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TNF-a Upregulates Sclerostin Expression in Obese Mice Fed a High-Fat Diet

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Introduction

- It has been demonstrated that low bone mass and high fracture risk are associated with obesity in both animals and humans.
- Obesity may increase adipocyte differentiation and fat accumulation while decreasing osteoblast differentiation and bone formation in the bone marrow.
- Obesity is linked to chronic inflammation and is characterized by increased production of inflammatory cytokines, such as tumor necrosis factor-a (TNF-a), and the activation of the inflammatory signaling network.

- In addition, TNF-a induces the mitogen-activated protein kinase (MAPK)-dependent expression of sclerostin in human osteoblasts, indicating that sclerostin plays a role in inflammatory disease-induced bone loss.
- In the study, the author examined whether obesity-induced bone loss is associated with sclerostin expression.

Materials and Methods

- Animals: C57 mice,high-fat diet (HF)/control diet (CON) for 12 weeks
- Trabecular morphology by uCT:

Tb.BMD(小梁骨密度)；BV/TV（骨体积分数）；Tb.Th（小梁厚度）；Tb.Sp（小梁分离）；Tb.N（骨小梁数）；ConnD（连通密度）

- Immunohistochemistry、ELISA
- Cell culture： MLO-Y4 cells
- RNA interference and transient transfection
- RT-PCR、WB、Chromatin immunoprecipitation (ChIP)

