Author's response to reviews

Title: Antioxidant enzymes activities in obese Tunisian children

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Author's response to reviews: see over
Dear Sir

Would you please find here-attached the revised version of an article submitted to the Journal of Pediatric Obesity. It is an original research paper which deals with investigating the obesity-associated changes in the antioxidant enzymatic response during the childhood period. The effects of different parameters including gender, age, blood pressure, biochemical data, etc. are also discussed. This work, as a part of a post-doctoral research project, was performed in the pediatric department (hospital of Mahdia) in collaboration with the research group “Trace elements, free radicals, antioxidant mechanisms and human pathologies”.

We would like to thank the editor and the reviewers for their useful comments and suggestions which aimed to improve the quality of the manuscript. These comments were addressed in the manuscript (modifications are underlined) and a point-by-point response to the concerns is given below.

Looking forward to a favorable opinion, we would like to thank you for the interest you would give again to this work.

Best regards,

Dr. Sonia SFAR
Reviewer 1

MAJOR COMPULSORY REVISIONS

Abstract: A sentence that summarizes the main conclusion of the study is needed. The conclusion stated by the authors is not derived of the data of the present work.

The conclusion of the abstract has been revised as: “The obesity-associated oxidant stress developed even in the childhood period. In addition to the complications of increased BMI, obesity itself can be considered as an independent risk factor of free radical production resulting in an increased antioxidant response.”

Background: I think that the aim of the work was to investigate the effect of childhood obesity on cell antioxidant enzymes. In this manner, the aim exposed by the authors “defining the early oxidant damage and the opposite response in obese children” is out of the scope of the present paper.

The sentence describing the aim of the work has been revised as: “The present work aimed at investigating the effect of childhood obesity on the antioxidant enzyme activities (SOD, GPx and CAT).”

Methods:
1. Subjects:
a. I do not understand what the authors mean when they say “healthy educated children” Did they mean “healthy controlled children”?

“Healthy children” has been used instead of “healthy educated children”

b. The number of obese girls + boys are 54 (instead of 53), and the number of control girls+ boys are 52 (instead of 53)

The number of obese and control children has been revised in the subjects paragraph and the abstract. (54 obese and 52 controls)

c. This is a study in a scholar population. How was made the selection of the subjects to obtain the same number of obese and control children?

As it was described in the text “The recruitment was performed between January 2012 and March 2012, from 25 elementary schools…”, the recruitment campaign was performed in 25 elementary schools. For every obese child, we considered two normal-weight children as controls (in case of technical analysis problems) at the same age, in the same day and in the same school. Only one control child for each obese child was considered in the final analysis.
2. Statistical analysis:
   a. A verification of normality of data is desirable before the application of parametric test.

Indeed, we have performed the normality test (Shapiro-Wilk (n<2000)). Here are the main results:

<table>
<thead>
<tr>
<th></th>
<th>Girls</th>
<th>Boys</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>0.94</td>
<td>0.11</td>
</tr>
<tr>
<td>Age</td>
<td>0.136</td>
<td>0.76</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>0.38</td>
<td>0.56</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>0.43</td>
<td>0.175</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.235</td>
<td>0.278</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.382</td>
<td>0.84</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.146</td>
<td>0.36</td>
</tr>
<tr>
<td>GPx</td>
<td>0.167</td>
<td>0.206</td>
</tr>
<tr>
<td>SOD</td>
<td>0.223</td>
<td>0.18</td>
</tr>
<tr>
<td>CAT</td>
<td>0.932</td>
<td>0.52</td>
</tr>
</tbody>
</table>

We can see that the significance values are superior to 0.05. The data can be then considered as normal.

b. Due the wide range of age it could be preferable to test association between variables using partial correlation adjusted by age.

We have also performed to partial correlation test. The results are almost the same. Only the SOD activity can be linked to the BMI, for both gender. We have added the paragraph “Partial correlation test adjusted by age has been also performed. The obtained results show that only the SOD activity is significantly correlated with the BMI, for girls (r= 0.19, p<0.01) and for boys (r=0.32, p=0.05).” at the end of the “results” section to develop this idea.

3. Results
   a. It is preferable to express the blood pressure as z-score referred to the height, using standard methodology as it is recommended by The Fourth Report on Blood Pressure in Children (National high blood pressure education program working group on high blood pressure in children and adolescent. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. Pediatrics 2004;114:555–76).

   Blood pressure z-scores referred to the height were calculated according to the standard method described in (Pediatrics 2004; 114:555–76). (Table 1). Also the sentence “As recommended in [15], the blood pressure z-scores were calculated in reference to the height.” was added to the statistical analysis paragraph and the sentence “Similarly, systolic and diastolic z-scores referred
to the height show no significant variation between obese and normal-weight children.” was added to the 2nd paragraph of the “results” section.


