Author's response to reviews

Title: Gene expression profiling in whole blood identifies distinct biological pathways associated with obesity

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Author's response to reviews:

November 9, 2010

Dear Dr. Demichelis,

We wish to re-submit manuscript # 1774792093408751 by Ghosh, et al., entitled ‘Gene expression profiling in whole blood identifies distinct biological pathways associated with obesity’ for reconsideration for publication in BMC Medical Genomics. We thank the reviewers for their helpful suggestions. We have revised the manuscript based on the additional recommendations of Reviewer 1 and have highlighted the areas of revision in red text in the body of the manuscript. Below are our point-by-point responses to the reviewers’ comments. We look forward to a favorable re-consideration of this work.

Sincerely,

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Responses to reviewer #1 (Robert Koza):

-Minor Essential Revisions

Authors should briefly and concisely address how variations in hematocrit among
individuals, especially between lean and obese, may affect interpretation of expression data in the discussion.

We agree with this comment and have added the following section to the Discussion on page 14 of the manuscript:

A gender-based sub-analysis demonstrated relative stability of the "apoptosis" and "oxidative phosphorylation" pathway ranks in both genders; in contrast, the "ribosome" pathway differed significantly in rank between females and males, suggesting a gender-specific effect (Additional File 7). Since a majority of genes upregulated in the obese subjects are highly expressed in erythrocytes and reticulocytes, we scaled the gene expression data independently by the expression of two erythrocyte-specific transcripts, hemoglobin D (HBD) and erythrocyte membrane protein, band 2 (EMPB2) and subjected the scaled data to gene-set enrichment analysis. Of the three pathways found to be differentially upregulated in the obese subjects, the "ribosome" pathway remained the top differentially expressed pathway (with the scaled data) whereas the "apoptosis" and "oxidative phosphorylation" pathways were no longer significantly enriched, with either of the scaled datasets. These findings suggest that an increase in erythrocyte/reticulocyte numbers in the obese (differential hematocrit) is a possible explanatory mechanism for the observed increase in transcript levels for "apoptosis" and "oxidative phosphorylation" in the obese subjects. The results for the "ribosome" pathway, in contrast, suggest a significant upregulation of the transcripts for the component genes of this pathway in the obese subjects, even after adjustment for erythrocyte-specific gene expression.

On page 15, we have also added a sentence as follows:

However, our results clearly demonstrate that inter-individual variations in hematocrit, especially between obese and lean subjects, may affect interpretation of expression data and should be considered as an important co-variate in future studies.