

POINT-BY-POINT ANSWERS TO REVIEWER AND EDITOR:

Manuscript NO.: 59150, Basic Study, entitled :

Vanadium-dependent activation of glucose transport in adipocytes by catecholamines is not mediated via adrenoceptor stimulation or monoamine oxidase activity

REVIEWER #1:

*General comment*) " The results of the present study confirm the role of vanadium as a potential anti-hyperglycemic agent. The authors demonstrate, through different well-carried out experiments, that some amines at certain concentrations are capable to stimulate glucose uptake in the adipose tissue only in the presence of vanadium, and that this mechanism could be due to a possible salvage pathway to regulate or prevent the excess of lipotoxicity. "

Thank you for your careful perusal.

*Minor concern 1)* " Statistical analyses. Please indicate the value of p and the post-hoc test employed."

These suggestions have been taken into account: Materials and Methods (M & M) are now modified by including the name of the post-hoc test: it was the Dunnett's multiple comparisons test that was used. Indeed, data of hexose transport were expressed as nmoles of 2-DG uptake, or as relative to baseline, or even as percentage of insulin stimulation. Irrespective of the unit used, data were distributed normally, since -for example- the percentages were not % of proportion (fixed between 0 and 100), and several experimental values exceeded 100% (conditions of overstimulation) while others were lower than 0% (conditions of strong inhibition). Thus, we assumed that ANOVA applied as well as its post-hoc tests, for which when comparing different means to a control mean, the Dunnett's test is appropriate. The mention to "paired t test" remains in the revised version of M & M since this applies for figure 2, in which the effect of vanadium was tested on other factors affecting glucose uptake in strictly parallel conditions, (now clarified in the legend). The requested changes have been performed in the text of M & M and they appear as red font in the text. Regarding the indication of P values, the authors respectfully disagree with the referee since we found that the P values are indicated in the legend of figures 3, 4, 5, 7, 8, 9, 10 and 11; while in figs 2 and 6 it is specified that the factors studied (i.e. vanadium and RX 821002, respectively) were without significant influence on the measured parameters, thereby with  $P \gg 0.05$ . As the authors suppose that the figure 1 was the concern, since it was lacking of such indications or asterisks, we have now indicated in the legend that the seven most efficient amines exhibited significant activation at  $P < 0.001$ . In fact, we think that there are too numerous columns (100) in the figure 1 to add stars to each significant condition. Let us repeat here that, as mentioned in the body text of Results, collected data were retrieved and displayed according to the rank order of the effect observed with 1mM of each amine in the presence of vanadium. So, the ordered list used for Y-axis of fig 7 in itself is almost sufficiently indicative of the hits, which are located in the upper part.

*Minor concern 2)* " Material and methods: The authors indicate a total  $n=53$ . Did the authors use the same animal to test the 25 amines? How was distributed the 53 animals for the different experiments? "

Corrected sentences focused attention on the number of rats used for each part of the screening approach. The first part of screening compared the effects of two doses of each of the 25 amines tested, with/without insulin or vanadium; while the second part aimed at deciphering the mechanism involved in the stimulation of hexose uptake observed with catecholaminergic hits.

Although all the corrected sentences seem to answer to the reviewer's comments and to describe in a clearer manner the experiments we performed, we present below an exhaustive

list of the rats used, giving clearly the distribution of the 53 rats, but in such a manner that we thought that I cannot be presented in the published version of a basic research article, for the sake of concision:

Rats (n)	test	figure/text	cumulated cases
3 cases	for each of the 5 blocks of 5 amines	(fig 1 )	15
4 cases	for +/- vanadium on controls and inhibitors	(fig 2 )	19
4 cases	for 3 inhibitors of AOs	(fig 3 )	23
4 cases	for a dopaminergic antagonist	(fig 4 )	27
4 cases	for noradre ou adre + RX antagonist	(fig 6 )	31
4 cases	for 2 alpha-adrenergic antagonist	(fig 7 )	35
4 cases	for 3 beta-adrenergic antagonist	(fig 8 )	39
4 cases	for test of prazosin 10 $\mu$ M	(not shown, in text)	43
4-6 rats	for alpha + beta-adrenergic antagonists	(fig 5 )	49
4 cases	for lack of effect of other adrenergic blockers	(not reported)	53

The last set of beta-adrenergic blockers, which led to inconclusive results was not included in the reported results, save for its negative and positive controls (insulin, amines, vanadium, which contributed to a minor part to the results retrieved in fig 1, and reaching n = 53 observations). This latter pharmacological test in rat adipocytes was omitted in our report since all co –authors agreed that the studies performed with the " $\beta$ -less" mice were much more demonstrative about the effects of catecholamines + vanadium on glucose handling by adipocytes. As the number of these mice used for the study was not concerned by reviewer's remarks (4  $\beta$ -less and 13 WT, as indicated in p 5), the corresponding passage of manuscript was not modified.

#### SCIENCE EDITOR:

*The manuscript describes a basic study of the vanadium-dependent activation of glucose transport in adipocytes. The topic is within the scope of the WJD. The study is of interest, especially in the expansive field of diabetes and metabolic syndrome, entities that are growing worldwide and demands new mechanistic knowledge and new therapeutic alternatives.*

These editor remarks are entirely justified. Thank you for your careful perusal.

*However, the value of P and the post-hoc test employed should be indicated. The questions raised by the reviewer should be answered.*

OK, see above. Corrections/modifications are indicated in red in the R1 version.

*This is an invited manuscript. The topic has not previously been published in the WJD. 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.*

Each figure is now uploaded as .pptx . The probable position for insertion in the text is now highlighted with red font in the revised version.

**COMPANY EDITOR-IN-CHIEF:** *I have reviewed the Peer-Review Report, the full text of the manuscript and the relevant ethics documents, all of which have met the basic publishing requirements, and the manuscript is conditionally accepted with major revisions. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report and the Criteria for Manuscript Revision by Authors. Before final acceptance, authors need to correct the issues raised by the editor to meet the publishing requirements..*

All required changes have been done by adding only a minimal number of sentences to keep Ms as concise as possible.