Supplementary Materials

Supplementary Materials and Methods

Animals, groupings, and treatment

A total of 12 healthy male tree shrews (six months old) were used in this study. The animals were purchased from the Institute of Medical Biology, Chinese Academy of Medical Sciences, Kunming, China. The tree shrews were provided with special feed and apples, with adaptive feeding for two weeks in a clean environment (temperature: 23–25 °C, relative humidity: 40%–70%, illumination time: 12 h, light intensity: ≤900 Lx, noise: ≤60 dB) (Ma et al., 2015). The animals were then randomly assigned to the experimental SAON group (n=6) or control group (n=6). Animals in the SAON group received one intravenous injection of LPS (300 μg/kg), followed by three intraperitoneal injections of MPS (130 mg/kg) over a 24 h interval, with subsequent intraperitoneal injections of MPS (130 mg/kg) two times per week until sacrifice. Animals in the control group were injected with the same volume of saline at the same time points. The tree shrews were sacrificed at 12 weeks post-induction via an intraperitoneal injection of pentobarbital sodium (100 mg/kg).

Biochemical analysis of blood samples

After the tree shrews were sacrificed, samples of blood were taken and incubated at room temperature for 2 h; after which, they were centrifuged at 3 000 r/min for 10 min at 4 °C. The serum fractions were collected for biochemical analysis. Enzyme linked immunosorbent assay (ELISA) kits (Shanghai Enzyme-Linked, China) were used to measure the levels of bone alkaline phosphatase (BALP; Cat. No. ml 627904), bone GLA protein (BGP; Cat. No. ml 625695), N-terminal propeptide of type I collagen (P1NP; Cat. No. ml 6038002), and C-terminal propeptide of type I collagen (P1CP; Cat. No. ml 6036832) in tree shrew serum.

Micro-CT scanning and analysis

After the tree shrews were sacrificed, the right femoral heads and femoral necks were removed as regions of interest (ROI), with micro-CT testing (Skyscan 1272, Belgium) then performed at the National & Regional Engineering Laboratory of Tissue Engineering, Third Military Medical University (Chongqing, China).

Bone mineral density (BMD), bone tissue volume fraction (BV/TV), bone surface/volume ratio (BS/BV), trabecular number (Tb. N), trabecular thickness (Tb. Th), and trabecular separation (Tb. Sp) of the femoral heads and femoral necks were determined separately with a CT analyzer.

Histological observations

After micro-CT scanning, the right femoral heads were fixed in 4% paraformaldehyde for 72 h, and then decalcified by soaking in 25% formic acid for 3 d. Cross-sections of paraffin-embedded tissue samples were cut for hematoxylin-eosin (H&E) and TUNEL

staining. An H&E staining kit was purchased from Beijing Solarbio Sciences & Technology Co., Ltd. (China; Cat. No. G 1120). In brief, the sections were de-paraffinized, washed for 2 min, and then stained with hematoxylin for 1 min. Subsequently, the samples were washed with purified water and differentiation solution for 6 s at room temperature, then counterstained with eosin for 1 min, washed with absolute ethanol, sealed with neutral gum, and finally examined by microscopy. An *in-situ* cell death detection kit (Beyotime, Shanghai, China, Cat. No. C1086) was used to perform TUNEL assay according to the manufacturer's instructions. Green fluorescent apoptotic cells were viewed under a fluorescence microscope (Nikon Ci, Japan).

Scanning electron microscopy observations

The left femoral heads were removed from both groups and bone tissues were fixed in 2.5% glutaraldehyde for 48 h. The tissues were then dehydrated with a gradient alcohol series, dried with tert-butanol, vacuum-plated, and examined by scanning electron microscopy (S-3400N, Hitachi, Tokyo, Japan).

Statistical analysis

All data were analyzed using SPSS Statistics for Windows v17.0 (SPSS, Inc., Chicago, IL, USA), and results are expressed as mean±standard deviation (*SD*). Student's *t*-test was used to analyze statistically significant differences between groups. *P*-values of <0.05 were considered statistically significant.

