Author’s response to reviews

Title: Determinants of deranged thyroid function parameters in children admitted for management of diabetic ketoacidosis/diabetic ketosis

Authors:

Peng Shao (shaopeng_2000@163.com)
Shujuan Guo (yemaishuqian@126.com)
Guimei Li (lgmusa2015@163.com)
Daogang Qin (lcrsmyykqdg@126.com)
Sen Li (lisendr@126.com)
Luan Ying (laurayinger@163.com)

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Author’s response to reviews:

Dear Editor,

Thank you very much for considering our manuscript entitled “Determinants of deranged thyroid function parameters in children admitted for management of diabetic ketoacidosis/diabetic ketosis” for publication in BMC Endocrine Disorders. We thank the editor and reviewers for providing the constructive comments and suggestions, which have improved the manuscript. All items raised have been addressed during revision, and our responses are detailed below. We hope the revised draft will be considered satisfactory for publication of this manuscript.

Again, thank you for your consideration.

With best wishes,
Guimei Li

Responses to editor
Thank you very much for considering our manuscript. I have a question: the signature of our hospital has changed from "Department of Pediatrics, Shandong Provincial Hospital Affiliated to Shandong University" to "Department of Pediatrics, Shandong Provincial Hospital, Cheeloo College of Medicine, Shandong University", which has been marked in red in the manuscript. Do we need to fill a 'Request for change in authorship' form? If it is required, would you please send the form to me?

Responses to Reviewers
We thank you for the comprehensive and thoughtful review, which has helped improve the manuscript significantly. We have addressed all points raised by the reviewers and revised the manuscript accordingly. All changes to the text have been highlighted with red font in the manuscript. These changes, as well as responses to all questions, are detailed below:

Reviewer 1

Abstract:
1. Please define all acronyms (i.e AG, TG, PA etc).
2. Consider softening the conclusion regarding nutritional status.
Response: Thank you so much for raising the issues. According to your suggestions, we have defined all the acronyms and revised conclusion as “Lower levels of free thyroid hormones and occurrence of ESS was associated with a higher degree of acidosis in children with DKA/DK” (Page 2, Lines 12-15 and Page 3, Lines 1-2).

Background:
1. Please change celiac sprue, to Coeliac disease (in the whole document please)
Response: Thank you so much for the suggestion. We have changed all the celiac sprue with coeliac disease in revised manuscript.
2. Remove last sentence of paragraph one, “Absolute insulin.....” Doesn’t add to the paper
Response: Thank you so much for the suggestion. We have removed the sentence accordingly.
3. Para 3: Please reference the statement ESS is reportedly associated with a higher risk of mortality
Response: Thank you so much pointing out the issue. We have added four references to the statement in revised manuscript (Page 4, Line 13-16).
4. Para 4: sentence one: check grammar (editor please)
Response: Thank you so much for pointing out the issue. We have revised the sentence to ensure the correctness of grammar and the context.

Methods:
1. Define DK
Response: Thank you so much for pointing out the issue. We have defined the DK as diabetic ketoacidosis in revised manuscript (Page 5, Line 8 and Page 6, line 1).
2. Please clearly define the threshold for ESS. You have stated when FT3 /FT4 is lower etc - but please provide laboratory values for this.
Response: Thank you so much for the suggestion. We have defined the threshold for ESS in revised manuscript (Page 6, Line 3-6).

Results:
1. Probably a message for the editor: There are a lot of tables here. I would advise having the important findings tabled, and then providing all the other tables as supplementary.
Response: Thank you so much for the suggestion. We have reorganized the tables to make sure just the necessary data being present in revised manuscript.

Discussion:

1. My main concern is the conclusion drawn that the lower albumin level means the child was more malnourished. In the setting of shock and DKA, there are other reasons for the albumin to decrease and affect hepatic production or renal loss, and therefore this review feels that you should at most provide a speculation that this might be the case. For example, the BMI is not significantly different between the groups, and while one might think there is a hint of difference, the ESS group were sicker, and more dehydrated (see Cr). The demonstration that the Na was lower as well - (is this a corrected sodium?). Low sodium levels in the setting of DKA is a strong predictor for poor outcome and cerebral oedema.
Response: The reviewer’s comment has been considered carefully. In revised manuscript, the explanation on the low serum albumin or pre-albumin in DK/DKA with ESS patients has been corrected (Page 14, Line 19-22 and Page 15, Lines 1-8). We agreed that the malnutrition might not be a key underlying mechanism, for there was no significant difference in BMI between ESS and euthyroidism groups. The albumin synthesis in hepatocytes depends on the sufficient insulin secretion. It has been demonstrated that the insulin deficiency led to diminished liver albumin production while the insulin infusion in diabetic patients enhanced live albumin synthesis. So the absolute lack of insulin in T1DM could account for the reduction of serum albumin and pre-albumin in the present study. Moreover, as the albumin deficiency arises with critical ill condition while the DKA/DK children patients tend to be in severe status, the diminished albumin in ESS as compared to euthyroidism might indicate the former are in an inferior situation and subject to more intensive treatment.
As for the serum sodium, it was lower in ESS patients, but when corrected by using blood glucose concentration it showed no significant difference between the ESS and euthyroidism groups. So this could be a pseudohyponatremia due to the water being osmotically drawn into the vascular space in hyperglycemia. Although the low serum sodium concentration has been shown indicative of poor outcome or cerebral edema in DKA, the present study did not reveal the relationship of it with ESS (Page 15, Lines 17-22).