Results

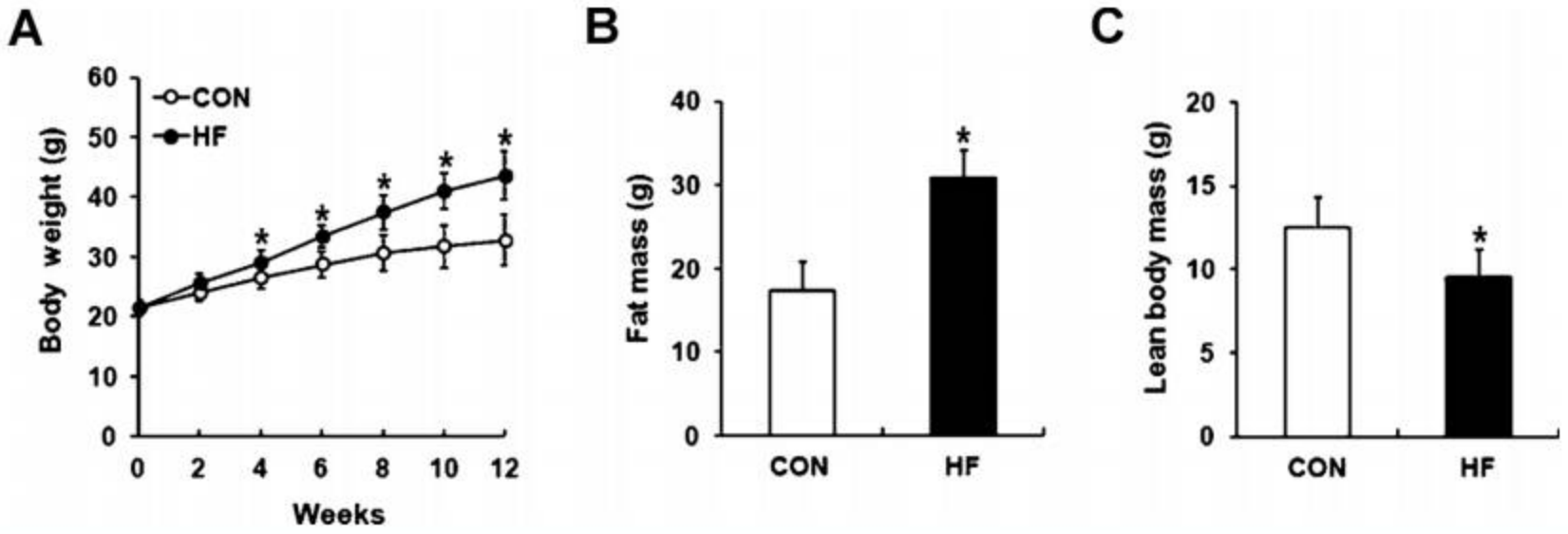
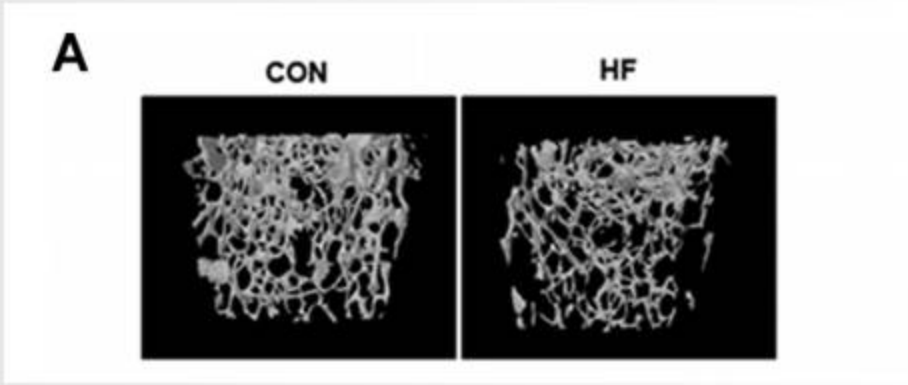
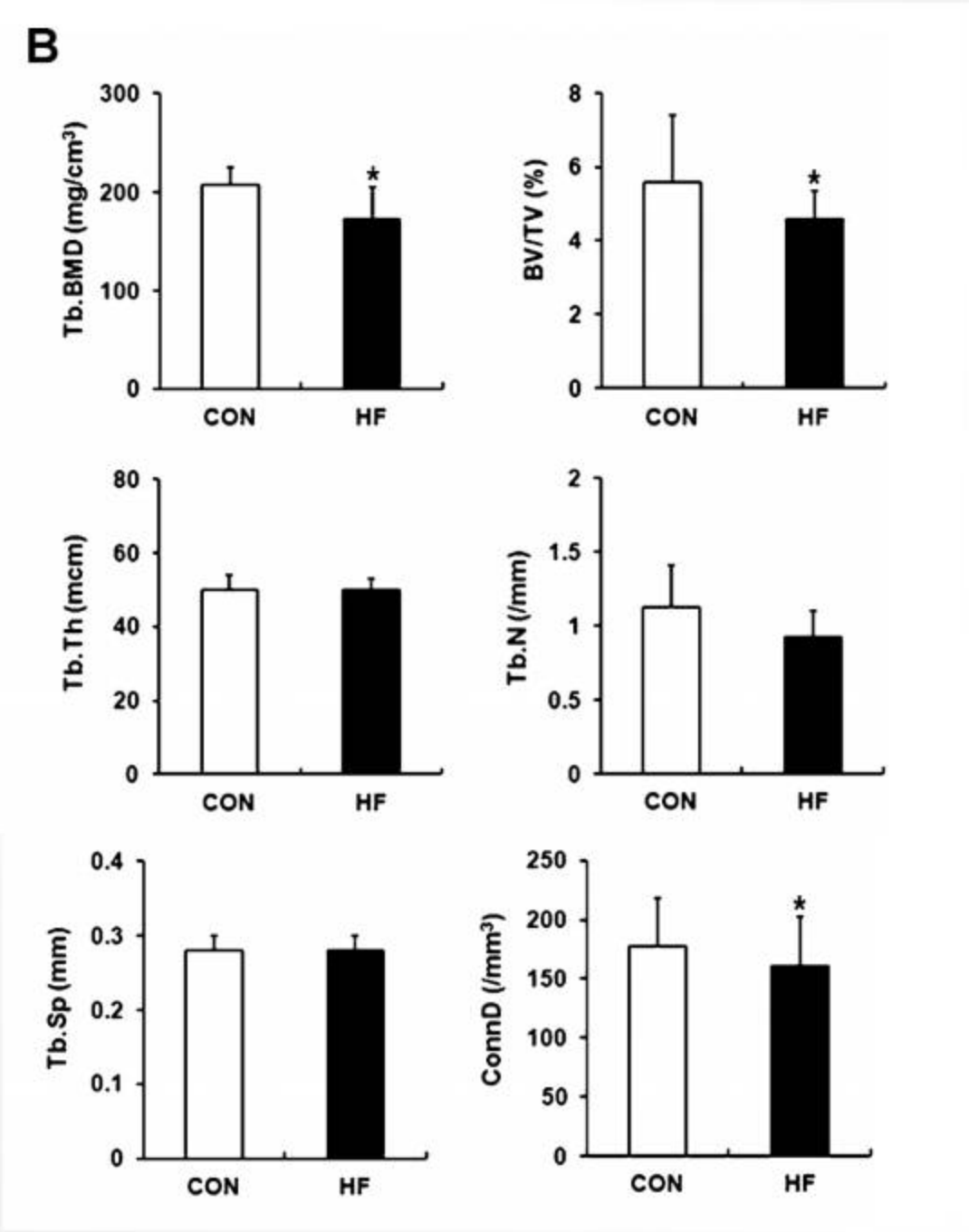


