Author’s response to reviews

Title: Early Commitment of Cardiovascular Autonomic Modulation in Brazilian Patients with Congenital Generalized Lipodystrophy

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Version: 1 Date: 04 Oct 2017

Author’s response to reviews:

Oct 4th, 2017

Dear Editor,

Prof. Michel Noutsias

Dear Reviewers,

Prof. Josivan Lima

Prof. Jesper Fleischer
Initially, we would like to thank you for the suggestions for improvement of our manuscript. We have made all suggested changes. We have highlighted the modifications in yellow and blue to denote changes made following the reviewers 1 and 2, respectively. We fully respond each topic as detailed below.

REVIEWER REPORTS:

Josivan Lima (Reviewer 1): This is an original article on an interesting topic and deserves to be published after some modifications.

TITLE

The title is adequate, and it says what was studied. OK

ABSTRACT

Page 3
Line 17 - change to "...including the commitment of cardiovascular autonomic modulation..."
Correction performed

Line 31 - change "compared" to "paired"
Correction performed

Line 58 - include the value of correlation ($r = ?, p = ?$)
Information included
BACKGROUND

It is appropriate, identifying the problem and justifying the study.

We modified this as requested by the other reviewer but in our understanding, without making any compromise of informed content

Page 4

Line 24 - "guideline" does not need to be in italic. The abbreviation HRV appears for the first time here and needs to be defined at this point.

Correction performed

Line 38 - Please, include a reference after "nervous system".

Segment excluded after suggested revision by the other reviewer

Line 41 - Change "testes" to "tests".

Segment excluded after suggested revision by the other reviewer

Line 58 - Please, include a reference after "the disease".

Reference included

Page 5

Line 12 - Please, include a reference after "important role".

Reference included

Line 31 - Refer to a more recent article, like this:

Reference included

METHODS

Page 6
Line 34 - Do you mean "who were regularly seen in our service"?

Information included in the methods section: 2 to 3 visits per year

Line 51 - Presence of acromegaly is not a diagnostic criterion. Otherwise, if there is acromegaly, the patient should be excluded.

Correction performed

Line 58 - Can you cite the specific mutation of each patient?

Information included: Table S1 (supplementary material)

Page 8
Line 4 - Change pot-prandial to postprandial

Correction performed

Line 9 - Change chat to chart

Correction performed

Page 10
Line 44 - Change reproducibility to reproducibilities.

Correction performed

Lines 38 and 43 - Use only one of the two writes: coefficient E/I or E/I coefficient.

Correction performed
RESULTs

It would be interesting to compare the frequency of CAN according to triglyceridemia tertiles (1st tertile vs. 3rd tertile) in each of the groups.

We compared the frequency of CAN according to triglyceridemia tertiles in the CGL group: 1st tertile (n=4): 1 case, 2nd tertile (n=3): 2 cases, 3rd tertile (n=3): 2 cases. However, we did not include this data in the manuscript because of the small size of each subgroup. The association between triglyceride levels and CAN assessment parameters can be observed through the correlation analyses presented in Tables 4 and S4. We did not have the triglyceride values among type 1 DM patients.

Likewise, I would like to know the frequency of CAN in patients with and without nephropathy.

Information included in the results section: “The frequency of CAN in patients with and without nephropathy was 67% (4/6) and 25% (1/4); p= 0.524, respectively”.

DISCUSSION

Page 15, Line 34 - Put the sensitivity value before the "and". Exclude the second "and".
The authors state (page 17, lines 46-47) that low leptin has an inhibitory effect on the sympathetic system. So, it has not been clear to me why basal heart rate is higher in patients with CGL.

The consequences of severe hypoleptinemia on ANS are not well understood. A priori, hypoleptinemia leads to the inhibition of the sympathetic ANS. However, the pronounced hyperinsulinemia characteristic of CGL may justify the increase of basal HR, because insulin per se increases serum catecholamine levels as well as reduces parasympathetic control of HR and is therefore associated with the development of CAN. This information was included in the discussion section.

Page 19, Line 31 - Change "a group are associated" to "a group is associated"

Correction performed

REFERENCES

The citation of references in the text is correct (numbered consecutively in the order of their appearance in the text and identified by Arabic). I just recommend the paper below to be cited:


Reference included

TABLES

Table 1
For a better understanding of the individual characteristics of each CGL patient, I suggest including a "CAN (yes / no)" column.

Information included

Table 2
Line 15 - Change MBI to BMI

Correction performed
Line 32 - Change Pioglitazon to Pioglitazone

Correction performed
Line 33 - Change Losartana to Losartan

Correction performed
Line 36 - Change "Uso de insulina" to "Use of insulin"

Correction performed
Include a column with duration of diabetes, comparing if there is any difference between the CGL and DM1 groups.

Information included

Line 37 - Fasting glycemia was not available in the DM1 group? Do you have (for all 3 groups) capillary blood glucose before the test? If so, you could include them instead of fasting blood glucose.

