Overall responses to peer-reviewing of the submitted manuscript #33411

The manuscript has been revised as requested by one reviewer while the other considered that our report could be published without additional improvement. The queries of the editor have been taken into account. All the changes/implementations are highlighted in the revised typescript. We therefore hope that the enclosed revised version is now in agreement with the scope and style of Word Journal of Diabetes.

Specific responses to the comments of reviewer #02446585 :

1. linguistic revision is required throughout the manuscript.

Re: Thank you for your careful perusal. Our revised manuscript has been improved by all coauthors and by Anais Briot, one of our colleagues who is not English native, but who spent six years at the Univ. of California - Los Angeles and has acquired a good grasp of knowledge regarding scientific report editing and proofreading. We acknowledged her editorial support in the revised manuscript while we will not provide any certificate verification from any official or private proofreading & editing service.

2. *The results section should describe the specific finding details with out any comment or discussion, which should be presented in the discussion section.*

Re: Thank you for raising this concern. As the reviewer suggests, all the sentences of the Result section that contained a quoted reference have been moved to Introduction or Materials & Methods. Moreover some conclusive sentences at the end of chapters have been moved to Discussion.

3. *3. In table 2, statistical evaluation of the results from 3 experiments is questionable.*

Re: Thank you very much for your comment. We respectfully agree that the putative antilipolytic action of the molecule obestatin can been considered as poorly investigated (in term of quantity of samples) in the study reported in Table 2. However, it must be mentioned that the effect of another molecule, well-known for its antilipolytic action and used in parallel as a "golden reference" - namely the alpha2-adrenoceptor agonist UK 14304 - was clearly inhibiting the lipolytic action of isoprenaline in our small sample of three individuals. Thus, under conditions in which the reference agent was significantly exhibiting antilipolytic action, no such influence was detected for obestatin at the three concentrations tested. When we considered that the antilipolysis of the reference was detectable while not the unknown, it was meant that the latter was not antilipolytic. Please consider that we - and other researchers - have previously and repeatedly confirmed on samples of larger size that UK 14304 is a strong inhibitor of glycerol release. Among these reports, let us quote as the most recent:

* Carpéné et al. "Short-term and rapid effects of lysophosphatidic acid on human adipose cell lipolytic and glucose uptake activities."AIMS Molecular Science, 2016, 3(2): 222-237.
In this paper, we report that 1 µM UK 14304 totally abolishes the glycerol release induced by 100 nM isoprenaline on 14 different human adipocyte preparations.

Another example of the works we published a decade ago, using UK 14304 antilipolytic action as the reference for complete inhibition of isoprenaline-induced glycerol release is:

* Bour et al. " The imidazoline I2-site ligands BU 224 and 2-BFI inhibit MAO-A and MAO-B activities, hydrogen peroxide production, and lipolysis in rodent and human adipocytes. " Eur. J. Pharmacol., 2006, 552, 20-30. In this work, complete blockade of lipolysis by UK14304 was evidenced on 8 individual cases.

That was for the pertinence of our test and its supported interpretations. Regarding the statistical aspect of our data processing, and as far as we know, the two-tailed *t*-test is applicable to compare two samples of size three. Historically, the very first demonstration of

the *t*-test by the statistician "Student" was in an application to sample sizes of size four. Of course, with such limited sample size, only large, clear-cut effect will be statistically significant (as for UK 14304). That is maybe the questionable aspect of statistical evaluation raised by the reviewer Please note that authors were aware of such limitations since they used the term "clear-cut antilipolysis" in the corresponding section of Results. The reviewer is therefore right in suggesting that we have under-evaluated the probability that obestatin could moderately impair lipolysis, in such a manner that would have been evidenced only in a cohort study.

Moreover, would the reviewer notes that what we named n = 3 actually reflects a larger number of observations since glycerol determination was performed in duplicate for each of the three adipocyte preparations, and for each of the three doses tested. In all, it can be advanced that table 2 illustrates that obestatin did not enable to detect alteration of isoprenaline lipolytic effect in a lumped sum of eighteen measurements while a total of six determinations (three duplicates) was sufficient to confirm the antilipolytic effect of the alpha2-agonist of reference. This was therefore a negative observation that did not require further efforts and this preliminary approach prompted us to spare time and energy in avoiding additional observations.

Nevertheless, we respectfully agree with the referee that a number of observations larger than 3 helps in definitely describing the obestatin action since human material exhibits inter-individual variations. However, to answer to the reviewer comment by adding complementary observations, it will be necessary to perform other assays of lipolytic activity in novel human adipocyte preparations Unfortunately, the accessibility to subcutaneous human adipose tissue is a time-consuming task: sufficient material cannot be collected in a short time revision period since the flux of patients undergoing lipectomy and giving informed consent is aleatory. As no other additional experiment was requested, we did not initiate such experiments. Additionally, there is already in Figure 2 another illustration of the lack of strong antilipolytic effect for obestatin at two concentrations. This was evidenced against 5 nM isoprenaline in a total of 7 cases with only approx. 10 % inhibition of lipolysis and therefore confirmed our preliminary observations of Table 2. Lastly, please, note that even with n = 10, obestatin did not reveal any interference with insulin stimulation of glucose uptake.

Specific responses to the comments of reviewer #02446524 :

Excellent work done.

Re: Thank you very much for your fair review of our manuscript. We sincerely acknowledge your careful perusal and your satisfactory perception of our message. In our revised version it still appears clearly that obestatin induces several effects in human adipocytes, the amplitude of which is more limited than its insulin-like effect previously reported on cultured murine preadipocytes and confirmed in our study. Thus, in human adipocytes, obestatin is not a valuable insulin-mimicking agent and this confirms a part of previous observations made in other models though being opposite to another reports claiming insulin-like properties for this ghrelin-related peptide.