May 3, 2018

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 38999-minireview.doc).

Title: Role of osteoprotegerin / receptor activator of nuclear factor kappa B / receptor activator of nuclear factor kappa B ligand axis in nonalcoholic fatty liver disease

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The manuscript has been improved according to the suggestions of the Editor as well as of the Reviewer:

- We have included in the revised manuscript a figure (Figure 1) depicting the role of OPG/RANKL/RANK system in physiological and pathophysiological conditions.

1) There are no studies on OPG knockout/transgenic mice in NAFLD. Nonetheless, we have summarized the very few OPG knockout/transgenic mice studies involving metabolic syndrome components' derangements associated with NAFLD:

   "**ANIMAL STUDIES**

   The development of transgenic technologies in mice has led to advances in knowledge of the role of OPG/RANKL/RANK system in bone metabolism and cardiometabolic functions. Concerning cardiometabolic disorders, Hao et al. showed that OPG$^{-/-}$ mice exhibited a significant increase in systolic blood pressure since early stages of life, and that

this rise was in parallel with the osteoporotic change in these mice. OPG-/- mice also presented a greater heart weight/body weight ratio than age-matched wild-type mice, suggesting that OPG plays a role in preserving heart structure. Kiechl et al. developed hepatocyte-specific RANK knockout ($RANK^{LKO}$) mice and compared them with wild-type mice. While $RANK^{WT}$ mice experienced insulin resistance after 4 weeks of a high-fat diet (NFD), $RANK^{LKO}$ mice did not. A very recent study demonstrated that mice lacking β-catenin specifically in osteoblasts exhibit postnatally decreased bone mass, increased glucose level, decreased insulin production, decreased fat accumulation and increased energy expenditure. OPG overexpression normalized not only the decreased bone mass but also the decreased fat accumulation and increased energy expenditure";

2) We have discussed the tissue specific effects of OPG:

"**ROLE OF OPG/RANK/RANKL SYSTEM IN BONE AND OTHER TISSUES**

The wide variety of cells and tissues in major organ systems such as the skeletal, vascular, and immune systems as well as other systems producing OPG, RANKL, and RANK support their role in the function of these organs (Figure 1). Typically, the OPG/RANK/RANKL axis regulates remodeling of bone as well as differentiation and activation of osteoclasts, and thus, the crucial equilibrium between formation and resorption of bone. RANKL binds to RANK on osteoprogenitor cells and controls osteoclastogenesis and bone resorption. OPG acting as a soluble decoy receptor, negatively regulates this interaction and competes with RANK, preventing RANKL-RANK interactions.

While OPG is expressed in the vessels of healthy mice, RANK and RANKL are not detected in the arteries of healthy adult mice. In contrast, RANKL and RANK have been discovered in the calcified arteries of $OPG^{-/-}$ mice and RANK expression occurred simultaneously with the appearance of multinuclear osteoclast-like cells[37]. These findings suggest that vascular OPG protects against RANK/RANKL induced osteoclast formation. In humans, RANKL and RANK are often undetected in the non-diseased vessel, while OPG is expressed in normal arteries. However, early as well as advanced human atherosclerotic lesions of carotid arteries and abdominal aortas manifest both RANKL and OPG immunoreactivity and mRNA expression.

Immune cells express OPG, RANKL, and RANK and these are believed to regulate

inflammatory and immune responses. Binding of RANKL to RANK augments dentritic cells' survival, enhances the immunostimulatory capacity of dentritic cells, and modulates activated T-cells. In particular, RANKL/RANK signaling in the immune system controls the development of thymocyte-mediated medulla, and the development of self-tolerance in T cells as well as the number of regulatory T cells (Treg). RANKL also regulates the production of proinflammatory cytokines in macrophages. An important function of OPG in the immune system is related to the cytotoxic ligand TRAIL, a potent activator of apoptosis. Binding of OPG to TRAIL inhibits cell apoptosis.

Yet, OPG, RANKL, and RANK have been demonstrated to be expressed in normal brain of rodents. Notably, in normal brain, RANKL/RANK signaling has been related to fever and body temperature control. The stimulation of RANKL/RANK signaling obtained by the deletion of OPG or the administration of RANKL has been demonstrated to prevent the exacerbation of infart volume as well as cerebral edema through the inhibition of the production of pro-inflammatory cytokines.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

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