Doctoral thesis review

Title: Regulation of lipogenesis in human adipose tissue: Effect of metabolic stress, dietary intervention and aging.
Candidate: Veronika Šrámková, MSc.
Affiliation: Charles University, Third Faculty of Medicine & Université Toulouse III Paul Sabatier

The candidate has focused on the regulation of lipogenesis in human adipose tissue, which is the cutting-edge topic of high importance in the field. The thesis challenges four novel hypotheses (aims) related to aging, stress of endoplasmic reticulum, calorie restriction or very low calorie diet and (*de novo*) lipogenesis, and is based on two published papers (one as the first author and one as a co-author) and two extensively discussed related projects. The results provide valuable and novel data on metabolism of human adipose tissue.

This thesis complies with the general rules for doctoral theses at Charles University. It is written in English, well arranged, appropriately illustrated and contains only minor grammatical mistakes. The introduction is very polished, but the results and discussions are less readable. I would appreciate a paragraph deciphering the contribution of the candidate to the collective work. I assume that her primary objective was mRNA measurement and differentiation of human adipocytes. The discussion of Projects A and C is identical to the text published in the paper. I believe that the published text represents the consensual point of view of all authors, while I would very much appreciate the personal interpretation of the results by the PhD candidate herself.

Questions:

1) **Introduction**: Cellular senescence is the phenomenon by which the cells cease to divide. As the adipocytes are terminally differentiated cells and their half-life is relative long, does the senescence affect more mesenchymal stem cells or preadipocytes in adipose tissue? Could be the stress-induced premature senescence of preadipocytes reverted?
2) **Project A:** Thapsigargin is primarily considered as an inhibitor of SERCA2, thus interfering with calcium signaling. Did you considered effects on calcium flux, signaling, or SERCA levels?
3) **Project A:** Regarding the radiolabeling experiment, why did you use both 14C(U) glucose and 1-14C acetic acid? The advantage of this dual labeling is not explained. Also, you show a difference in 14C-labeled glycerol (Fig 1B), but it is not mentioned how did you measure this enrichment. How did you separated the labelled glycerol from other metabolites? Besides true neutral lipids, the difference in fatty acids and others (presumably phospholipids, sphingolipids?) seems to be important (Fig 1C). Could you comment on this with respect to cellular localization of various lipogenic processes and incorporation of the tracers?
4) **Project A:** You discussed that “chronic ER stress seen in obesity could have an impact rather on the newly recruited preadipocytes and thus could impair necessary renewal of AT”. With respect to the adipocyte half-life, how would you estimate the impact of such effect on adipose tissue of adult obese population?
5) **Project B**: You speculate that “GFD15 is a cytokine that might be induced by various cellular stresses, including mitochondrial dysfunction that may contribute to senescence and aging”,

related to Figures 14 & 15. Did you quantify any markers of mitochondrial dysfunction in your human samples?

6) **Project C:** You discuss that "the higher expression of CD36 observed after 2 days of calorie restriction could provide AT better FFA absorption capacities for the anticipated refeeding phase". Why would these cells synthesize more CD36 when all the other enzymes needed for downstream fatty acid (re)esterification into neutral lipids were downregulated? How would the cell handle the excess fatty acids? Did you measure protein levels of the involved enzymes? Did you explore also alternative pathways for fatty acids, e.g. sphingolipid synthesis in the ER?
7) **Project D:** You discuss a possible explanations for the discrepancy between 2-deoxyglucose uptake data, unchanged Glut4 expression and Akt phosphorylation. Why wouldn't you consider to use the golden standard 2DG uptake methods corrected for nonspecific transport of 14C-mannitol?
8) In general, many results are based on mRNA measurement and data normalized per several reference genes. Could you comment on how you selected the reference genes, especially between old and young subjects?

This thesis is well focused on basic research in human medicine. All studies were performed at the laboratories of the Charles University and the University in Toulouse, in well known groups in the field of lipid research. I would value highly the work on human material, especially the work with (pre)adipocytes in the culture and clinical studies with elderly patients. Some hypotheses were confirmed, some rejected, all specific aims met and results published. The candidate clearly demonstrated the ability of independent scientific work and thus I recommend this thesis to PhD defense.

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