The reference « Chatepie A, Maurage Ch, Marchand S, Ployet JL: Pédiatrie en poche. DOIN Edt, France, 2003, pp 37-45. » has been added.

c. The authors confirm the normal health status (absence of comorbidities?) in children by the results of glucose, total cholesterol and triglycerides. However, a frequent comorbidity in obesity is insulin resistance and this condition is not rule out in the present study.

Indeed, we have concluded that all the recruited children had normal health status on the basis of a questionnaire and results of biochemical analysis. In addition, chronic diseases including diabetes, hepatitis, epilepsy and renal diseases are considered as exclusion criteria (paragraph 2.1 page 4): ”Chronic diseases like diabetes, hepatitis, epilepsy, and renal diseases, were included in the exclusion criteria.”

4. Discussion:

a. There is a contradictory sentence (Page 8, first paragraph “Cases of exogenic obesity were not considered…” In fact the inclusion criteria in the present study was exogenic obesity.

“Cases of exogenic obesity were not considered …” was substituted by “Cases of endogenic obesity were not considered …”


A paragraph has been set at the end of the discussion to take into account the previous recommendation “The antioxidant enzyme activity…the markers of the antioxidant enzyme activity”

c. The discussion can be simplified and shortened.
The sentences “Increased fat deposition may be then a result ... consequently cause lipid peroxidation [20].”, “Total cholesterol content of erythrocytes and other cells ... could be caused by cholesterol-rich nourishment.” and reference [30] have been erased.

5. References:
a. Only three references (of de 30 total) are dated in the last five years. Please change to more recent references.

More recent references are used for [1], [5], [7], [20], [22], [33]. Reference [31] has been added to the discussion.

6. Tables
a. Please homogeneize the format. In Table 1 are “controls” in Table 2 are “normal – weight”. In Table 2 add the number of subjects in each group. The same remark in Table 3.

“Controls” and “obese children” have been respectively used instead of “normal- weight” and “obese” in table 2. The size of each group (boys ,girls, obese and controls) has been added in table 2 and 3.

MINOR ESSENTIAL REVISIONS:
Page 4 , line 5 “crushing’s syndrome”. Is Cushing’s syndrome?

The mistake has been revised

General:
I strongly recommend the manuscript to be reviewed by a professional proofreader in order to improve the attention to smoother phrasing and finer points of style.

Linguistic improvements have been performed. Main revisions are listed below:

Abstract: L1 “…in obese adults, has an important role in the …” is used instead of ““...in obese adults, plays a critical role in the ...”
L3 “This study aimed at evaluating the ...” is substituted by “The main objective of this study is to evaluate the...”.

Background: L2 “…that involves the interactions between...” is used instead of “…that involves interactions between...”

Statistical analysis: L2 “The results...” is used instead of “The findings...”
Results: P7 1st paragraph L7 “In addition, obese children have higher waist and hip ...” is used instead of “Obese children also show higher waist and hip...”

Conclusions: L3 “In particular, it is still...” is used instead of “In particular, it still...”
L7 “… resulting from obesity developed even in the childhood period.” is substituted by “…resulting from obesity appeared at an early age.”
Reviewer 2

Minor essential revision:
1. in the part of methods: the Authors should mentioned that the obese children and the control group were divided into two subgroup: at age: 6-8,5 years and 9-12 years and why they decided to divide the groups. (p.3)

Because of the age-related variations of growth characteristics (mainly height, weight and BMI) in the age interval 6-12 years, the study population was divided into two subgroups for both obese and normal-weight groups. A sentence was added to the methods section in order to explain this idea (page 4).

2. p.4-Blood pressure was measured using a mercury sphyngomanometer- the Authors should write more precisely-f.e. the measurements were done after 30 minutes of resting, on both arms, and they taken into the consideration the highest values of pressure.

The text “The measurements were done after 30 minutes of resting, on both arms. The highest value of pressure was considered for every child.” was was added in page 4.

3. p. 5 : instead of anti-radical enzymes activity: better will be antioxidant enzymes activity (because the title is antioxidant enzymes activity in obese children)

The paragraph title was revised to address with this remark (page 5).

4. the Authors did not mention how they determined cholesterol, triglicerydes and glucose levels ( if they were determined these parameters in plasma??? or in serum ???)

Cholesterol, triglycerides and glucose levels are commonly determined in plasma. Therefore, the sentence “In addition to the assays described below, other biochemical measurements including fasting plasma glucose, plasma total cholesterol and plasma triglycerides were performed.” was added to the section “blood samples collection” (page 5).

5. instead of cholesterol the Authores should use the term- total cholesterol

The term total cholesterol was used instead of cholesterol (page 7, 8, 10 - tables 1 and 3).

6. The Authors should also metioned that SOD acivity was determined in erythrocytes (p.5)
The sentence was revised in page 5 to take into account this suggestion.