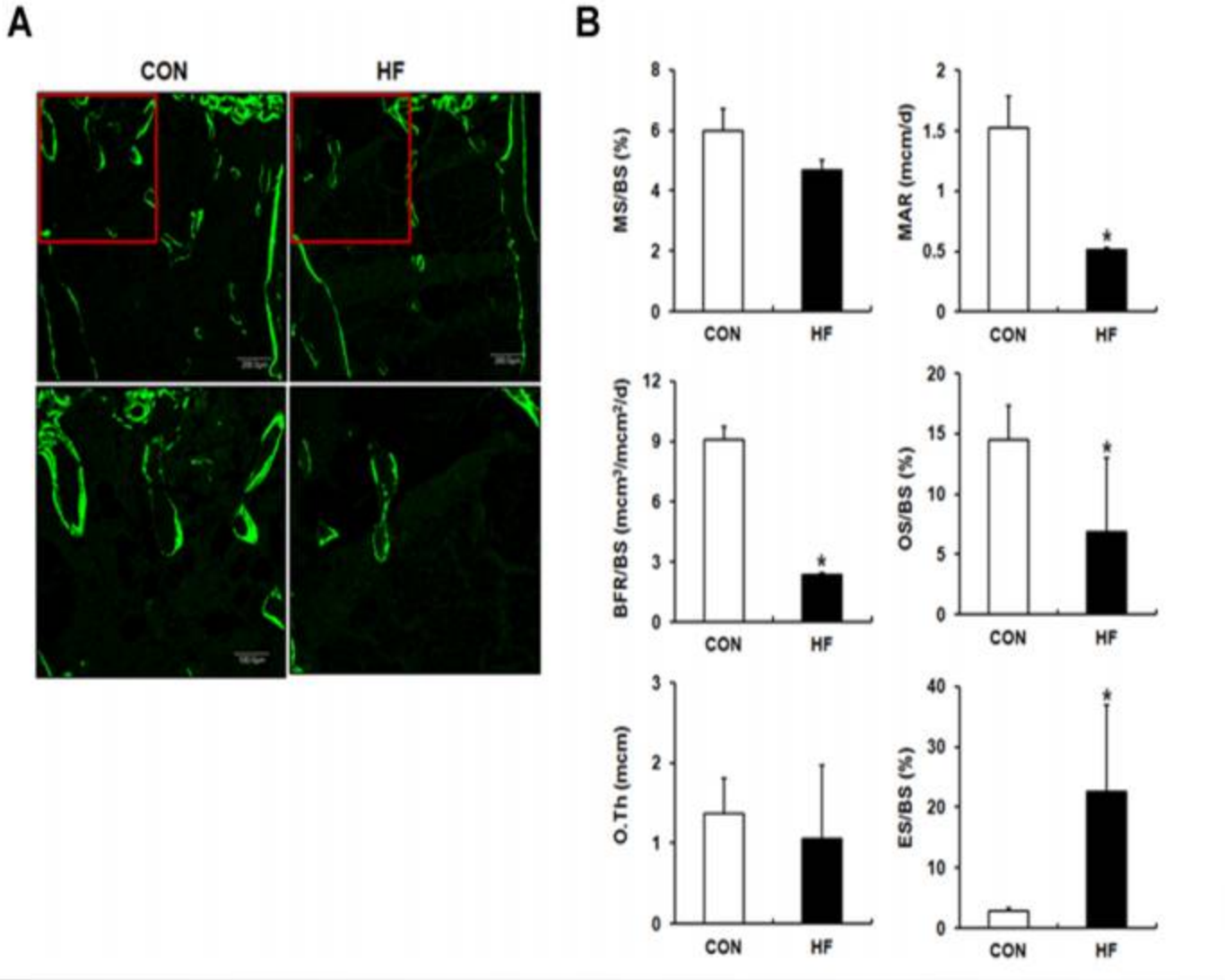
Fig. 1. The high-fat diet increased body weight and body fat mass while decreasing body lean mass. A: Body weights of the high-fat diet-fed (HF) or control diet-fed (CON) mice over 12 weeks. For both groups, n = 10. *Significant difference versus CON ($P < 0.01$). B, C: Total body fat mass and lean mass at Week 12 from whole body DEXA scans. *Significant difference versus CON ($P < 0.01$). Values are means ± SD.

- HF mice had higher body weights than CON mice from Week 4 of treatment until the end of the experiment.
- The total body fat mass of HF mice was higher than that of CON mice.

