REPLY ON REVIEWERS' COMMENTS-WJCP

Reviewer-1

1. **Reviewer:** The studied children aged bet 6-12 years, is non homogenous group. Getting close to puberty as we called prepubertal age has great influence on oxidant and antioxidants status among children which can affect the findings – major covariant

Reply: The age of most children (93%) involved in the study of my Manuscript is in between 6-8 years, the age of the remaining children is between 8-12. The same range is for normal children. Therefore, the effect of age is cancelled due to the same range of age for both overweight, obese children and normal weight. In addition, **in the following studies, their Authors** considered 6-14 years old are children:

i) **Codoner-Franch P, Boix-Garcia L, Simo-Jorda R, Castulo-Villaescusa CD, Maset-Maldonado J, Valls-Belle V. . Is obesity associated with oxidative stress in children? International Journal of Pediatric Obesity. 2010; 5: 56-63: In ABSTRACT: Methods. The study population included 68 children (30 girls, 38 boys), between 6 and 14 years of age.**

ii) **Moln ár D, Decsi T, Koletzko B. educed antioxidant status in obese children with multimetabolic syndrome. International Journal of Obesity 2004; 28, 1197–1202: SUBJECTS: In total, 16 control children (age: 16.2±1.1 y, BMI: 20.7± 1.9 kg/m^2, body fat (BF): 25.6±5.7%; mean±SD), 15 obese children (age: 13.4± 2.1 y, BMI: 34.2±3.1 kg/m^2, BF: 36.9± 5.8%) and 17 obese children without MMS (age: 14.4±2.3 y, BMI: 30.4±6.2 kg/m^2, BF: 36.3±5.8%) were included in the study**

2. **Reviewer:** Did not use the right assays For example measuring reduced glutathione only, the ideal to measure both reduced and oxidized glutathione and reported as a ratio (reduced/oxidized), because the total glutathione can vary from one child to another

Reply: I measured the Glutathione-dependent enzymes, i.e. glutathione peroxidase (GSH-Px) and glutathione reductase (GSSG-R) which are sensitive for any change in GSH/GSSG ratio. Reduced glutathione (GSH) is an abundant cellular thiol and is more sensitive for oxidative stress and plays an important role in the free radical scavenger **[Bains JS, Shaw CA. Neurodegenerative disorders in humans: the role of glutathione in oxidative stress-mediated neuronal death. Brain Research Reviews 1997; 25(3): 335–358].** In addition, the impairment of glutathione metabolism weakens the defense mechanism against cellular oxidative stress [**Dorvala J, Hontela A. Role of glutathione redox cycle and catalase in defense against oxidative stress induced by endosulfan in adrenocortical cells of rainbow trout (Oncorhynchus mykiss). Toxic Applied Pharma 2003; 192 (2): 191–200].** The determination of these oxidative markers in combination provides information about the interplay between oxidant and antioxidant processes and an estimate of an individual's overall oxidative status. [**Olusi SO. Obesity is an independent risk factor for plasma lipid**

peroxidation and depletion of erythrocyte cytoprotectic enzymes in humans. Int J Obes Relat Metab Disord. 2002; 26: 1159-1164 & **Codoner-Franch P, Boix-Garcia L, Simo-Jorda R, Castulo-Villaescusa CD, Maset-Maldonado J, Valls-Belle V. . Is obesity associated with oxidative stress in children? International Journal of Pediatric Obesity. 2010; 5: 56-63].** GSH is the most important intracellular hydrophilic antioxidant, and cells containing low levels of GSH have been reported to be much more sensitive to oxidative damage [**Guha P, Dey A, Sen R, et al. Intracellular GSH depletion triggered mitochondrial Bax translocation to accomplish resveratrol-induced apoptosis in the U937 cell line. J Pharmacol Exp Ther 2011;336: 206–214**]. Thus, these markers (GSG-Px, GSSG-R and GSH) are important indicators of oxidative stress.

3. **Reviewer:** The author stated measuring Total protein among studied groups and did not show the results

   Reply: As I pointed out in "materials and Methods" Section, the concentrations of both **plasma oxidized low-density lipoproteins (ox LDL)** (ox LDL is expressed in mg/**g protein**) and **Advance oxidation protein products (AOPP)** (μmol/**g protein**) are related to g protein

4. **Reviewer:** Graph B missing the significance mark

   Reply: The reviewer is right, this is a writing mistake and I have corrected in the revised manuscript

5. **Reviewer:** Some concern about statistics findings in table SOD Con. In the three groups as follow: Normal weight (n) Mean + SD (66) 224.6 ± 21.8 Over Weight (n) Mean + SD t-test and P value (83) 252.7 ± 19.4 9.02?, 0.001a Obese (n) Mean + SD t-test and P value (64) 217.1 ± 18.4 12.32?, 0.001a,b You can see the mean bet Control and obese very close (224 vs.217) with wide range of SD in both groups (21 vs. 18), which means obvious overlap between both groups and decrease the difference between both groups to be NOT Significant, while they stated as statistical significant. No need to include T value in the table

   Reply: This is a mistake, and after submitted the present manuscript to the Editor, I noted this point, then I submitted other issue of my manuscript but the Editor refused the new one. Therefore, this mistake has corrected in the revised manuscript and I have checked the calculation of all results. In addition, I have removed the **T test** from the tables and the tables and graphs include **ED** instead of SD.

6. **Reviewer:** The author as a clinician is missing many basic knowledge of his study, especially in the assays and its interpretation. For example, he is using term measuring enzyme activity, while he did measure conc. only as started in his method, which is different from enzyme activity assay, that apply for SOD, Catalase, etc.

