Author’s response to reviews

Title: A Novel Mutation in the Glycogen Synthase 2 Gene in a Child with Hypoglycemia Due to Glycogen Storage Disease Type 0

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Author’s response to reviews: see over
To: 
Scott Edmunds, PhD
Senior Scientific Editor
BMC-series Journals

Dear Professor Edmunds

We are resubmitting the manuscript entitled “A Novel Mutation in the Glycogen Synthase 2 Gene in a Child with Glycogen Storage Disease Type 0”. We are grateful for the comments presented by the referees that helped us to improve this manuscript. All criticisms were considered and revised accordingly to the suggestions.

ANSWERS TO EDITOR COMMENTS:

1. On page 5 you state that no ethical approval was obtained for the fasting studies since the investigations were part of normal diagnostic protocols. Unfortunately a fasting study in the child with GSD0 is within the standard of care, but investigations into adult carriers of a genetic disease would not fall under the standard of routine diagnostic protocols. A fasting study in the unaffected sibling with no mutations is therefore for research purposes, and needs IRB approval and consent. Therefore approval from the IRB needs to have been obtained. If this is not the case please make sure this is done retrospectively in a revised manuscript.

   As requested, the protocol of investigation was sent to the Institution’s Ethics Committee. We did not do that before, because during the investigation of the affected child, the parents were concerned about the possibility that the other members of the family (themselves and the two daughters) could also present some disorder in the glucose metabolism, especially due to the positive family history for type 2 diabetes (maternal grandmother). Thus, they requested the biochemical investigation for the whole family. All the biochemical tests were performed after a 12-hour fasting, as a routine check-up. None of the family members were submitted to prolonged fasting.

2. The manuscript should also include a statement to this effect in the ‘Acknowledgements’ section, in follows: “Written consent was obtained from the patient or their relative for publication of study”.

   An Acknowledgement Section was included in the revised version of the manuscript.
ANSWER TO REVIEWER COMMENTS

REFEREE 1

1. In background, please describe a little more about the properties of hepatic glycogen synthase including the KDalton, when GYS2 gene was cloned, and how many exons, etc. In addition, how many cases with GSD0 have been reported in your home country?

As suggested, additional information about hepatic glycogen synthase and about GYS2 were inserted in the revised version of the manuscript. We also stated that, to our knowledge, this is the first documented case of GYS2 mutation in Brazil.

2. The glucose profile after moderate alcohol challenge study may be repeated again in the father who experienced hypoglycemia after alcohol intake; otherwise, the result was unable to suggest haploinsufficiency.

Nowadays, the father does not consume alcohol. We requested an alcohol challenge but he refused for religious reasons. However, we managed to improve the information regarding the antecedents of hypoglycemia: the father presented some episodes of hypoglycemia after moderate alcohol ingestion (5 to 7 ethanol doses, approximately 90 grams of ethanol) during adolescence. In two occasions, he was taken to the hospital and had documented hypoglycemia (capillary blood glucose concentrations between 2.5 and 2.7 mmol/L) and improvement of the symptoms after glucose infusion.

3. Please specify the gender of patient and his or her glycated hemoglobin level.

Female patient (information added on the revised version of the manuscript) with HbA1c of 5.2 %.

4. The context of discussion is disproportional to the results found and please shorten the discussion not more than 2 pages in the present form.

The discussion was shortened, as requested.

5. Minor suggestions
5.1 The title of manuscript should be concise and informative. Please delete “hypoglycemia due to”
5.2 All data should be expressed in SI units.
5.3 Please delete irrelevant data including TSH, free T4, LH and FSH.
5.4 In background, the statement of “an invasive approach that can be inconclusive since assays to measure the activity of the enzyme GS are not performed routinely in clinical laboratory” is not appropriate because the authors may send it to research laboratory elsewhere.
5.5 The first paragraph of discussion may be moved to Introduction.
5.6 Redundancy is not uncommon in the manuscript, e.g.,
   a. At 2 years of age, diabetes mellitus was suspected because of healing difficulties at foot wound.
   b. Tanner stage
   c. Further studies are needed to investigate this issue.

In summary, this paper requires substantial re-writing to improve the fluency of the text.

