Author's response to reviews

Title: Zinc Transporter Gene Expression is Regulated by Pro-Inflammatory Cytokines- A Potential Role for Zinc Transporters in Beta-Cell Apoptosis?

Authors:

Lærke Egefjord (le@studmed.au.dk)
Jens Ledet Jensen (jlj@imf.au.dk)
Claus Heiner Bang-Berthelsen (clbb@steno.dk)
Andreas Brønden Petersen (Andreas.Petersen@studmed.au.dk)
Kamille Smidt (kcjs@farm.au.dk)
Ole Schmitz (os@farm.au.dk)
Allan Ertman Karlsen (aek@novonordisk.com)
Flemming Pociot (fpoc@steno.dk)
Fabrice Chimienti (f.chimienti@mellitech.com)
Jørgen Rungby (jr@farm.au.dk)
Nils Erik Magnusson (nm@farm.au.dk)

Version: 2 Date: 17 December 2008

Author's response to reviews: see over
December 17, 2008

Cover letter BMC

To the Editors

Re: MS: 8643934092342772

Zinc Transporter Gene Expression is Regulated by Pro-Inflammatory Cytokines- A Potential Role for Zinc Transporters in Beta-Cell Apoptosis?

Lærke Egefjord, Jens Ledet Jensen, Claus Heiner Bang-Berthelsen, Andreas Brønden Petersen, Kamille Smidt, Ole Schmitz, Allan Ertman Karlsen, Flemming Pociot, Fabrice Chimienti, Jørgen Rungby, Nils Erik Magnusson.

Dear Sirs,

Thank you very much for reviewing the above manuscript.

We have addressed the criticisms raised by the reviewers and revised the manuscript accordingly:

**BMC:** We have added a Competing interests section between the conclusions and Authors´ contributions

**Reviewer 1:**

i) Figure 2 has been changed and includes legends on the figure.

ii) The discussion now includes a section on intracellular zinc concentrations and cell death. A reference has been added (46).

**Reviewer 2:**

i) Figures and tables have been changed:

Figure 2 and 3: Sample numbers and SEMs have been added.
Figure 4: In this figure, which primarily describes the stability of the house keeping genes, we feel that SEMs would disturb more than clarify. Appropriate p-values for this figure are given in the results section. Sample numbers have been added and legends have been included on the figure.

A new table 2 has been prepared: This table shows the fold changes on a log2 scale and SEMs and sample numbers have been included.

Tables 2 and 4 contain p-values and are not estimated parameter values. As such no SEMs can be applied.

Table 5: Sample numbers and SEMs have been added.

Figure and table legends have been changed accordingly.

ii) The manuscript has been carefully revised with respect to the quality of the written English.

iii) Regarding the measurement of protein levels (major point 1): We agree that further studies should focus on protein levels as well. At present there is a lack of suitable antibodies and it is our expectation that this may be the case for some time. The title of the paper as well as the content reflects the caveat that gene-expressions are not the full truth. The amount of data presented here does suggest that (at the genomic level) cytokines affect this particular part of beta cell metabolism. We will continue our efforts to study also protein levels, however for the purpose of this paper which focuses on genomic changes we hope that gene-expressions will suffice. In the results section we have specified fold changes for the more sensitive transporter ZnT8.

iv) Regarding the mechanism for cytokine-induced alterations in gene expression (major point 2): Our aim was to study the consequences of cytokine exposure in a controlled way. Therefore cytokine receptor activation studies were not included. We do agree that this is an excellent subject for further studies. However, we feel that the present data fully supports the conclusion that changes in zinc transporter expression could be induced by the cytokines. In the discussion section we have addressed the need for further studies to determine the potential role for zinc transporters in cytokine mediated beta apoptosis.

v) Regarding the molecular mechanisms underlying apoptosis (major point 3): This has been clarified as also suggested by reviewer 1, by adding to the discussion and with a new reference as well.
Finally, we have carefully reviewed the statistics which have all been performed by our statistician, the second author of the paper, if the editor or the reviewers wishes, we will be happy to supply the documentation showing the statistical calculations.

We hope the corrections described above will satisfy you.

Yours sincerely

Nils E. Magnusson