POINT-BY-POINT ANSWERS TO REVIEWER AND EDITOR:
Manuscript NO.: 64514, Basic Study, entitled :
High doses of catecholamines activate glucose transport in human adipocytes independently from adrenoceptor stimulation or vanadium addition
by: Carpéné C, Grolleau J-L, Boulet N, Morin N.

REVIEWER #1:

General comment) " This study investigated catecholamines activate glucose transport in human adipocytes; "

Thank you for your careful perusal.

1) "The introduction and discussion were too much redundant, which need more concise expression."

These suggestions have been taken into account: the introduction is shorter, having 10 lines less in the revised version. The requested changes have been performed in the text of Ms and they appear as red font, except for the deletions.

2) "The insulin resistance of adipocytes in each group should be evaluated;".

The authors do not understand the referee's comments, what is meant by insulin resistance of adipocytes? In the legend of Fig 1 it is clearly stated that baseline of 2-DG uptake is increased with 100 nmol/L insulin from 0.45 ± 0.04 to 1.29 ± 0.12 nmol /100 mg cell lipids / 10 min. This is the mean ± SEM from adipocytes preparations from 10 individuals, which is highly similar to the values reported in Fig 3, obtained from 7 other individuals. This corresponds to a three-times increase of basal glucose transport in response to insulin, as concisely noted in the first paragraph of the Results section. This three-fold increase over basal uptake (also visualized in figure 6 for 12 additional individuals) belongs to the normal range of human adipocyte responsiveness to insulin, which is very different from the ten-fold increase found in adipocytes from laboratory young rats or the six-fold increase found in mice. We are aware of these interspecific differences since we reported in figure 3 a comparison between glucose transport activity in mouse and human adipocytes, in which the response to insulin is illustrated by a red circle, and since we commented them in Discussion. Whether the referee means insulin responsiveness of each group of the subjects tested for successive exploration of the catecholamine effect on glucose transport, the authors argue that in each group we have detailed the baseline and the insulin-stimulated levels of 2-DG uptake, even when they have been set respectively at 0 and 100 %. Let us also comment that, as stated in Materials and Methods, the range of the Body Mass Index was relatively narrow for the total of 34 subjects included in this preclinical study since we reported in M & M that it was: " 25.04 ± 0.65 kg/m2 (range: 21-41)". Please confer to our previous analysis to see the link between insulin responsiveness of human subcutaneous adipocytes and BMI (Carpéné et al, Medicines, 2019, 6, 89; doi:10.3390/medicines6030089) to see that, in non-diabetic individuals, there is a decrease in insulin responsiveness of adipocytes, but when BMI remains lower than 30, a minimal two-fold increase of baseline remains obtained with insulin, and although it is lower than the maximal seven-fold increase observed in the younger and slim women undergoing reconstructive surgery, this is far from being overt insulin resistance. The Ms is therefore not modified regarding this unclear comment.

3) " it need some experiments on signal transduction;".

The authors respectfully disagree with the comments of the referee. The test of the waste metabolites of catecholamine catabolism, namely pyrocatechol and benzoquinone (figs 3, 4), as well as the test of the sensitivity to amine oxidase inhibitors (fig 5) belong to experiments aiming at deciphering the signal transduction. Nevertheless we added in the text of Results,

