Faculty, Health and Biomedical Sciences

Omega-3 fatty acid-rich fish oil supplementation prevents rosiglitazone-induced osteopenia in aging mice.

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Abstract

Rosiglitazone is an effective insulin-sensitizer, however, associated with bone loss mainly due to increased bone resorption, and bone marrow adiposity, and decreased bone formation.
We investigated the effect of the co-administration of fish oil (FO) rich in omega-3 fatty acids (FAs) on rosiglitazone (RSG)-induced bone loss in aging C57BL/6 mice and the mechanisms underlying potential preventive effect. Mice fed the iso-caloric diet supplemented with fish oil exhibited significantly higher levels of bone density in different regions compared to the other groups. In the same cohort of mice, reduced activity of COX-2, enhanced activity of alkaline phosphatase, lower levels of cathepsin k, PPAR-γ, and pro-inflammatory cytokines, and a higher level of anti-inflammatory cytokines were observed.
Moreover, fish oil restored rosiglitazone-induced down-regulation of osteoblast differentiation and up-regulation of adipocyte differentiation in C3H10T1/2 cells and inhibited the up-regulation of osteoclast differentiation of RANKL-treated RAW264.7 cells.
We finally tested our hypothesis on human Mesenchymal Stromal Cells (MSCs) differentiated to osteocytes and adipocytes confirming the beneficial effect of docosahexaenoic acid (DHA) omega-3 FA during treatment with rosiglitazone, through the down-regulation of adipogenic genes, such as adipsin and FABP4 along the PPAR /FABP4 axis, and reducing the capability of osteocytes to switch toward adipogenesis. Our findings demonstrate that fish oil may prevent rosiglitazone-induced bone loss by inhibiting inflammation, osteoclastogenesis, and adipogenesis and by enhancing osteogenesis in the bone microenvironment. Further clinical studies will be undertaken to establish this treatment regimen for the successful treatment of diabetic patients with rosiglitazone without adverse side effects on bone.

Background

RSG is an oral antidiabetic drug of the thiazolidinediones (TZDs) class which acts by increasing insulin sensitivity in peripheral tissues through binding to the nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR-γ) and affecting gene expression of glucose transporters to facilitate glucose uptake and used to treat T2DM. TZDs are the only class that specifically targets insulin resistance. PPAR-γ receptors are present on osteoblasts and adipocytes. RSG also directs the differentiation of MSCs toward the adipocyte lineage in BM, and since there is an inverse relationship between osteoblasts and adipocytes in the BM microenvironment (both derived from BM-MSCs), any stimulation of adipogenesis will reduce the number of osteoblasts leading to the formation of fatty BM, and imbalance bone remodeling eventually causing osteoporosis. The negative effects of RSG on bone metabolism including the significant reduction in bone mineral density (BMD) and the increase in fracture risk by twofold, are among the safety concerns that led to its withdrawal.

Considered that the safety of this powerful therapeutic agent is still under strict scrutiny, there is an imperative need to develop preventive strategies to reduce RSG-mediated bone loss in T2D patients. Both T2D and osteopenia are considered to be inflammatory diseases and fish oil (FO) rich in omega-3 fatty acids (FAs) are natural anti-inflammatory compounds. Several studies suggest that omega-3 fatty acids-rich fish oil exerts an anti-osteoporotic effect by attenuating inflammation and osteoclastogenic bone resorption. Therefore, we hypothesize that fish oil co-treatment attenuates rosiglitazone-induced bone loss in aging mice by its pro-osteogenic and anti-inflammatory, and anti-bone resorbing capacity.

Objectives

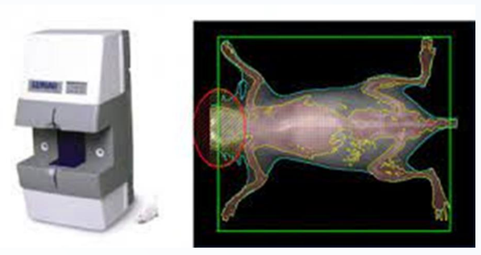
- In vivo animal study to determine whether fish oil co-treatment can attenuate rosiglitazone-induced bone loss in aging mice.
- Ex vivo tissue analyses to determine the effect of combined treatment on inflammation, bone formation, and bone resorption markers as part of the mechanisms of action.
- In vitro cell culture experiments to determine the effect of combined treatment on osteoclastogenesis, osteoblastogenesis and adipogenesis.
- In vitro human adipose-derived MSCs (AT-MSCs) culture to: **(1)** study the RSG-induced changes in the adipogenesis and osteogenesis to **(2)** test the effect of DHA omega-3 FA co-treatment in restoring the RSG-induced changes in the osteogenic and adipogenic setup by studying the gene expression modulation of linage specific markers and differentiations assays.

