| *Tyrosine hydroxylase* | Cell Signaling, 2792S | rabbit | Western Blot | 1:1000 | 5% BSA, overnight, 4°C |
| *Anti-rabbit IgG* | Abcam, ab97051 | goat | Western Blot | 1:50000 | 5% BSA, 1hr RT |

**Table S3.** Primer sequences used for qPCR.

|  |  |  |
| --- | --- | --- |
| **Gene** | **Forward sequence** | **Reverse sequence** |
| *NPY* | 5’ CAGATACTACTCCGCTCTGC 3’ | 5’ AAGGGTCTTCAAGCCTTGTT 3’ |
| *Gapdh* | 5’ CAACTCCCTCAAGATTGTCAGCAA 3’ | 5’ GGCATGGACTGTGGTCATGA 3’ |