Capillary blood glucose before the test was included in table 2

Legend - HDL is described twice. Change "NA: not applicable" to "NA: not available"
The analysis of the CGL group alone showed statistical significance between some variables, as the text in the results section: “When the CGL group was analyzed alone, the 30/15 coefficient showed an inverse correlation with the triglyceride levels (r= -0.778; p=0.008) and the E/I coefficient showed an inverse correlation with ACR (r= -0.769; p=0.009) and triglycerides (r= -0.818, p=0.004). The LF component was inversely correlated with HbA1c (r= -0.636; p=0.048), ACR (r= -0.063; p=0.048) and triglycerides (r= -0.648; p=0.042), and the LF/HF ratio showed a positive correlation with HOMA-IR (r=0.673; p=0.033). After the Bootstrap reassembly technique was applied in CGL group, the variables that maintained the correlation included the E/I coefficient and triglycerides (IC95%: -1.000; -0.036) and ACR (IC95%: -0.945; -0.301); LF component and triglycerides (IC95%: -0.950; -0.198); and LF/HF ratio and HOMA-IR (IC95%: 0.231; 0.884). A correlation between serum leptin levels and results of the autonomic tests was not observed in this group”.

We have also included the following comment in the discussion: “This may be mentioned that correlation analyses do not necessarily suggest a causality”.

Correction performed

Table 3

Lines 10 e 11 - Change "CAN (%)" to "CAN % (n)"

Correction performed

Line 32 - Exclude "Sistema simpatico"

Correction performed

Table 4

To increase the number of patients, the CGL and controls groups were evaluated together. But as CGL have higher insulin resistance, higher triglyceridemia values, lower HDL, higher A1c, the correlation is there probably due to selection bias. It is necessary to emphasize this in the discussion and to affirm that the analysis of the CGL group alone did not show statistical significance. Also, remember that correlation evaluates only association between two variables and not causality.
Table S3

Why is the number of type 1 diabetes only 14 and not 20?

In this table, the CAN frequency was not different in the CGL and DM1 groups.

This table refers to a comparison only between a subgroup of patients with lipodystrophy with DM (n=7) to a subgroup of patients with type 1 diabetes (n=14) after matching for age, gender, glycemic control and duration of diabetes.

FIGUREOK

Jesper Fleischer (Reviewer 2): This manuscript could be of interest to the readers.

The authors investigated the the prevalence of cardiovascular autonomic neuropathy (CAN) in patients with congenital generalized lipodystrophy (CGL) compared with individuals with type 1 diabetes and healthy subjects.

Comments:

1. The introduction is too long. The focus should be on CGL and not on the known description/history of CAN. Please update with this in mind.

Modified as requested

2. In the methods section, The HRV parameter (VLF) represents very slow fluctuations and should be avoided in short-term recordings (e.g. ≤ 5 minutes). Please remove or describe the limitation of this parameter in discussion section.

Information included in discussion:
“We emphasize that the VLF component has been recommended less often since its interpretation in short recordings (5 min or less) is less clear and this could be a study limitation. However, this parameter was used in combination to the others HRV tests and CAN diagnosis was done according to recommended in CAN Subcommittee of the Toronto Consensus Panel Statement. In addition, the two patients who presented alteration of VLF coefficient had criteria for CAN diagnosis due to alterations in the others HRV tests, minimizing the impact of this limitation.”

3. In the methods section - In regards to age-dependent reference value shown on (page 11, line 55-), the authors refer to an article number 26. However, this reference (number 26) have no description of age-dependent reference values?

Initially, we had used the reference values adopted by a national study (Brazilian Type 1 Diabetes Study Group) that evaluated patients with type 1 diabetes and its complications conducted by Gomes et al. and published in a thesis of Tannus (2014). However, the data referenced by the authors in the thesis were not found in the literature. We decided to perform a new literature review and we use values published by Ziegler et al. (1992) and Spallone et al. (2011). After the new classification, we observed the same prevalence of CAN in CGL and control groups and lower frequency among type 1 diabetes, but without promoting changes in results (statistical analyses). However, we have observed a lower frequency of alteration in the Valsalva’s coefficient due to the lower cutoff recommended in the latter articles. We believe that this does not compromise the interpretation of our findings. We emphasize that the differences in the cardiac autonomic modulation found between the groups are important, especially the commitment observed in the CGL group (table 3).

References:


4. In the methods section - Please describe in detail how the orthostatic test was performed. It is a response from supine to standing?

Information included in the methods section:

“Orthostatic test (30/15 coefficient): after rest in supine position…”

“Orthostatic or postural hypotension test: after a 30 minutes rest in supine position…”

5. In the result section the authors found at a positive correlation between leptin and the 30/15. How was the statistical adjustment of insulin resistance and triglycerides performed?

A partial correlation analysis was performed between the leptin and coefficient 30/15 variables controlling the effect of triglyceride and homa-ir variables through R3.3.1 software.

6. Table 1 is very confusing. I would recommend that you delete Table 1 and update Table 2 with the most important variables from table 1.

We decided to keep the table because it allows us an individualized view of all CGL cases, and also considering the recommendation of by reviewer 1 for the inclusion of a "CAN (yes / no)" column. However, if necessary, we can keep it as supplementary material.
If improvements to the English language within your manuscript have been requested, you should have your manuscript reviewed by someone who is fluent in English. If you would like professional help in revising this manuscript, you can use any reputable English language editing service. We can recommend our affiliates Nature Research Editing Service (http://bit.ly/NRES_BS) and American Journal Experts (http://bit.ly/AJE_BS) for help with English usage. Please note that use of an editing service is neither a requirement nor a guarantee of publication. Free assistance is available from our English language tutorial (https://www.springer.com/gb/authors-editors/authorandreviewertutorials/writinginenglish) and our Writing resources (http://www.biomedcentral.com/getpublished/writing-resources). These cover common mistakes that occur when writing in English.

We used an English language editing service (American Journal Expert) prior to the submission of the first version of the manuscript, but now we did another review fluent in english (his mother-language) that made some modifications.

Thank you again for all the suggestions.

Best Regards,