Study Design

In the current study, we have conducted three separate sets of experiments to evaluate the efficacy and elucidate potential mechanisms of omega-3 fatty acids rich fish oil in the prevention of rosiglitazone-mediated bone loss:

a. In vivo, we investigated the protective effects of FO against RSG-mediated bone loss in aging C57Bl/6 mice (12 mo old), a strain susceptible to obesity, hyperglycemia, and insulin resistance when fed omega-6 rich-corn oil (CO)-based diet. Mice were treated for 6 months with experimental diets. Dual Energy X-ray Absorptiometry (DEXA) was used to determine the treatment effect on bone mineral density (BMD).

Ex vivo bone marrow and splenocytes cells isolated from different experimental groups were tested for osteoclastogenic, osteoblastogenic bone markers and inflammatory markers



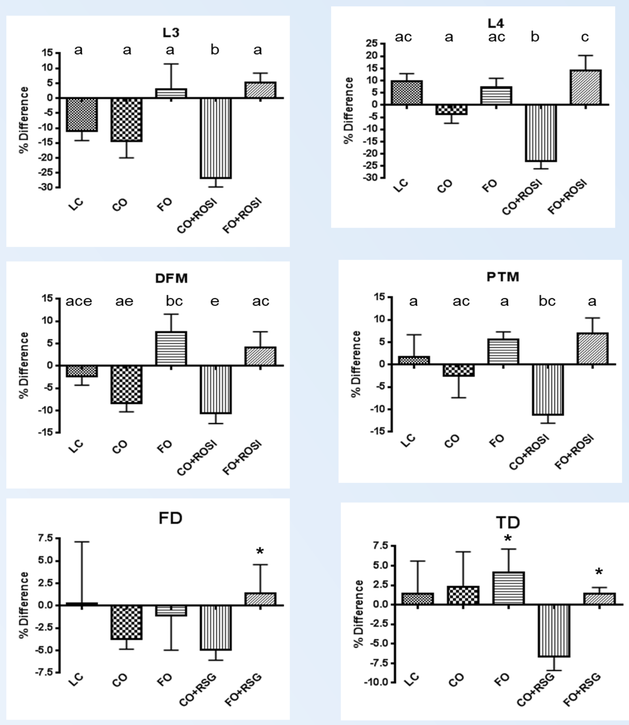
b. In vitro, we explored possible mechanisms by which the FO components, EPA and DHA, might reduce RSG-associated bone resorption. In particular, we evaluated the RANKL-stimulated osteoclast differentiation in RAW264.7 cells and the osteoclastic activity on sperm whale dentin slice, induced by parathyroid hormone-related protein (PTHrP) in young mouse bone marrow (BM) cells. Osteogenesis and adipogenesis induced by bone morphogenic protein-2 (BMP-2) in C3H10T1/2 cells in-vitro were also examined.

c. Finally, as an assay of human readout, we tested the effect of DHA on human adipose tissue-derived MSCs (AD-MSCs) during differentiation towards osteocytes and adipocytes in vitro, showing how gene expression is modulated.

