Author’s response to reviews

Title: Different Skeletal Effects of the Peroxisome Proliferator Activated Receptor (PPAR) Alpha Agonist Fenofibrate and the PPAR Gamma Agonist Pioglitazone

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Version: 2 Date: 15 January 2009

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Dated: Our ref.: Your letter dated: Your ref.:
15.01.2009 2009/US

Dear Editor,

Enclosed you will find the revised manuscript: Different Skeletal Effects of the Peroxisome Proliferator Activated Receptor (PPAR) Agonist Fenofibrate and the
PPAR\# Agonist Pioglitazone.

The manuscript has been revised according to the comments from the reviewers, see enclosed. We hope that it is now acceptable for publication.

Yours sincerely

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Answers to reviewer 1

1. We have previously performed a pilot study where we investigated the effect of Wyeth 14643 compared to fenofibrate and pioglitazone on BMD in intact, female rats (Syversen U et al, PPAR-Alpha Agonists Increase Bone Mineral Density in Female Rats. In: Abstract at ASBMR 25th Annual Meeting. Minneapolis, Minnesota, USA 2003). We found a similar increment in BMD with the two PPAR alpha agonists. A paragraph about this, and the reference is now included in the text.

2. We find a bell-shaped curve which is a common observation in proliferation studies.

3. Lean body mass also includes liver weight. As expected, the fenofibrate group has an increase in liver weight. When we correct for this, we still find a significantly higher lean mass in fenofibrate rats and a lower lean mass in the pioglitazone rats. As proposed by the reviewer, we have made a correlation analysis between lean mass (corrected), BMD and cortical area. For all groups together there is a significant positive correlation between lean mass and BMD, as well as cortical area. The data on liver weight and corrected lean mass, and correlation analyses are now included in the results, and the discussion has been changed accordingly.

4. The gender of the rats is now included in the text. The cause of death in the pioglitazone group was aspiration in connection with gastric gavage in 3 of the rats, in one of the rats no reason could be found. These data are now included in the result section. We have no explanation why this happened only in the pioglitazone rats. No difference in the wellbeing of the rats was observed.

5. The data from the human preosteoclasts are briefly mentioned in the discussion, page 24, last line in the first paragraph.

6. We find that fenofibrate increases several markers of osteoblast differentiation, while pioglitazone only increases CD44 which is an unspecific marker. In preosteoblasts it may be a marker for proliferation or cell attachment.
1. The paragraph on adipokines has been made a little shorter and rephrased. Since the data on adipokines and bone are conflicting, we find it difficult to make an illustrative graph.

Answer to reviewer 2

General comments

Pioglitazone is a well described compound which is currently used in the clinic for treatment of type 2 diabetes mellitus. Fenofibrate belongs to the class of fibrates, which are also thoroughly described compounds, and has been used for many years in the treatment of hypertriglyceridemia. Fenofibrate has been found to be mainly a PPAR alpha agonist, while bezafibrate is a pan-agonist. A paragraph on this is included in the discussion, and a reference is included.

Concerning the molecular mechanism, both PPAR alpha and PPAR gamma agonists have been reported to inhibit NF-κB activation which is crucial in osteoclast differentiation. In our studies, however, we did not find any direct effect on in vitro osteoclast differentiation, and we therefore chose not to include this in the discussion.

Page 3: Corrected.

Page 6: BMD and BMC are now defined. The location for the Hologic is included in the text. The histomorphometry was performed in undecalcified bone, and was evaluated by two persons.

Page 7: Corrected.

Page 8: Corrected.

Page 9: Corrected.

Page 10: Corrected.

Page 11. FixDenat is part of the kit, this is now made more clear in the text. DEPC has been replaced by “nuclease free water”.

As the reviewer correctly states, there were several papers published in the 1990s demonstrating that beta-actin is reduced in MC3T3-E1 cells after administration of hormones that induce stellate cell morphology etc. There are also numerous articles published on MC3T3-E1 cells that use beta-actin as the only housekeeping gene.

In our experiment we observe an enhanced osteocalcin/beta-actin ratio after fenofibrate stimulation. Either this is due to increased osteocalcin expression, reduced beta-actin expression, or both, our conclusion is still that fenofibrate stimulates differentiation. The observed increase in plasma osteocalcin in the fenofibrate group supports this conclusion. We have used GAPDH as a reference gene in some of the differentiation studies with the same results as with beta-actin.

Page 12. M-CSF is now defined. Six parallels mean “six parallel wells”. This is now changed in the text.

Page 13. The osteolyse assay was a kit from Lonza. Primary human osteoclast
precursor cells. were also purchased from Lonza. This is now more clearly stated in the text.
Page 19. This is now more thoroughly discussed.