data about the separate effect of each amine oxidase inhibitor tested. As these inhibitors were not able to inhibit the catecholamine action, neither MAO nor SSAO supported the observed insulin-like effect. When the inhibitors are ineffective, it is hard to tell about signal transduction, but the authors want to remind that in human adipocytes, the effect of benzylamine on glucose transport is depending on its oxidation by SSAO (Morin et al, J Pharmacol Exp Ther 2001; 297: 563-572; PMID: 11303044) and this is somewhat signal transduction, reinforced by the fact that mice genetically invalidated for SSAO cannot exhibit such insulin-like effects of benzylamine (Jargaud et al, J Physiol Biochem. 2021; 77, 141-154. doi 10.1007/s13105-020-00756-y).
Although it was suspected that the effects of the catecholamine on glucose transport were not depending on adrenoceptor activation since occurring mainly at millimolar doses (figs 1, 5), we also tested adrenergic agonists and antagonists to verify whether the hypothesis of adrenoceptor-independent action could be ruled out. Into our opinion, this is also pharmacology of signal transduction and was reported in figure. Unfortunately, these mechanistic investigations could not depict the involved mechanism. Therefore examining signal transduction of G-coupled proteins was not applicable here. Only the passages corresponding to the tests of ROS-mediated mechanisms (fig 7) showed a probable mediation by ROS. To answer to the reviewer a more constructive comment is necessary: what kind of ROS-mediated signal transduction or oxidative stress should have been explored (NF kappa, MAPKs, Keap1-Nrf2-ARE, PI3K-Akt, or redox-sensitive phosphatases?) taking into account that all the signalling cascades leading to gene regulation requires more longer ROS exposure than the time required to activate glucose transporter activation. For sure, a major limitation of our study is its lack of a complete description of the transduction cascade leading to rapid glucose transport activation by adrenaline, but this was clearly stated in the previous version and is still reported in the revised Ms. This defect in mechanistic approach cannot hamper to disseminate soon the descriptive message that human adipocytes behave differently from rodents adipocytes, for which we already reported that catecholamines activate glucose uptake in a vanadate-dependent manner (Fontaine et al. World J. Diabetes, 2020, 11 (12): 622-643).

REVIEWER #2:

General comment) *" Dear Author, I have evaluated manuscript titled "High doses of catecholamines activate glucose transport in human adipocytes independently from adrenoceptor stimulation or vanadium addition" for publishing to World Journal of Diabetes. The authors investigated adrenalin and noradrenalin stimulation on glucose transport as 2-DG uptake with and without vanadium in both of rodents (mouse or rat) and human adipocytes. "*

Thank you for your careful perusal.

1) "The title of the manuscript completely reflect of the study, but I think authors may change the title more striking than before; may be added or changed in line with research results."

These remarks are entirely justified. However, we cannot find clearer and falsely more striking bullet sentence to entitle our present work since it's true that we demonstrate a stimulation of glucose transport by catecholamines in human adipocytes, while it is occurring only with high doses, under in vitro conditions that are probably extra physiological. Regarding the signalling of this catecholamine action, we could not depict the exact cascade of events but can ascertain that there was no mediation by adrenoceptor activation, or by amine oxidase oxidation, while there was no potentiation by the phosphatase inhibitor vanadium.

2) "The abstract summarize and reflect the work sufficiently"

Thank you.

3) "Key words reflect the focus of the manuscript"

Thank you.

4) "Yes, the manuscript adequately describe the background, present status and significance of the study. "

Thank you.

5) "The manuscript describes applied methods in adequate. "

Thank you.

6) "The research are achieved by the experiments in this study. In human adipocyte, there were investigated response to insulin with 100 µmol/L adrenaline or noradrenaline activated 2-DG uptake, and dosage quantity of adrenalin, noradrenalin together with natural amines incubation with or without vanadium. Also, there were presented the results of pargyline, semicarbazide, and benzylamine or methylamine effects on the 2-DG uptake in human adipocyte. "

Thank you for careful perusal.

7) "The manuscript interprets the findings adequately and appropriately, and highlighting the key points concisely. The findings and their applicability are stated clear. The discussion is accurate and the paper's scientific significance is sufficient. "

Thank you.