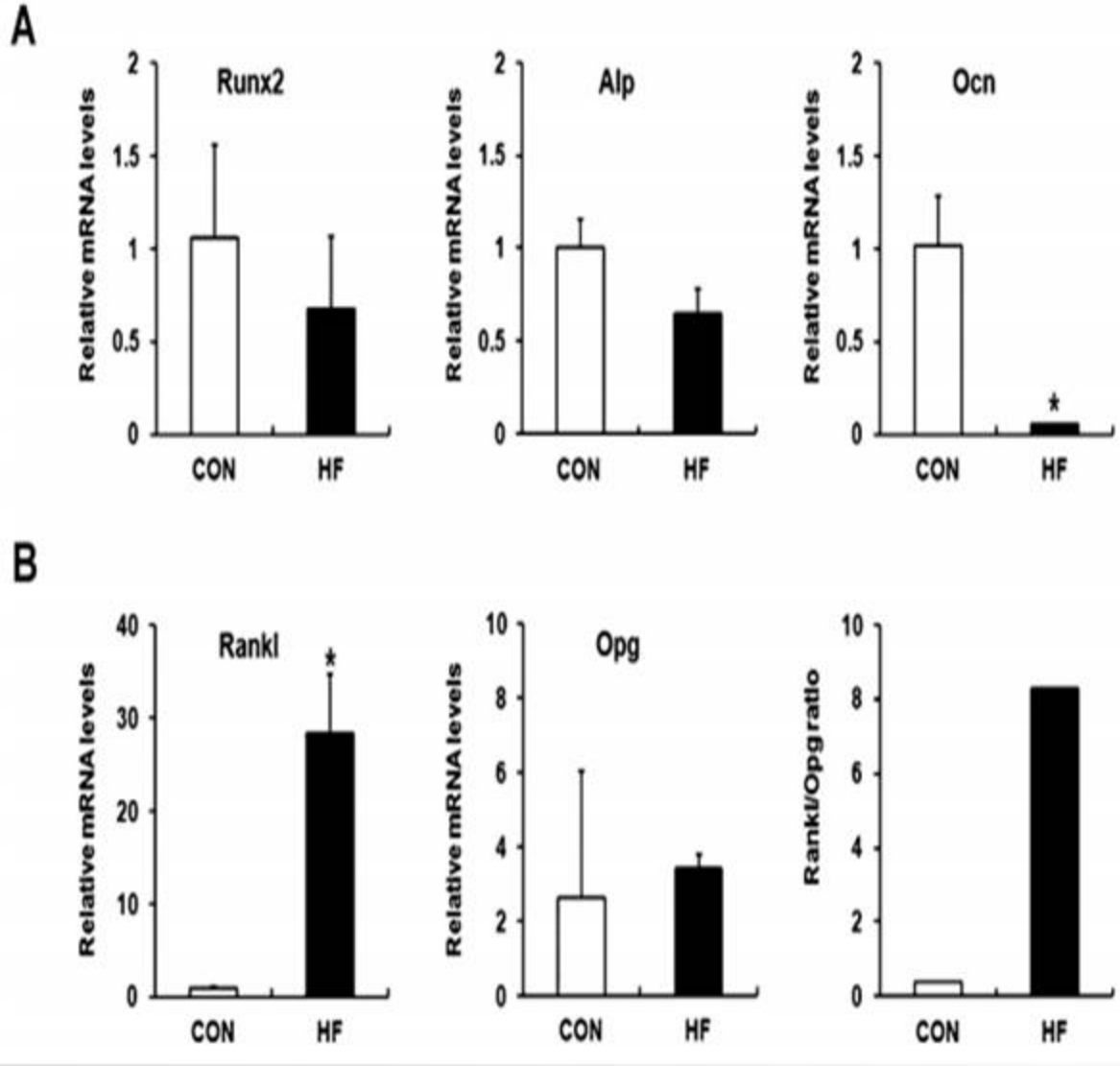


- uCT measures revealed that HF mice had 17.0% lower cancellous vBMD (Th.BMD) in the distal femur compared to the CON mice
- Significant decreases in the cancellous BV/TV and ConnD were also observed in the HF mice compared to the CON mice.

