Author's response to reviews

Title: Long-term interdisciplinary therapy reduces endotoxin level and insulin resistance in obese adolescents

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Author's response to reviews: see over

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To
Nutrition Journal

Dear Editor-in-Chief,

Thank you very much for your comments regarding our manuscript Nutrition Journal - MS: 7236345536685056 - Long-term interdisciplinary therapy reduces endotoxin level and insulin resistance in obese adolescents”. We have carefully revised the manuscript, taking into account the comments and criticisms of the referees. Please find attached the revised version and the response to the referees. We hope the article has been sufficiently improved for publication in the Nutrition Journal.

Sincerely,

Dr. Fabio Santos Lira
RESPONSE TO REVIEWERS

The authors would like to thank both reviewers for their excellent suggestions, which greatly improved the quality of our manuscript.

Reviewer #1:

In the present study Lira and colleagues sought to determine the role of long-term (1 year) of interdisciplinary therapy on endotoxin level and insulin resistance in obese adolescents. The authors found that the long-term of interdisciplinary lifestyle therapy reduced the energy intake, endotoxin level and increased the adiponectin level and insulin sensitivity. The role of interdisciplinary lifestyle therapy on the endotoxin levels is interesting and relevant. The study was well design and the methodological approaches are adequate. However, some point needs to be addressed.

Major concerns:

The effects of lifestyle therapy in some parameters, such as, glucose, Insulin, HOMA-IR, inflammatory profile and adiponectin levels in obese subjects were expected, as demonstrated by several well controlled studies. I believe that the result regarding the endotoxin level is interesting, however, this date is isolated and need to be extended.

Answer: As the reviewer suggested, we insert more information’s about endotoxin in the discussion section. For review see page 11: The chronic endotoxemia, have promoted glucose intolerance and hepatic insulin resistance, suggesting its role as a link between innate immunity, inflammation, and insulin resistance (Park et al 2011).
The correlation analysis about fat dietary with endotoxin, insulin, adiponectin and HOMA index in the figure 1 is inconsistent, since the analysis of the fat intake was superficial. What kind of fat the subjects consumed? Maybe this positive correlation is also observed with the other nutrients or other parameters such as, body weight or fat mass. This figure is not necessary.

**Answer:** As the reviewer suggested, we performed correlations between endotoxin and body weight, fat mass, adipokines, carbohydrate, protein and total caloric intake. We observed positive correlation between endotoxin levels and visceral fat (r=0.37; p<0.05), endotoxin levels and visceral/subcutaneous fat ratio (r=0.34; p<0.5), endotoxin levels and insulin levels (r=0.29; p<0.05), and negative correlation between endotoxin levels and adiponectin level (r= -0.29; p<0.05). No correlations were observed between others parameters and endotoxin levels.

Sabine Thuy et al (The Journal of Nutrition, 2008) have showed positive correlation between endotoxin and carbohydrate intake, we no observed the same profile, and it’s correlations with inflammation and metabolic syndrome, reinforcing the hypothesis that fat intake is closely related to increased systemic endotoxin in the present study.

The values regarding the control (lean) group could be important to compare the IL-6, endotoxin and adiponectin levels with obese subjects.

**Answer:** We agree with the suggestion of the reviewer. However, our interdisciplinary program have as aim explore potential therapeutic in adolescents obese, which is known exhibit increased inflammatory status, conform demonstrated by works of our group (Tock et al 2006; Caranti et al 2007; Carnier et al 2008; Lofrano-Prado et al 2009; de Piano et al 2010; Carnier et al 2010; de Lima Sanches et al 2011; Lira et al 2011; de Mello et al 2011; Campos et al 2012). In future studies, we will examine the profile of inflammatory in lean group and compared with obese subjects. However, for review in the text see page 4: Recently, our group has shown that long-term interdisciplinary lifestyle therapy is effective in controlling the psychological and physiological
alterations that are commonly observed in obese patients (Tock et al 2006; Caranti et al 2007; Carnier et al 2008; Lofrano-Prado et al 2009; de Piano et al 2010; Carnier et al 2010; de Lima Sanches et al 2011; Lira et al 2011; de Mello et al 2011; Campos et al 2012).

It has been demonstrated that the lifestyle intervention reduces the inflammatory profile; however, this date was not observed in the present study. Please comment this issue.

**Answer:** We agree with the suggestion of the reviewer. In the present study we observed tendency in reduces IL-6 levels (p<0.06), however, our data exhibit make clear significant decreases endotoxin levels. Our group (Carnier et al 2008; de Mello et al 2011; Campos et al 2012; da Silva et al 2012) have showed reduced inflammatory status after changes lifestyle in interdisciplinary program. The number little subjects, least in part, may be associated with reduced statistic power in present study, which observed only tendency in reduction of serum IL-6 levels.

**Minor points:**

In the introduction section (3rd paragraph), the authors described: “Recently, our group has shown that long-term interdisciplinary lifestyle therapy…”, however no reference was mentioned.

As the reviewer suggested, we insert references. For review see “Recently, our group has shown that long-term interdisciplinary lifestyle therapy is effective in controlling the psychological and physiological alterations that are commonly observed in obese patients (Tock et al 2006; Caranti et al 2007; Carnier et al 2008; Lofrano-Prado et al 2009; de Piano et al 2010; Carnier et al 2010; de Lima Sanches et al 2011; Lira et al 2011; de Mello et al 2011; Campos et al 2012)”.

What is “insulinA” in the figure 1B.

As the reviewer suggested, we adjust the axis.
The p values were demonstrated in the tables, please, remove the p values from the result section.

As the reviewer suggested, we removed p values.

The reference used in the second paragraph is insufficient. Please use more references.

As the reviewer suggested, we insert the references (Pedersen BK, 2009 and Ropelle et al., 2010).

Please remove the "minus" symbols in the first paragraph in the results section.

As the reviewer suggested, we removed symbols.
Reviewer #2:

The submitted paper describes the metabolic and anti-inflammatory effects of an integrated approach to obese adolescents. The paper is well written, and the results are interesting. However, a number of issues should be considered.

Major compulsory revisions

1. In the discussion section, the authors state that their intervention program was able to reduce fat intake, which was sufficient to reduce endotoxin concentrations and insulin resistance. Although it is acknowledged that fat intake, and specifically saturated fatty acids, is involved in the development of obesity and insulin resistance, it appears that the authors do not provide data on the relative contribution to the outcome measured of the different factors included in the program. Therefore, the authors should consider to review their claim that the effects observed are mainly from the reduction of fat intake.

Answer: As the reviewer suggested, we insert more information’s about different factors included in program in the discussion section. For review see page 12 “In the present study, we observed that interdisciplinary therapy was able to decrease the fat intake, which was sufficient to reduce endotoxin concentrations and insulin resistance”.

2. The paper reports the results of only 18 patients out of 44 who started the program. Therefore, the program appears effective but the drop out/failure rate is quite high (more than 50%). It would be interesting to know more about the reasons for failing, whether patients dropped out early or at the end of the program, etc.

Answer: In the present study, from 39 adolescents that finished the program (54 started) we selected 18 obese adolescents that lost more than 5% of fat mass (range sample: 5.4% at
22.50% fat mass). This selection reduced the number of the sample, because not all obese adolescents reduced more than 5% fat mass.

The adherence in this program is relatively high and the desistance levels are around 25%. Furthermore, it is important to note that the treatment of obese adolescent populations is difficult, and the success depends on, at least in part, the participation of parents and/or guardians.