Author's response to reviews

Title: NPC1 in Human White Adipose Tissue and Obesity

Authors:

Clara Bambace (clara.bambace@gmail.com)
Ingrid Dahlman (ingrid.dahlman@ki.se)
Peter Arner (peter.ärner@ki.se)
Agne Kulyte (agne.kulyte@ki.se)

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Lipid Laboratory
Department of Medicine
Karolinska Institutet
Stockholm, Sweden

To the Editor

\textit{BMC Endocrine Disorders}

Dear Editor,

Thank you for giving us the opportunity to resubmit our manuscript “NPC1 in Human White Adipose Tissue and Obesity” (1998960968850175).

We have performed the revision of the manuscript according to the comments from the reviewers. As you can see from the point-by-point response below, we have addressed each query and indicated how the manuscript has been altered accordingly. We believe that we have been able to address all the comments of the reviewers and we hope that you will find this improved manuscript adequate for publication in \textit{the BMC Endocrine Disorders}.

Yours sincerely,

Agné Kulyté, PhD
Response to reviewers: “NPC1 in Human White Adipose Tissue and Obesity”

Reviewer William S Garver:

1. The values in Table 1 are represented as mean ± SD. Although I suspect values in Figures 1 and 2 are also represented as mean ± SD, this should be included into the two figure legends.

   The correction has been added to the figure legends.

2. In the second paragraph of the discussion, it is written there is a discrepancy between decreased NPC1 gene dosage in mice which predisposes to weight gain and increased NPC1 gene expression in adipose of obese humans. I would like the authors to reconsider this point of view in light of our findings presented at the annual obesity meeting (Obesity 18:S54, 2010). In brief, we reported increased NPC1 gene expression (measured by increased amounts of NPC1 protein) in livers of obese wild-type mice fed a high-fat diet (I can send the results if authors are interested). These results are similar to the authors. Therefore, as proposed by the authors, the increased NPC1 gene expression in white adipose tissue of obese humans may be compensatory. The reference to expression of leptin served as a good example.

   We are grateful for the comment and we have revised the paragraph in the discussion accordingly. A new reference has been added (Garver et al., 2010) and the reference list has been revised accordingly.

Reviewer Kikuko Hotta:

1. The detail method of adipocyte differentiation should be described. And the data of day 0 or before differentiation data should be indicated in figure 2 C.

   We have described main points of the differentiation procedure of human mesenchymal stem cell-derived adipocytes in the Methods and Procedures section. The method has been published in full earlier and we added the new methodological reference (van Harmelen et al., 2005). Specifically, we do
not collect stroma-vascular fraction at day 0 or before differentiation because at that time point the fraction contains other cell types, nonadhering material and is not enriched with fat cell precursors. First point during differentiation is therefore day 4 when rosiglitazone (a stimulator of adipocyte differentiation) is removed from the differentiation medium.

2. **In Figure 2D, the explanation of 3 panels and “loading” are necessary.**

   Figure legend has been revised to explain the Western blot panels. Description of the Western blot quantification has been revised in the Methods and Procedures section. Specifically, NPC1 protein was normalized to the bovine serum albumin, the most abundant protein present in our protein lysates prepared from mature human adipocytes and representing the right proportional to the amount of NPC1 protein on the blot in a linear fashion.

3. **In the Result section, 3rd paragraph, Pearson’s coefficient should be indicated. The figures for correlation analysis may be useful for readers.**

   The Pearson’s coefficients have been indicated in the Result section and Figure 3 showing correlation analysis has been added.