The title of the manuscript was changed; all data were expressed in SI units; the irrelevant data were excluded; the comments about liver biopsy were excluded; the first paragraph of Discussion was moved to Introduction and the redundant sentences were deleted.
REFEREE 2

MAJOR COMPULSORY REVISIONS:
1. Much more information is required about the parents if a relationship between haploinsufficiency and clinical manifestations is going to be established.
   • What is the mother’s weight and BMI? What was the hemoglobin A1c and fasting insulin level in the mother? In GSD 0, the fasting insulin should be normal while it would be possibly elevated if the post-prandial hyperglycemia were due to developing diabetes.

   We believe that the post-prandial hyperglycemia could be attributed to haploinsufficiency because the mother does not present clinical and laboratorial findings suggestive of insulin resistance:
   - BMI = 24.3 kg/m²
   - Basal insulinemia = 5.6 µU/mL
   - HOMA IR = 1.08 (cut-off value for HOMA-IR to identify insulin resistance in the Brazilian population: > 2.71¹)
   The HbA1c is 6.3%.

   • What happened to the ketones in the parents with fasting?
   The parents were submitted to a maximum period of fasting of 12 hours and presented a ketonemia slightly increased: 0.8 mmol/L (mother) and 1.2 mmol/L (father) in the absence of hypoglycemia (4.8 and 4.7 mmol/L, respectively).

   • Much more information is needed about the father’s hypoglycemia if it is going to be attributed to haploinsufficiency. How low was the blood sugar and how did he present? When was the hypoglycemia in relationship to the drinking and how high was the EtOH concentration?

   We managed to improve the information regarding the antecedents of hypoglycemia: the father presented some episodes of hypoglycemia after moderate alcohol ingestion (5 to 7 ethanol doses, approximately 90 grams of ethanol) during adolescence. In two occasions, he was taken to the hospital and had documented hypoglycemia (capillary blood glucose concentrations between 2.5 and 2.7 mmol/L) and improvement of the symptoms after glucose infusion. The measurements of EtOH concentration are not available because this biochemical test is not routinely performed in emergency rooms in Brazil.

2. How were the glucose and lactates measured? Were they plasma glucose concentrations or meter readings?
Glucose and lactate concentrations were measured by immunoenzymatic methods.

3. If the glucose intolerance in the mother was due to GSD 0, why didn’t the lactates go up? This finding should be addressed.

   We believe that the absence of postprandial hyperlactatemia does not rule out an impact of haploinsufficiency because this biochemical feature may be absent even in some patients with GSD0 ². This aspect was addressed in the revised version of the paper.

4. Studies have been done looking at mutations in the GYS2 gene in people with type 2 diabetes. It would be very important to compare these findings with the published literature on this topic.

² Molecular Genetics and Metabolism 87: 284-8, 2006
A comment regarding the association of polymorphisms in the GYS2 gene and NIDDM was inserted in the revised version of the paper.

5. Both of the sisters were hypoglycemic at 2 hours, yet only one of the girls had a mutation. Was this related to the GYS2 gene? Please discuss the hypoglycemia in the siblings.
   The observed hypoglycemia after glucose overload is unlikely to be related to GYS2 mutation because it happened in both siblings. Since plasma glucose nadirs below 2.8 mmol/L are usual in healthy persons after OGTT \(^3\), we decided to withdraw the biochemical findings of the two sisters, because they were not bringing useful information.

6. Why was the patient with GSD0 hypoglycemic at the beginning of the fast?
   The table was corrected to transmit the accurate information: the test began as soon as the patient woke up, after a fasting period of 5 hours.

MINOR ESSENTIAL REVISIONS:
7. There are a number of grammatical and spelling errors throughout the document (i.e. "toa" in the title of Table 3). Please review the text carefully.
   The text was revised.

DISCRETIONARY REVISIONS:
8. It may be useful to have more of a family history. Did they have European ancestry that could explain the common mutation?
   Yes. They have Portuguese and Spanish ancestry. This information was inserted in the revised version of the paper.

9. The authors may want to discuss the rise in the glucose concentration after glucagon.
10. Do carriers for other forms of GSD develop hypoglycemia? The authors may want to discuss clinical manifestations due to haploinsufficiency in other diseases.
   We opted not to discuss these topics because Referee 1 requested that the length of discussion was shortened.

   We really appreciate the chance of this work to be considered for revision. Hoping to hear from you very soon, I remain

   Truly yours.

   Maria Lúcia Corrêa-Giannella, MD, PhD

\(^3\) Diabetes & Metabolism 26: 337-51, 2000