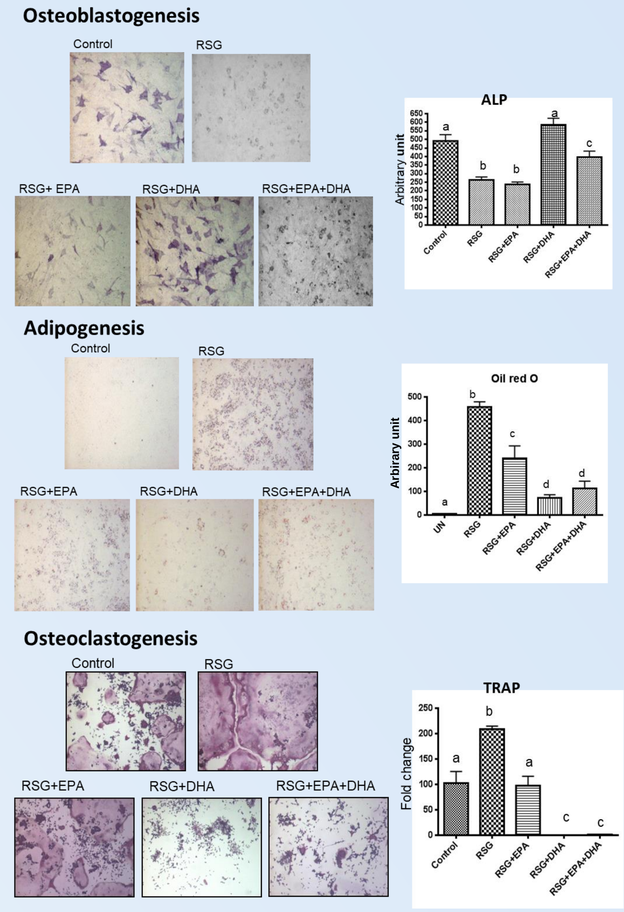
Abbreviations: FO, Fish oil; CO, Corn oil; RSG, Rosiglitazone; L, Lumbar; DFM, Distal Femoral Metaphysis; PTM, Proximal Tibial Metaphysis; FD, Femoral Diaphysis; TD, Tibial Diaphysis; SPL, splenocytes. ALP, Alkaline Phosphatase; COX, Cyclooxygenase; PPAR, Peroxisome proliferator activated receptor; MSC, Mesenchymal stromal cell; DHA, Docosahexaenoic acid; EPA, Ecosapentaenoic acid; IL, Interleukin; TNF, Tumor necrosis factor

Results

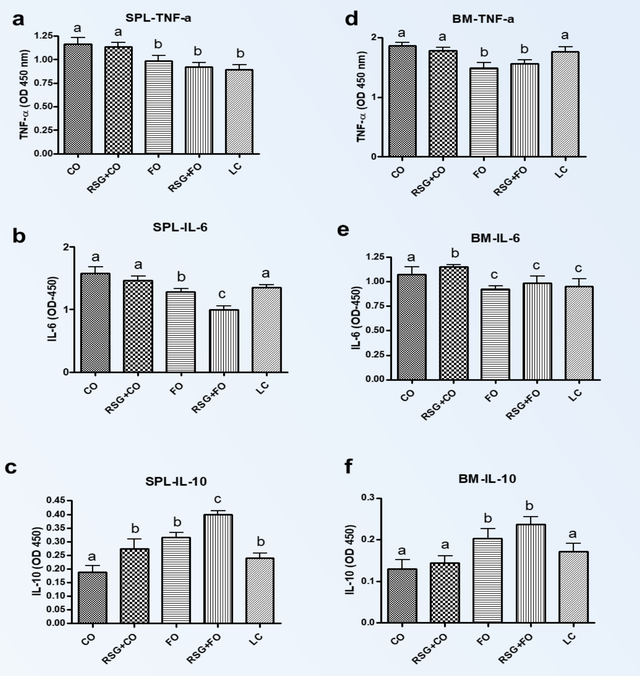
Effect of rosiglitazone alone or in combination with fish oil (FO) on bone mineral density (BMD) in aging mice



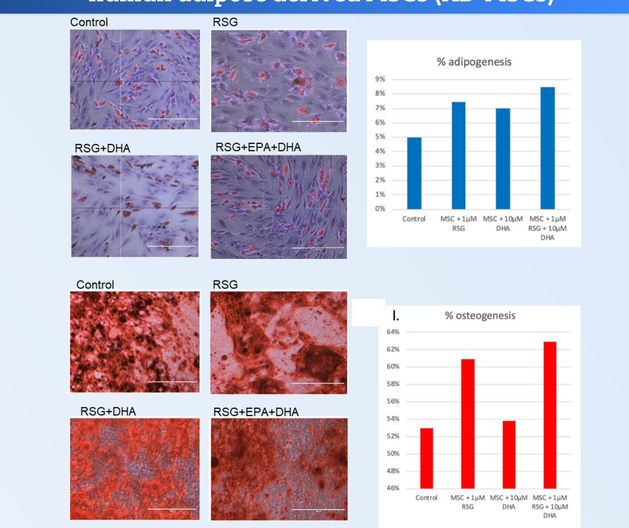
Effect of rosiglitazone alone or in combination with active components of fish oil (FO), EPA and DHA on osteoblastogenesis, adipogenesis, and osteoclstogenesis



