POINT-BY-POINT ANSWERS TO REVIEWER AND EDITOR:
Manuscript NO.: 66383, Basic Study, entitled :
Increased monoamine oxidase activity and imidazoline binding sites in insulin-resistant adipocytes from obese Zucker rats

REVIEWER #1:

General comment) " The manuscript 02817363 theoretically studied the activation of glucose uptake activation by hydrogen peroxide in adipocytes using radiolabeled non-metabolizable analog of glucose [3H]-2-DG for hexose uptake and [3H]-glucose for lipogenic activity. The study found that the adipocytes from obese the Zucker rats exhibit increased monoamine oxidase (MAO) activity and imidazoline binding site number. But, perhaps a number of issues should be clarified to improve the overall quality of this manuscript. "

Thank you for your careful perusal.

Concern 1) " Semicarbazide-sensitive amine oxidase (SSAO) and monoamine oxidases (MAO) were highly expressed in adipocytes, and hydrogen peroxide was generated after SSAO and MAO activated. And Carpéné et al (2007) had found that fat deposition was reduced by combined inhibition of monoamine oxidases and semicarbazide-sensitive amine oxidases in obese Zucker rats. According to the published literature, whether were the results of the increased monoamine oxidase (MAO) activity and imidazoline binding site number also obtained the same decreased fat deposition or glucose uptake? If yes, is there any innovation for this manuscript? Reference Carpéné C, Iffiú-Soltesz Z, Bour S, Prévot D, Valet P. Reduction of fat deposition by combined inhibition of monoamine oxidases and semicarbazide-sensitive amine oxidases in obese Zucker rats. Pharmacol Res. 2007, 56(6):522-30."

The doubt raised by the reviewer is unfounded. In one of our previous works quoted as 'Reference' by the reviewer, we treated Zucker obese rats with amine oxidase inhibitors for 3-5 weeks. There were several groups of **obese rats treated** with either pargyline (MAO inhibitor) or semicarbazide (SSAO inhibitor) that were **compared to untreated obese only**. We found that inhibition of amine oxidase activity was accompanied by a limitation of body weight gain, and that the antilipolytic action of tyramine (a substrate of both MAO and SSAO in rodents) was reduced in the treated rats. We proposed therefore that the blockade of the antilipolytic action of tyramine, or any other MAO/SSAO substrate, was involved in a greater mobilization of fat stores, and therefore in the slimming effect of MAO and SSAO pharmacological inhibitors in obese rats. No treatment was performed/studied in the lean rats. Moreover we have never compared the amine oxidase activity in the adipose depots of obese and lean Zucker rats.

While the obese rats are homozygotous for the recessive mutation of a gene impairing leptin signalling and rendering the animals tremendously hyperphagic, their heterozygotous littermates remain lean and without insulin resistance. We therefore **compared in the present study the obese and lean littermates** of this useful animal model of genetic obesity, and we reported in the present manuscript that **we found a greater MAO activity in the adipose depots of the obese rats than in the age-matched lean controls. This spontaneous difference was seen without any prior pharmacological treatment**. In the present study, the *in vivo* treatment is not performed with pharmacological amine oxidase inhibitors as in our previous study, since it is the substrate tyramine that was administered to obese and lean rats and reduced the hyperglycemia and hypertriglyceridemia of the obese only. this treatment was applied to additional rats than those used for phenotyping the obese vs the lean littermates, regarding insulin responsiveness, and amine oxidase expression. Thus, even if we used the same animal model for both studies, the "innovation" of this submitted work is that the genetically obese littermates spontaneously express larger amount/activity of MAO and of binding sites related to these enzymes than the lean ones, especially in adipose tissue. Consequently, the fat cells of obese Zucker rats are likely more prone to exhibit biological

responses to MAO and SSAO substrates and inhibitors. Moreover, the amine oxidases appeared to be linked with glucose consumption in fat cells from both lean and obese rats. Indeed, in the adipocytes, the substrates of MAO and SSAO were able to activate glucose consumption and its incorporation into lipids, which can be considered as an insulin-like anabolic effect. The enclosed revised manuscript allow us proposing that such larger MAO abundance in adipose tissue might contribute to the larger fat deposition found in the obese and insulin-resistant rats, and has to be added to the long list of phenotypic differences between lean and obese Zucker rats.

The confusion of the reviewer has been taken into account. It was probably induced by the terms "increase / increased" regarding amine oxidation. During revision, in several occurrences, we have changed the word "increase" or "increased" when comparing between lean and obese littermates the MAO expression, the oxidase activities, or the substrate effects on glucose utilization, by "larger, higher or greater"(e.g in legend of fig. 6). Currently, we don't know/prove where/when is the increase; we just observed that the MAO activity is larger in obese than in age-matched lean rats. Anyhow, this might prompt investigators to ask whether a similar difference exists between non-obese and obese individuals, and might constitute a relevant target for combatting obesity.

Point 2) " Amine oxidase expression was also established a human preadipocyte cell strain from a patient with Simpson-Golabi-Behmel syndrome (Bour et al., 2007). How is the expression profile of amine oxidase encoding genes in insulin-resistant adipocytes from obese Zucker rats? Reference Bour S, Daviaud D, Gres S, Lefort C, Prévot D, Zorzano A, Wabitsch M, Saulnier-Blache JS, Valet P, Carpéné C. Adipogenesis-related increase of semicarbazide-sensitive amine oxidase and monoamine oxidase in human adipocytes. Biochimie. 2007, 89(8):916-25."