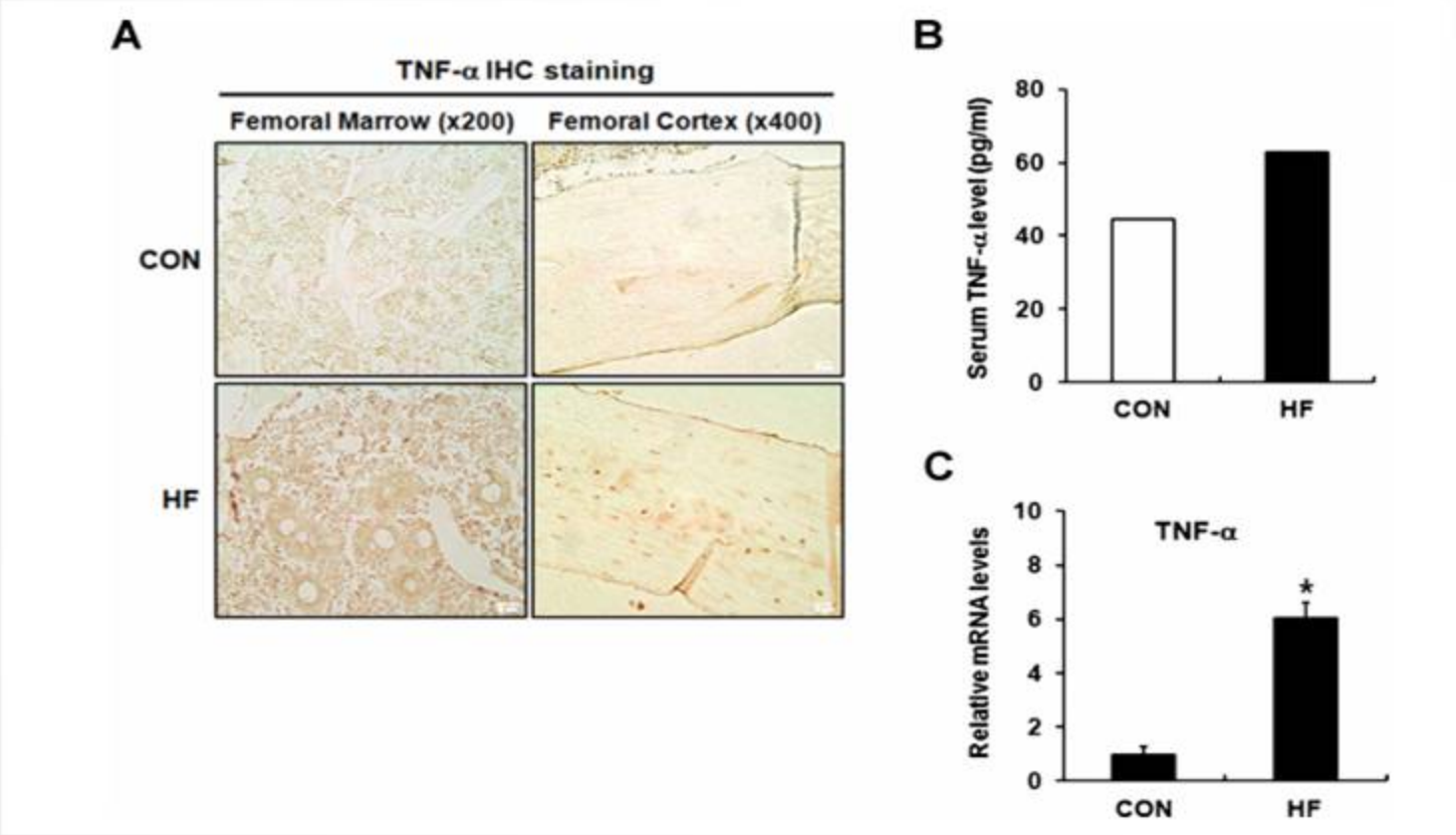




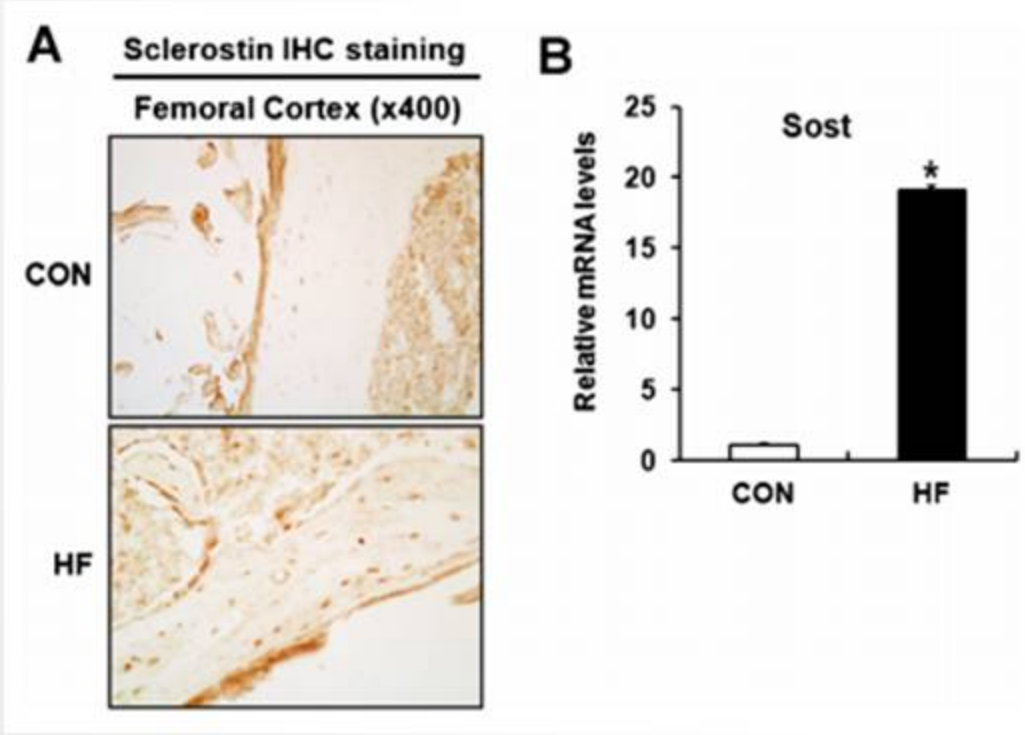
- Analysis of femur sections showed a threefold decrease in MAR in the HF mice compared to the CON mice.
- These results suggest that high-fat diet reduces BMD by both reducing bone formation and enhancing bone resorption.



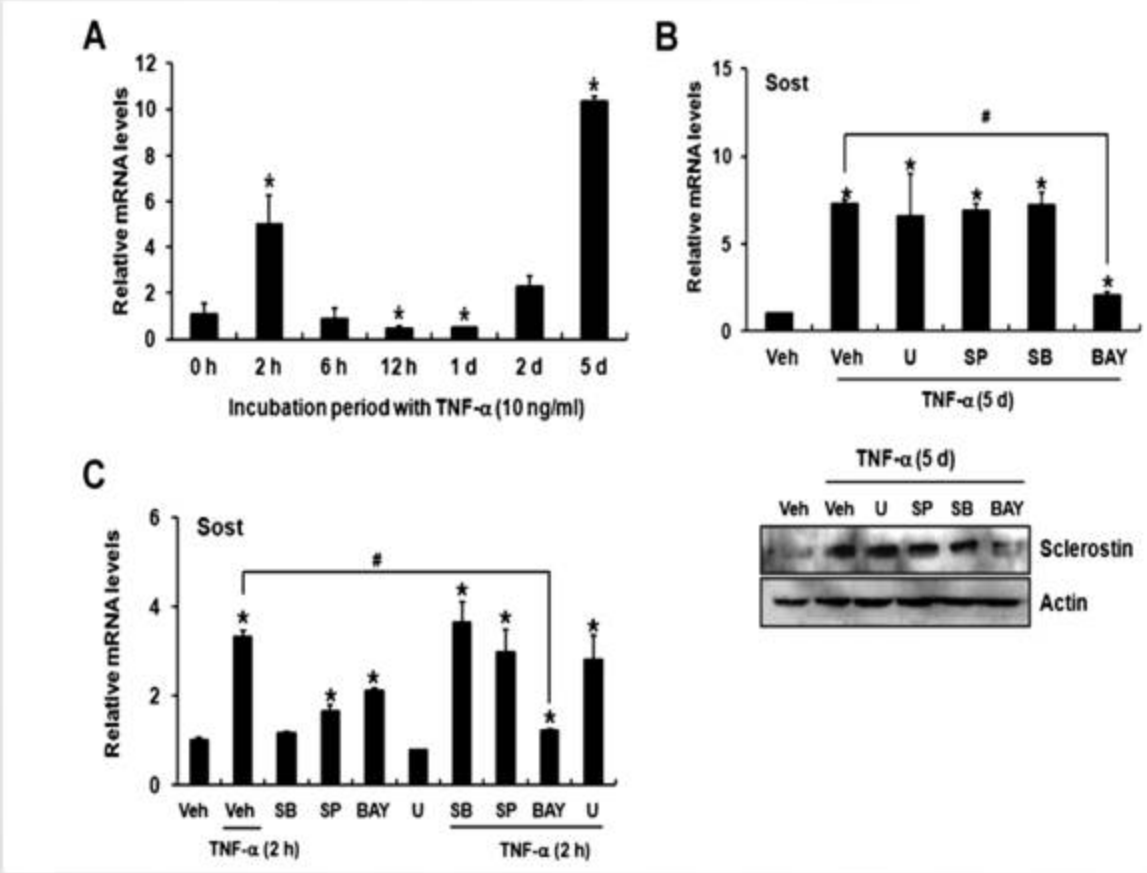
- To decipher the mechanisms of low vBMD in HF mice, the author examined the expression levels of osteogenic and osteoclastogenic markers in tibial bone tissue.
- RT-PCR analysis revealed the expression levels of these osteogenic markers were lower in the HF mice than the CON mice, but statistical significance was only observed for Ocn expression.
- A significant increase in Rankl expression was observed in HF mice,suggest that high-fat diet-induced obesity decreases osteoblastic bone formation while upregulating osteoclastic bone resorption.