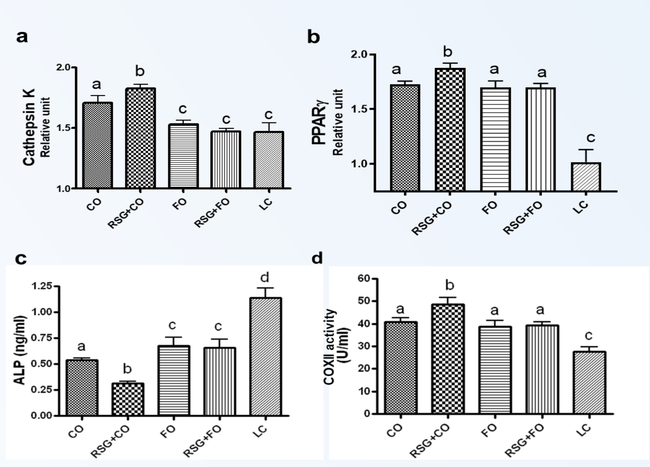
Effect of rosiglitazone alone or in combination with fish oil (FO) on inflammatory markers



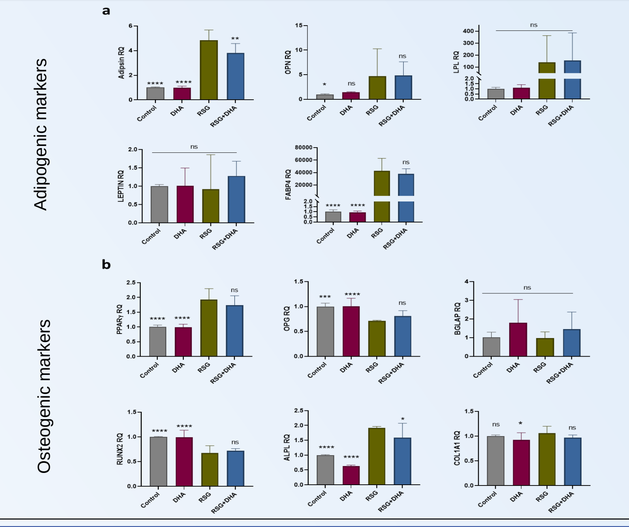
Effect of rosiglitazone alone or in combination with EPA and DHA on osteoblastogenesis, and adipogenesis in human adipose derived MSCs (AD-MSCs)



Effect of rosiglitazone alone or in combination with fish oil (FO) on bone markers



Effect of rosiglitazone alone or in combination with EPA and DHA on osteogenic, and adipogenic gene expressions in osteo-differentiated AD-MSCs.



Conclusion

- In vivo animal study data reveal that omega-3 fatty acids-rich fish oil co-treatment can prevent rosiglitazone-induced bone loss.
- Ex vivo bone marrow and splenocyte cell culture study data reveal that bone loss prevention by fish oil co-treatment is associated with increased osteoblastogenesis, decreased osteoclastogenesis, and decreased inflammation.
- In vitro osteoclast and osteoblast precursor cell culture study data reveal that active components of fish oil, EPA and DHA co-treatment revert rosiglitazone-induced reduction in osteoblastogenesis, induction in adipogenesis and induction in osteoclastogenesis.
- Human readout in vitro study using human adipose derived MSCs data reveal that active components of fish oil, EPA and DHA co-treatment can attenuate rosiglitazone-induced upregulation of adipogenesis, and down-regulation of osteogenesis by positively modulating adipogenic and osteogenic genes.
- Our impressive preclinical data warrant clinical studies to establish this treatment regimen for the successful treatment of diabetic patients with rosiglitazone without adverse side effects on bone.

References

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