Reply: The reviewer is right; I have corrected this point in the revised manuscript

7. **Reviewer:** His discussion is very poor and his findings analysis stands against his concept and findings in some situation. For example, he discussed that NO is increased in obese patients and did not show the data which is very significant findings for his study. Based on his hidden finding, he conclude that "The increased production of nitric oxide (NO) in obese children (data not shown) may act as an endogenous free radical scavengers that they react with superoxide radicals (O2?-) at a rate three times faster than the reaction of O2?- with superoxide dismutase (SOD, which may decrease SOD activity which occurs in our case"...which means the body does not need to produce more SOD, for the decrease the need for antixoidants as he stated. While in the previous paragraph he stated that "The depletion of the antioxidant activities in obese children may be attributed to the high production of ROS which may destroy these antioxidant enzymes"

Reply: I have measured NO concentration in other study which is under publication. Therefore, I have removed this paragraph from the revised manuscript

REVIEWER-2

1. **Reviewer:** The biggest problem with the paper was the language

   Reply: The English languish of the Manuscript is revised by a native speaker

2. **Reviewer:** The title must be revised: Evaluation of Oxidant-Antioxidant Status in Overweight and Morbid Obese Children. A Local Experiment from Saudi Arabia (not experiments)

   Reply: The new Title is: "Evaluation of Oxidant-Antioxidant Status in Overweight and Morbid Obese Saudi Children"

3. **Reviewer:** The results should not start with: Compared with controls, overweight and obese children had a significantly higher body mass index (BMI).This is not a result of the study as the authors are comparing the results in obese and non-obese children

   Reply: This expression is present in the Results Section in ABSTRACT, and I have corrected in the revised Manuscript.

4. **Reviewer:** In the discussion what is meant by inherited obesity must be explained.

Reply: Inherited obesity refers to inherit obesity from their obese parents or grandparents. I selected children of new obesity to avoid the effect of this inherited obesity on the present parameters

5. **Reviewer:** The first paragraph of the discussion must be re-written especially the sentence….. investigated the effect of the acquired fatness…

   Reply: This sentence means that the present study selected ONLY obese children who acquired their obesity via **excess feeding without exercise** not inherited obesity from their parents.

6. **Reviewer:** Many parts of the discussion are unclear.

   Reply: the English languish and the scientific discussion of the manuscript have completely revised

REVIEWER 3

1. **Reviewer:** Although this manuscript is therefore suitable for publication in WJCP, the author should rewrite some minor points as described below. 1. The statement in page 3, line 11 up to line 7 up is not matched with Page 6, line 18 and Table (1). Please check these descriptions.

   Reply: I have considered this point in the revised manuscript

2. **Reviewer:** The author measured reduced glutathione (GSH) values. GSH/GSSG ratios should be evaluated, if possible.

Reply: I measured the Glutathione-dependent enzymes, i.e. glutathione peroxidase (GSH-Px) and glutathione reductase (GSSG-R) which are sensitive for any change in GSH/GSSG ratio. Reduced glutathione (GSH) is an abundant cellular thiol and is more sensitive for oxidative stress and plays an important role in the free radical scavenger **[Bains JS, Shaw CA. Neurodegenerative disorders in humans: the role of glutathione in oxidative stress-mediated neuronal death. Brain Research Reviews 1997; 25(3): 335–358].** In addition, the impairment of glutathione metabolism weakens the defense mechanism against cellular oxidative stress [**Dorvala J, Hontela A. Role of glutathione redox cycle and catalase in defense against oxidative stress induced by endosulfan in adrenocortical cells of rainbow trout (Oncorhynchus mykiss). Toxic Applied Pharma 2003; 192 (2): 191–200].** The determination of these oxidative markers in combination provides information about the interplay between oxidant and

antioxidant processes and an estimate of an individual's overall oxidative status. [**Olusi SO. Obesity is an independent risk factor for plasma lipid peroxidation and depletion of erythrocyte cytoprotectic enzymes in humans. Int J Obes Relat Metab Disord. 2002; 26: 1159-1164** & **Codoner-Franch P, Boix-Garcia L, Simo-Jorda R, Castulo-Villaescusa CD, Maset-Maldonado J, Valls-Belle V. . Is obesity associated with oxidative stress in children? International Journal of Pediatric Obesity. 2010; 5: 56-63].** GSH is the most important intracellular hydrophilic antioxidant, and cells containing low levels of GSH have been reported to be much more sensitive to oxidative damage [**Guha P, Dey A, Sen R, et al. Intracellular GSH depletion triggered mitochondrial Bax translocation to accomplish resveratrol-induced apoptosis in the U937 cell line. J Pharmacol Exp Ther 2011;336: 206–214**]. Thus, these markers (GSG-Px, GSSG-R and GSH) are important indicators of oxidative stress.

3. **Reviewer:** Discussion should be rewritten more clearly. I think that the first paragraph should be moved to introduction and the second paragraph is not fit well in this paper.

   Reply: This paragraph has been revised and I think that it should be still in discussion section because it explains why I did measure it.

4. **Reviewer:** The columns in Figure 1B should be designated as other graphs 5.

   Reply: I considered this point in the revised manuscript

5. **Reviewer:** Page 8, line 9, and Check the word, "oxidation". oxidize?

   Reply: I have considered this point in the revised manuscript

REVIEWER 4

Reviewer: Although this is an interesting study, design is flawed and introduces several source of bias that compromises the main conclusion. The study does not add news in the field

Reply: I have not any reply unless I say to him that he should read the comments of the previous Reviewers