Author’s response to reviews

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

Authors:

Fabio S Lira FSL (fabioslira@gmail.com)
Jose C Rosa JCR (josecesar23@hotmail.com)
Gustavo D Pimentel GDP (gupimentel@yahoo.com.br)
Ronaldo VT Santos RVTS (ronaldo.thomatieli@unifesp.br)
June Carbier JC (june@unifesp.br)
Priscila L Sanches PLS (prisanches@unifesp.br)
Aliane de Piano AP (piano@unifesp.br)
Lian Tock (tock@unifesp.br)
Sergio Tufik (tufik@unifesp.br)
Marco T de Mello (tmello@pesquisador.cnpq.br)
Marliia Seelaender MS (seelaender@usp.br)
Claudia M Oller do Nascimento CMON (claudia.oller@unifesp.br)
Lila M Oyama LMO (lmoyama@unifesp.br)
Ana R Damaso ARD (damaso@unifesp.br)

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Author’s response to reviews: see over

Dr. Fabio Santos Lira
Rua Botucatu 862
2º andar, V. Clementino
04023 – 062 - São Paulo - SP.
Brazil
phone/fax: 5511-5573-9525
fabioslira@gmail.com

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 all 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:

1. 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 pages 10 and 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).
2. 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. This observation was very important to improvement our manuscript. As results found, 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.

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

**Answer:** We totally agree with the suggestion of the reviewer. However, our interdisciplinary program present as main aim explore potential therapeutic in adolescents obese, which is known exhibit increased inflammatory status, in according to demonstrated by several papers 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). However, in future studies, will be examine the inflammatory status in lean group (control group) comparing with obese adolescents. In the present study, we ought to verify the effects of long-term interdisciplinary therapy (nutritional counseling, exercise program, and
psychological and medical intervention) on some metabolic parameters such as IL-6, then we analyzed the differences between IL-6, adiponectin and endotoxin concentrations at baseline conditions and at the end of one year of intervention, revealing that this kind of treatment was effective to improve inflammatory status and to reduce consumption of dietary fat. Indeed, previously it was described substantially improvement in impaired metabolic, clinical and physiological parameters 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).

4. 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. In addition, we can suggested a significant improvement of inflammatory profile once adiponectin, the anti-inflammatory adipokine, increased significantly after the long-term intervention (12%) and reduction of HOMA-IR, an index that present association to inflammatory pathways, such as increase of IL-6 and TNF-alpha. Previously studies (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 in adolescents. Although in present study, the number low subjects, least in part, could be associated with reduced statistic power, we observed only tendency in reduction of serum IL-6 levels. Furthermore, serum adiponectin concentrations reduced significantly, hypothesizing the benefic effect of interdisciplinary treatment in pro-inflammatory pathways.

Minor points:
1. 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.

**Answer:** 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)”.

2. What is “insulinA” in the figure 1B.

**Answer:** As the reviewer suggested, we adjust the axis.

3. p values were demonstrated in the tables, please, remove the p values from the result section.

**Answer:** As the reviewer suggested, we removed p values.

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

**Answer:** As the reviewer suggested, we added excellent papers to reference this association (Pedersen BK, 2009 and Ropelle et al., 2010).

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

**Answer:** As the reviewer suggested, we removed the 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:** We totally agree with your comment about the claim that effects observed are mainly from the reduction of fat intake. We reconsider this claim once we could not affirm it, we only can suggest that based on the association between dietary fat intake, endotoxin and HOMA-IR an possible link between these variables and the pro-inflammatory pathways, that can be improved after the long-term intervention. This way, we rewrote the sentence in the discussion section, for review see pages 12-13.

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 opted to select only the adolescents who reduced more than 5% fat mass. This way, the subjects were 18 obese adolescents that lost more than 5% of fat mass (range sample: 5.4% at 22.50% fat mass). The adherence in this program is relative high and the desistance levels are around 25%. The main reasons for dropping out in our study were financial and family problems, followed by school and job opportunities. 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.