8) "In Figure 1, it should be corrected "control" word the following sentence; A significant influence of vanadium when compared to respective control was observed. It means basal or without vanadate ones? In Figure 3, in X axis, there are shown used concentrations of pyrocatechol, benzoquinone, P+van, and B+van as log(M); but in the result section, from µmol/L up to 1 mmol/L for pyrocatechol (as below); so there should be same measurements between manuscript and figure (also it should be corrected in each figure) for used each agents. "The metabolite pyrocatechol, formed by a benzene core carrying two hydroxyl substituents, was inefficient on glucose transport in mouse and in human adipocytes when tested alone from 1 µmol/L up to 1 mmol/L (Figure 3)." Also, Fig 3 should be re-drawn clearer and more uderstandable for readers, are the "c" significance which compares with each agent to basal one? In Fig 4, "c" significance shows different from basal for adrenaline or noradrenaline, but "c" shows the difference glycerol release between adrenaline and noradrenaline. So, it should be re-drawn given meaning under the Fig 4. In Fig 6, there are no using of RX 821002, blocking 2-ARs and pan-antagonist bupranolol for blocking the -ARs; so authors should add the results related to agents of RX 821002 and bupranolol into Fig 6. In the result following of Fig 6, there were written continuing sentence: "adrenalin at 100 µmol/L increased the basal 2-DG uptake by approximately twice, and this was not impaired by 10 µmol/L of each of the antagonists (basal: 0.34 ± 0.02, adrenaline: 0.66 ± 0.04; adrenaline + RX 821002: 0.61 ± 0.06; adrenaline + bupranolol: 0.58 ± 0.04 nmol 2-DG uptaken / 100 mg lipids /10 min; n = 4; NS)" But in the figures, there are no related figure about adrenalin, and antagonist, and adrenalin+RX 821002, adrenaline + bupranolol. If authors will not present the results in the figures, they should state in the manuscript. "

Legend of Fig 1 was corrected: as recommended by the reviewer, it is clear now that the statistical mark indicates a difference between condition with vanadate to the corresponding condition without vanadate.

Legend of Fig 3 was corrected accordingly to the remarks, clearly indicating that the agents were tested from 1 µmol/L to 1 mmol/L. Nevertheless, the X-axis remains in logarithmic scale, and the label 'log(M) agent' has not been changed. As noted by the reviewer, the "c" significance compares each agent to basal condition, and this is indicated clearer in the legend and better visualized in the novel figure 3 for 1 µmol/L insulin and 1 mmol/L benzoquinone.

For Figure 4, we apologize for having created a trouble to the reviewer, but we were very far from thinking that a reader could find a possible difference between adrenaline and noradrenaline since their dose-response curves are obviously superimposed, even at the highest tested dose of 10^{-3}M (= 1 mmol/L), in which the respective error bars are overlapping. Since it was difficult to put at the same place two statistical marks, we still use the brace to mark both catecholamines. However, the last sentence of legend was modified into " The doses of adrenaline and noradrenaline ranging between 1 µmol/L and 1 mmol/L induced a response different from basal at: c $P < 0.001$." to remove any doubt for the readers. Evidently, there was no difference between the lipolytic response to adrenaline and that of noradrenaline, while these results clearly indicated that pyrocatechol and benzoquinone are not lipolytic.

Regarding Fig 6, we are surprised that the reviewer associates the sentence copied from the body text of results to comments about this figure, which is limited to the test of adrenoceptor

agonists, and not antagonists. The sentence copied/ pasted by the reviewer is dealing with the lack of effect of antagonists and is containing only 4 mean values. This does not deserve to built a novel figure or table: the sentence was therefore better separated from the comments of figure 6, by a novel paragraph introduced as following: " In additional experiments performed to study the sensitivity to antagonists...". Moreover, the mention 'not shown' was added at the end of data given between parentheses. Accordingly, the subtitle of the section has been modified into two halves: " Is there a direct activation of hexose uptake in human adipocytes by adrenoceptor agonists, or a blockade by adrenergic antagonists?". Thus, as proposed by the reviewer, all these corrections are clearly stated in the revision and figure 6 is entirely commented.

9) "Yes, the manuscript meet the requirements of biostatistics. "

Thank you.

10) "Yes, The manuscript meet the requirements of use of SI. "

Thank you.

11) "Yes, the manuscript cites appropriately references. "

Thank you, nevertheless in response to editor we have deleted necessary references about the regulation of glucose transport in adipocytes by natural amines, to limit the auto-citations. This was also a consequence of shortening the introduction.

12) "The manuscript well, concisely organized and presented, its grammar accurate and appropriate."

Thank you.

13) "Yes, the author prepare the manuscript according to the appropriate research methods and reporting."

Thank you.

14) "Yes, Yes, it meet the ethic requirements. After these corrections, this manuscript may be accepted, Sincerely Yours."

Thanks for your positive perception of our message and your constructive comments.