- Because obesity is closely linked to the increased production of inflammatory cytokines such as TNF-a, the author examined the TNF-a levels in the serum and bone tissue of HF mice.
- High-fat diet increases TNF-a levels in serum and osteocytes.

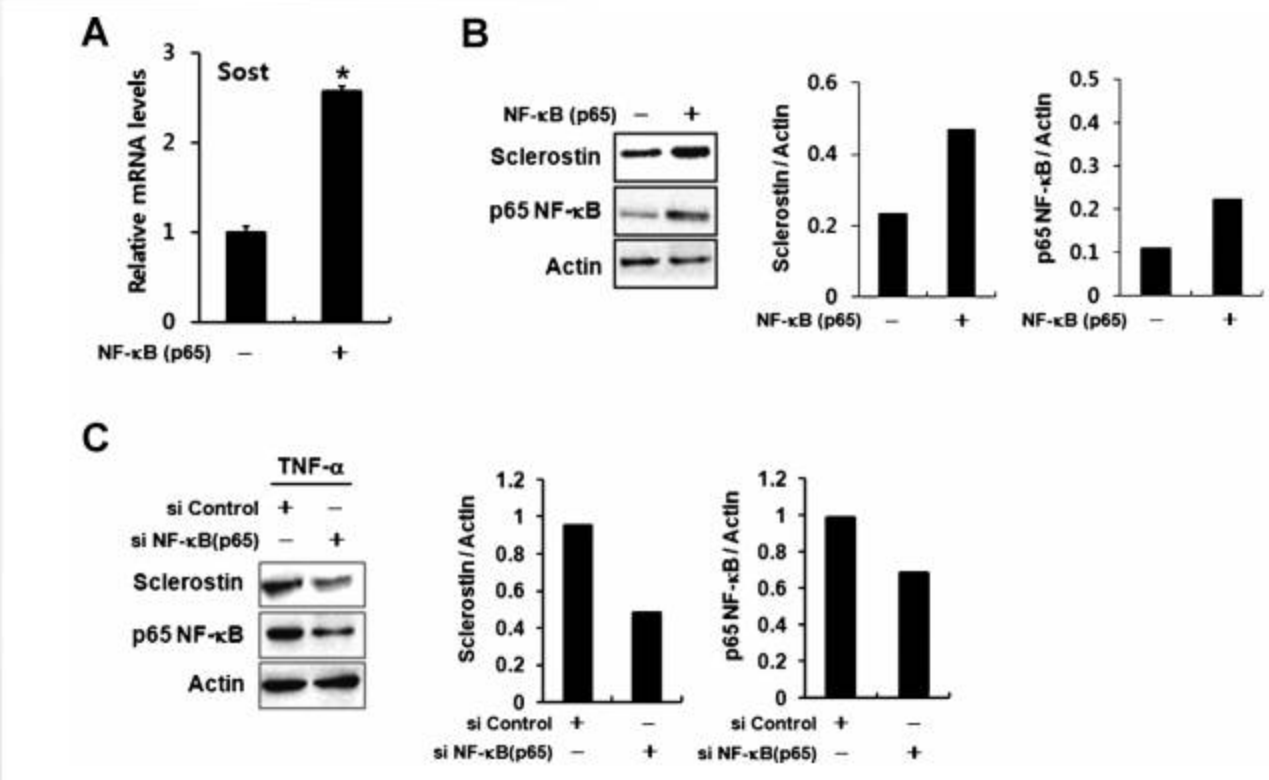


- These results indicate that high-fat diet-induced obesity results in increased sclerostin expression in bone tissues.