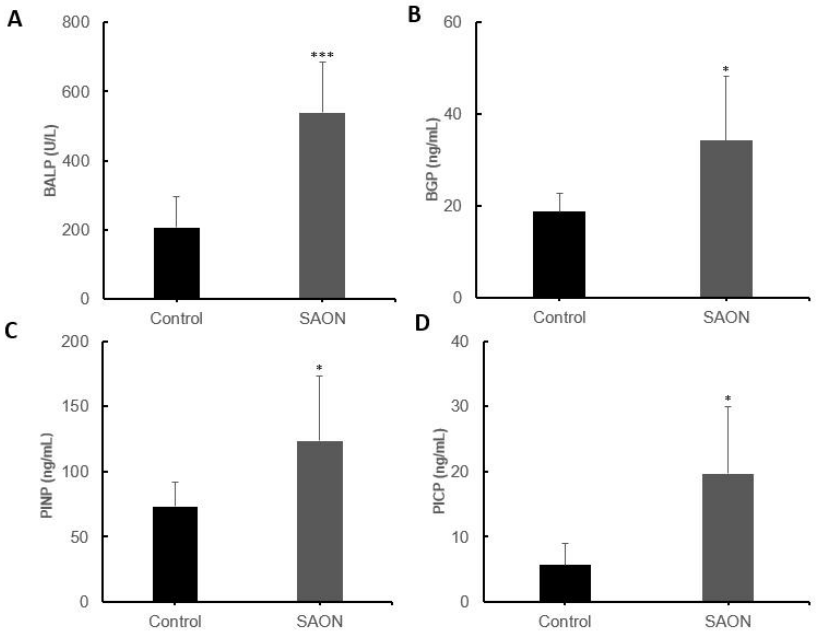
REFERENCES

Ma YH, Chen JQ, Hu ZF, Wu JW, Lv LB. 2015. A new breeding management system for tree shrews was introduced. *China Animal Husbandry and Veterinary Digest*, **31**(2): 75, 96. (in Chinese)

Supplementary Table S1 Micro-CT evaluation of control and SAON groups (*n*=6, X±S)

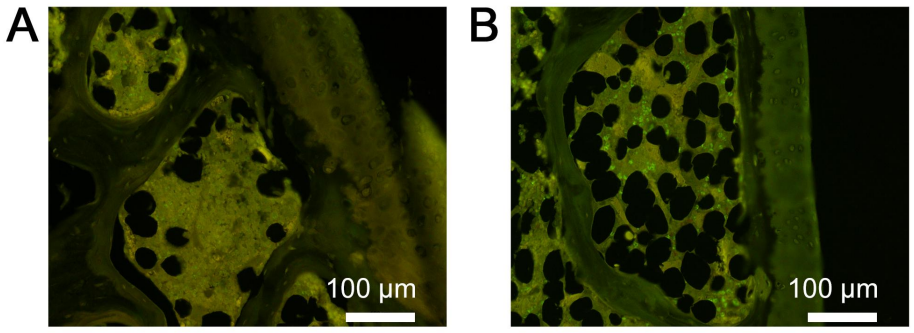
Group	BMD value	BV/TV (%)	BS/BV (/mm)	Tb. Th (mm)	Tb. Sp (mm)	Tb. N (/mm)
Control	0.5410±0.0333	45.43±3.87	28.72±4.69	0.11±0.01	0.21±0.01	4.28±0.22
SAON	0.4709±0.0492*	38.03±5.05*	32.11±5.53$^{\triangle}$	0.10±0.02$^{\triangle}$	0.25±0.02**	3.77±0.27**

BMD: Bone mineral density; BV/TV: Bone tissue volume fraction; BS/BV: Bone surface/volume ratio; Tb. Th: Trabecular thickness; Tb. Sp: Trabecular separation; Tb. N: Trabecular number. Compared with control group, $^{\triangle}$: $P>0.05$, *: $P<0.05$, **: $P<0.01$.



Supplementary Figure S1 Blood biochemical indicators in tree shrews from SAON and control groups

BALP (A); BGP (B); P1NP (C); P1CP (D) were detected. All data are presented as mean±*SD* (*n*=6). **P*<0.05 and****P*<0.001 vs. control group. BALP, bone alkaline phosphatase; BGP, osteocalcin; P1CP, procollagen type I C terminal propeptide; P1NP, procollagen type I N terminal propeptide.



Supplementary Figure S2 Photomicrographs of TUNEL reactions indicating presence of apoptotic cells in necrotic zones

A: There were more total cells in each tissue section, but fewer apoptotic cells of bone marrow and bone trabecula in control group.

B: There were fewer total cells in each tissue section, but more apoptotic cells of bone marrow and bone trabecula in SAON group.