

JoVE61884 "Isolation of viable adipocytes and stromal vascular fraction from human visceral adipose tissue suitable for RNA analysis and macrophage phenotyping"

Editorial comments

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

Response: The manuscript was proofread and modifications are highlighted with the track changes tool.

2. Please format the manuscript as: paragraph Indentation: 0 for both left and right and special: none, Line spacings: single. Please include a single line space between each step, substep and note in the protocol section. Please use Calibri 12 points

Response: The manuscript was formatted as required.

3. Please provide at least 6 keywords or phrases.

Response: We are now including 6 keywords.

4. Please ensure that the summary is between 10-50 word limit.

Response: Summary is 50 words.

5. Please ensure that the Abstract is between 150-300 words.

Response: Abstract is 202 words.

6. JoVE cannot publish manuscripts containing commercial language. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents.

For example: Agilent, FACS™ Lysing Solution, FACS Flow Sheath Fluid, FACS Aria III, FASCDiva software 6.0, etc.

Response: Commercial language was removed and referenced in the Table of Materials and Reagents.

7. Please include an ethics statement before the numbered protocol steps, indicating that the protocol follows the guidelines of your institution's human research ethics committee.

Response: Ethics statement and protocol number is now included in the revised version of the manuscript.

8. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as "could be," "should be," and "would be" throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a "Note."

Response: The manuscript is written in the imperative tense

9. The Protocol should contain only action items that direct the reader to do something.

Response: The manuscript includes only action items

10. Please ensure that individual steps of the protocol should only contain 2-3 actions sentences per step.

Response: Individual steps only contain up to 3 actions

11. The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections.

Response: the protocol does not include large paragraphs

12. Please ensure you answer the "how" question, i.e., how is the step performed?

Response: Each step contains a detailed description about how it was performed

13. 1: How do you identify different zones, etc.

Response: The visceral adipose tissue collection is one of the steps that we would like to record to be more explicit.

14. Please include gating strategy for FACS.

Response: Gating strategy was included in the manuscript.

15. There is a 10-page limit for the Protocol, but there is a 3-page limit for filmable content. Please highlight 3 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol.

Response: Steps were identified and highlight in the manuscript

16. Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account. The Figure must be cited appropriately in the Figure Legend, i.e. "This figure has been modified from [citation]."

Response: The figure 2 was replaced by a new original figure in the manuscript. Figure 3 and table 1 were removed.

17. Please remove the figure legend from the figures. Please include all the Figure Legends together at the end of the Representative Results in the manuscript text. Each Figure Legend should include a title and a short description of the data presented in the Figure and relevant symbols.

Response: Figure legends were removed and were included in representative results.

18. As we are a methods journal, please ensure that the Discussion explicitly cover the following in detail in 3-6 paragraphs with citations:

- a) Critical steps within the protocol
- b) Any modifications and troubleshooting of the technique
- c) Any limitations of the technique
- d) The significance with respect to existing methods
- e) Any future applications of the technique

Response: Discussion addresses a - e points.

19. Please do not abbreviate the journal titles in the references section.

Response: Journal titles in the references section are complete

20. Please sort the materials table in alphabetical order.

Response: Materials table is now in alphabetical order.

Reviewers' comments

Thank you for your comments which contribute to improving our manuscript. All modifications were made using the track changes in the manuscript.

Reviewer #1

Manuscript Summary:

Estrada-Gutierrez et al. describe a method for the isolation of adipocytes and macrophages from human visceral adipose tissue. Adipose tissue is harvested from the omentum of pregnant females at the time of Caesarean section. The authors' propose enzymatic digestion with collagenase and gentle centrifugation steps to preserve the floating adipocyte division and enable a good RNA yield. The stromal vascular fraction forms a cell pellet which has been characterised by flow cytometry. The methods are sufficiently described and comprehensible.

Major Concerns:

- The authors' declare they have isolated CD45-positive and CD45-negative macrophages using their flow cytometry strategy. The existence of CD45-negative macrophages is unsupported by existing literature. Current literature suggests macrophages are of the haematopoietic lineage, and it is unclear as to what the cell population is that the authors' have identified using their flow cytometry strategy. Further clarification is required, or simplification/omission of their method to remove the contentious flow cytometry speculations.

Response: As reviewer suggests, we eliminate the CD45 negative macrophages from the analysis. Then we are showing only a CD45 positive population in the corrected manuscript.

- The authors' suggest that their population of CD45-positive cells are 'recruited' macrophages and the CD45-negative cells are 'resident' macrophages. This is highly speculative and requires proof.

Response: This hypothesis was eliminated in the new version of the manuscript.

Minor Concerns

- Figure 2 appears to be a direct copy of a Figure in the authors' previous publication. Was this intentional? If so, I suggest their previous work should be cited here.

Response: The figure 2 was replaced by a new original figure in the manuscript.

- There are several minor English grammar mistakes throughout the text which require editing.

Response: The grammar mistakes were corrected.

- page 9: HLD-RA (4th line from the bottom) should be corrected to HLA-DR.

Response: HLD-RA was corrected to HLD-DR.

Reviewer #2

Manuscript Summary:

This manuscript described the detailed protocol for the isolation of mature adipocyte and immune cells from human omental adipose tissue. The authors also provided protocols for RNA isolation from mature adipocyte and immune cell Characterization using flow cytometry. The protocols that the authors provided are clear and pretty straight forward.

Major Concerns:

none

Minor Concerns

none