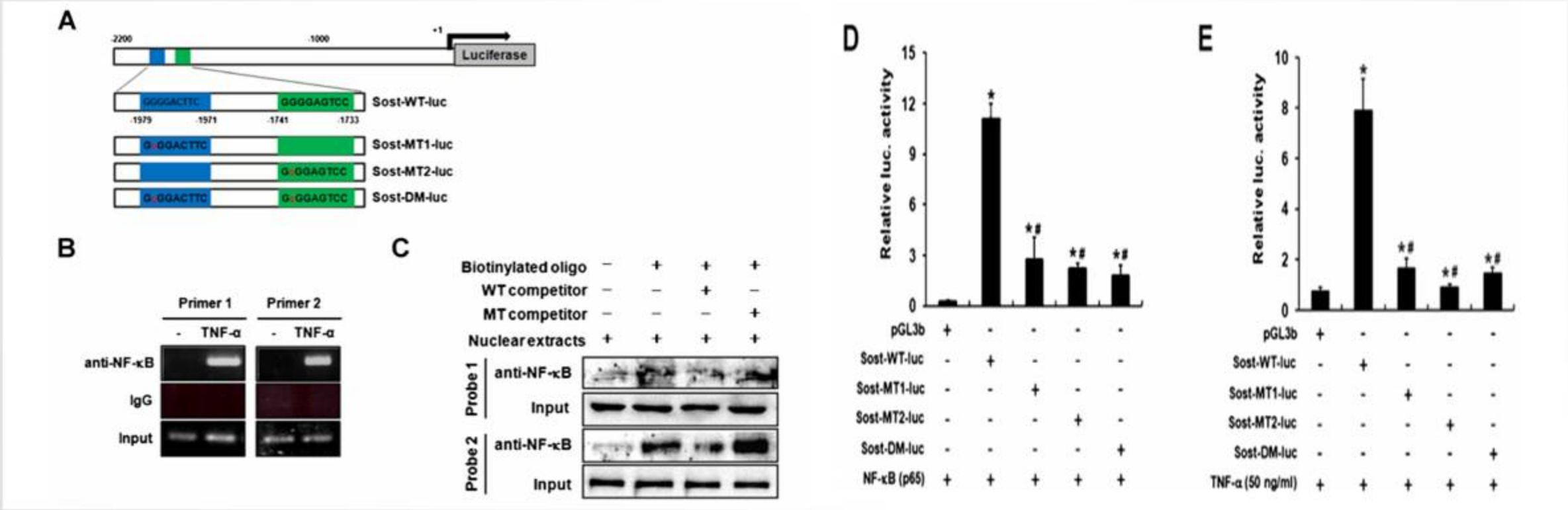


- To elucidate the regulatory mechanism of obesity-induced sclerostin expression, the author examined the effect of TNF-a on sclerostin expression.
- Real-time PCR and Western blot analysis demonstrated that TNF-a-induced sclerostin expression was blocked by the inhibition of NF-kB activation.

ERK inhibitor (U0126)
JNK inhibitor (SP600125)
p38 MAPK inhibitor (SB203580)
NF-kB inhibitor (BAY-11-7082)



- Increase in sclerostin expression in NF-kB transfected cells was confirmed by real-time PCR and Western blot analysis.
- The knockdown of NF-kB significantly decreased TNF-a-induced sclerostin expression.



- Consistent with the ChIP assay results, NF-kB bound to the oligonucleotides containing the NF-kB binding motif in the sost promoter in vitro.
- NF-kB binds directly to the sost promoter, thus inducing the transcription of the sost gene.

Discussion

- This experiment is the first to examine sclerostin expression in the context of a high-fat diet-induced obesity in growing mice.
- it is assumed that highly expressed sclerostin in the context of high-fat diet would antagonize Wnt/b-catenin signaling in the long bones, contributing to a high-fat diet-induced bone loss.
- These results of study indicate that NF-kB directly binds to and transactivates the sost promoter, thus increasing sclerostin expression.

- The main effect of high-fat diet-induced obesity on limb bones is likely to be a negative regulation of bone rather than mechanical load-induced bone gain.
- Growing mice fed a high-fat diet over 12 weeks exhibited significant bone loss in their femurs and increased TNF-a and sclerostin expression in osteocytes.
- The high-fat diet reduced bone mass by decreasing bone formation and increasing bone resorption. The data presented in this study demonstrated that TNF-a is a transcriptional activator for sclerostin.

- The findings from the present study support a model in which, in the context of obesity or other inflammatory disease increasing production of TNF-a, TNF-a enhances bone loss via the induction of sclerostin expression in an NF-kB-dependent manner.

Thank you