SCIENCE EDITOR:

1 Scientific quality: *The manuscript describes a basic study of the high doses of catecholamines activate glucose transport in human adipocytes independently from adrenoceptor stimulation or vanadium addition. The topic is within the scope of the WJD. (1) Classification: Grade B and Grade C; (2) Summary of the Peer-Review Report: The authors investigated adrenalin and noradrenalin stimulation on glucose transport as 2-DG uptake with and without vanadium in both of rodents (mouse or rat) and human adipocytes. However, the introduction and discussion were too much redundant, which need more concise expression. The questions raised by the reviewers should be answered;*

Thank you. All these points have been corrected in the R1 version. The authors were initially thinking that there was no limitation of space for their report. Many introductive sentences have been removed, and several in Discussion as well. But Results section required additional sentences to answer to reviewers' requests.

(3) Format: There is 1 table and 7 figures. (4) References: A total of 62 references are cited, including 6 references published in the last 3 years; (5) Self-cited references: There are 25 self-cited references. The self-referencing rates should be less than 10%. Please keep the reasonable self-citations that are closely related to the topic of the manuscript, and remove other improper self-citations. If the authors fail to address the critical issue of self-citation, the editing process of this manuscript will be terminated;

Thank you. The old self-references of the original version, namely: 11) Carpene 1998, 13) Lafontan 1985, 14) Carpene 1983 , 29) Carpene 1993, 30) Morin 2002, 31) Visentin 2003, 32) Les 2016, 33) Carpene 2003, 34) Carpene 2018, 37) Marti 2001, 39) Iglesias Osma 2005, 42) Carpene 2016, 44) Enrique Tarancon 2000, 45) Missaoui 2008, 46) Pizzinat 1999, 47) Fontana 2001, 48) Carpene 2006, 51) Fischer 1996, 61) Marti 2004 have been deleted, while some of them have been replaced by quoting equivalent reports or reviews from other research teams. In several cases, these references were irreplaceable and the corresponding passage of discussion has been deleted (e.g. work on amine oxidase substrates and tungstate).

In the revised version, the remaining quoted previous works of the corresponding author are: Fontaine 2020, Carpene 2019, Carpene 2020, Morin 2001, Mercader 2010, Marti 1998. Thus, the current proportion of auto-citation is lowered to 10%.

(6) References recommend: The authors have the right to refuse to cite improper references recommended by peer reviewer(s), especially the references published by the peer reviewer(s) themselves. If the authors found the peer reviewer(s) request the authors to cite improper references published by themselves, please send the peer reviewer's ID number to the editorialoffice@wjgnet.com. The Editorial Office will close and remove the peer reviewer from the F6Publishing system immediately.

Does not apply.

2 Language evaluation: *Classification: Grade B and Grade B.*
3 Academic norms and rules: *The authors provided the Biostatistics Review Certificate, The ARRIVE Guidelines and the Institutional Review Board Approval Form. No academic misconduct was found in the Bing search.*
4 Supplementary comments: *This is an invited manuscript. No financial support was obtained for the study. The topic has not previously been published in the WJD.*

OK, thank you.

5 Issues raised: *(1) The authors did not provide original pictures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor; and (2) The "Article Highlights" section is missing. Please add the "Article Highlights" section at the end of the main text.*

OK, see above. Corrections/modifications are indicated in red in the R1 version. The figures have been organized into a single PowerPoint file, and submitted as "64514-Figures.ppt" on the system as recommended. The probable position for insertion in the text is now highlighted with red font in the revised version. The "Article Highlights" section, which was located before acknowledgements, is placed now at the end of main text.

6 Recommendation: *Conditional acceptance.*

OK, thank you. We hope that we have addressed correctly all the required modifications/improvements in the enclosed R1 version. All required changes have been done by adding only a minimal number of sentences for answering to reviewers by keeping Ms as concise as possible

COMPANY EDITOR-IN-CHIEF: *I have reviewed the Peer-Review Report, full text of the manuscript, and the relevant ethics documents, all of which have met the basic publishing requirements of the World Journal of Diabetes, and the manuscript is conditionally accepted. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office's comments and the Criteria for Manuscript Revision by Authors.*

OK, thank you.