The reviewer is absolutely right. We have already reported that here is high expression of SSAO and MAO in fully differentiated adipocytes from patients with Simpson-Golabi-Behmel syndrome. However, again, the reported comparison was performed between non-differentiated and fully-differentiated adipocytes, and nothing was described about putative difference between non-obese and obese humans, an issue that was out the scope of the present study, but which deserves future investigations. Regarding gene expression, the tools we have developed were suitable for humans and for mice but not totally validated for rat genome, and we encountered some difficulties as already mentioned in the fourth paragraph of the Discussion. Consequently, we cannot add during the revision of this Ms any additional relevant data about *Maoa*, *Maob* or *Aoc3* rat genes. According to the reviewer's suggestion, it is sure that such data, at least in adipose tissue, would have supported further the larger MAO protein and oxidase activity found in obese when compared to lean rats, as displayed in figures 3, 4 and 5.

Point 3) " Would you please update the cited references using the latest ones? Fontaine J, Tavernier G, Morin N, Carpéné C. Vanadium-dependent activation of glucose transport in adipocytes by catecholamines is not mediated via adrenoceptor stimulation or monoamine oxidase activity. World J Diabetes. 2020, 11(12):622-643."

This suggestion has been taken into account, and for answering also to concerns raised by editors, the 4 following auto-citations have been removed and replaced by more recent:

*ex ref27 : Carpéné C, Gomez-Zorita S, Gupta R, Gres S, Rancoule C, Cadoudal T, Mercader J, Gomez A, Bertrand C, Iffiu-Soltesz Z. Combination of low dose of the anti-adipogenic agents resveratrol and phenelzine in drinking water is not sufficient to prevent obesity in very-high-fat diet-fed mice. Eur J Nutr. 2014; 53: 1625-1635.

*ex ref30 : Harant-Farrugia I, Garcia J, Iglesias-Osma MC, Garcia-Barrado MJ, Carpéné C. Is there an optimal dose for dietary linoleic acid? Lessons from essential fatty acid deficiency supplementation and adipocyte functions in rats. J Physiol Biochem. 2014; 70: 615-627

*ex ref 34 : Morin N, Visentin V, Calise D, Marti L, Zorzano A, Testar X, Valet P, Fischer Y, Carpéné C. Tyramine stimulates glucose uptake in insulin-sensitive tissues in vitro and in vivo via its oxidation by amine oxidases. J Pharmacol Exp Ther. 2002; 303: 1238-1247
*ex ref41 : Bour S, Caspar-Bauguil S, Iffiu-Soltesz Z, Nibbelink M, Cousin B, Miiluniemi M, Salmi M, Stolen C, Jalkanen S, Casteilla L, Penicaud L, Valet P, Carpéné C. Semicarbazide-sensitive amine oxidase/vascular adhesion protein-1 deficiency reduces leukocyte infiltration into adipose tissue and favors fat deposition. Am J Pathol. 2009; 174: 1075-1083

SCIENCE EDITOR:
1 Scientific quality / The manuscript describes a Basic Study of the Increased amine oxidation in adipocytes of obese rats. The topic is within the scope of the WJBC. (1) Classification: Grade C; (2) Summary of the Peer-Review Report: Perhaps a number of issues should be clarified to improve the overall quality of this manuscript. The questions raised by the reviewers should be answered. (3) Format: There are 2 tables and 9 figures; (4) References: A total of 73 references are cited, including 8 references published in the last 3 years; (5) Self-cited references: There are 8 self-cited references, The self-referencing rates should be less than 10%. Please keep the reasonable self-citations (i.e. those that are most closely related to the topic of the manuscript) and remove all other improper self-citations.

These editor remarks are entirely justified. We have removed 4 self-citations and total number of references is now of 72, as the moved quotes were replaced for the sake of clarity by the following ones:
doi 10.4331/wjbc.v11.i3.76
doi 10.4331/wjbc.v2.i10.215
doi 10.4239/wjd.v10.i1.23 .

2/ Language evaluation and 3/ Academic norms and rules and 4 Supplementary comments: no concern raised

OK.

5/ Issues raised:
(1) The authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s).

As it is difficult to encounter a single accounting document that justifies the recurrent fundings of a National institute like INSERM that are distributed yearly to Research Units, then dispatched monthly within research teams, the authors cannot provide a certificate corresponding to the duration of the studies. The approval document provided by the authors has been uploaded for the Institutional Review Board Approval. Whether this document is not considered at BPG, the editorial staff can delete if necessary the supportive foundations that were summarized as ' Supported by Institut National de la Santé et de la Recherche Médicale to INSERM Unit 1048, France. ' in the revised Ms.

(2) 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

These editor remarks are answered by providing the required .pptx files.
6/ Recommendation: Conditional acceptance.

OK, thanks.

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 Biological Chemistry, 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.*

All required changes have been done by adding in red font only a minimal number of sentences, and by removing the less useful self-citations, to keep Ms as concise as possible. Please, note that for R1 version of the Ms, the corrections made during revision are indicated in red.