2. Just line up the conclusion you've drawn at the end of the discussion with that in the abstract: i.e at the end of the manuscript you don't mention nutritional status - but you do in the abstract. This reviewer prefers that you don't conclude that nutritional status is the reason behind the low albumin and then the ESS.
Response: Thank you so much for the constructive comment. We have revised the explanation on the low serum albumin or pre-albumin in DK/DKA with ESS patients in revised manuscript (Page 14, Lines 19-21), removing the statement about the malnutrition.

3. Para 4: Please reference the statement "previous studies have shown that patients with ESS tend to me more critically ill compared to those with normal TFTs"
Response: Thank you so much for the suggestion. References[18,20-22] demonstrated the abnormal serum thyroid hormone levels presented in patients admitted with acute critical illness which are often associated with the severity of conditions (Page 13, Line 12).

Reviewer 2

Title:
1. The title claims this study is about children with diabetic ketoacidosis, but the methods section implies some of these children have DK, an abbreviation never spelled out. The title needs to accurately represent the manuscript.
Response: Thank you so much for highlighting this gap. In the current study, a total of 194 children qualified for the final analysis. Among them, 88 were adjudicated to the euthyroid group including 19 cases of DKA and 69 cases of DK, and 106 to the ESS group including 79 cases of DKA and 27 cases of DK. Based on the reviewer’s comment, the title of the manuscript has been revised as “Determinants of deranged thyroid function parameters in children admitted for management of diabetic ketoacidosis/diabetic ketosis”. The full name of DK has been given in revised manuscript (Page 5, Line 8 and Page 6, line 1).

Abstract:
1. Well written, although in the context of severe illness, albumin is not solely a marker of nutrition.
Response: Thank you so much for the suggestion. We have revised the abstract in revised manuscript, removing the statement on the malnutrition.

Background:
1. page 1 line 10-14. It is not well explained why Absolute Insulin Deficiency means that hospital admissions for hypoglycaemic and hyperglycaemic emergencies are frequent.
Response: Thank you so much for pointing out the issue. This statement was inaccurate, so we have removed it in revised manuscript.

2. Para 2. All statements should be referenced. What is "high mortality" in children less than 5 years of age? More generally is this paragraph necessary in a paper about sick euthyroid syndrome in DKA?
Response: Thank you so much for pointing out the issue. As you have mentioned, this paragraph was irrelevant to the main subject of the study. We have replaced it with a sentence “The occurrence of DKA is most caused by infection, stress, inappropriate diet or medications, which has a higher incidence in younger children” in revised manuscript (Page 4, Lines 8-10).

3. Para 3. An important statement of fact is not referenced.
Response: Thank you so much for pointing out the issue. We have provided the third reference for the statement (Page 4, Line 14).

The first sentence is not referenced and the context needs to be given - is this talking about children with DKA? Adults with sepsis? Who?
The second sentence is not referenced and the context is also not explained.
The third sentence is referenced but as written it is not clear what poor prognosis means for children with T1DM - death? Cerebral oedema, later development of microvascular complications? The authors need to be more specific.

Response: Thank you so much for pointing out the issues. The first sentence referred to ICU patients and now was moved to be the third sentence (Page 4, Lines 15-16). The second sentence has been referenced (Page 4, Lines 18). The original third sentence has been removed.

5. Para 6, Hu et al have presented a very similar observation to this manuscript. You comment that it was a retrospective case-control study which is potentially susceptible to recall and selection bias. Your study is this also. Are there any other important differences, flaws of the Hu study that your study improves on?

Response: Thank you so much for pointing out the issues. The present study was also a retrospective case-control study and as compared to the Hu et al’s report, the main advantage was that it analyzed the relationship of much more metabolic parameters to ESS in DKA. However, the potential susceptibility to recall and selection bias could be not improved significantly. We have reworded the text of introduction in revised manuscript (Page 5, Lines 6-9).

6. Para 7. Is it correct that this study looked at the risk of deranged thyroid function in general? It appears that you focussed solely on ESS, and actually did not look at the incidence of other thyroid disorders - such as those with an elevated TSH. This is fine, but Para 7 should accurately reflect the study.

Response: Thank you so much for the constructive comment. We have revised the text and narrowed the subjects to ESS children patients with DKA/DK (Page 5, Lines 5-9).

Methodology:
1. Para 2. The paper referenced for the criteria for T1DM mellitus diagnosis, gives criteria for a diagnosis of DM, but not specifically for Type 1 DM. The authors should specifically state how the diagnosis was made. i.e. were antibody levels taken into account, how were cases of t2dm or monogenic diabetes identified and excluded? (if they were).

Response: Thank you so much for raising the important issue. The diagnosis of T1DM in the present study was based on the American Diabetes Association criteria and ISPAD Clinical Practice Consensus Guidelines 2018: Stages of type 1 diabetes in children and adolescents. In our research, patients with obesity, acanthosis nigricans, fair islet function, family genetic tendency or monogenic hereditary disorders were excluded. We have added the reference to the text in revised manuscript (Page 5, Line 21).

2. DK is problematic. The abbreviation is not explained. The definition of positive urine ketones is not given. This appears to be a group of children with ketosis without acidosis. This is a very different group to those with DKA, and the metabolic derangement is much less severe. The title and abstract for this paper implies that all children had DKA. Some justification for including both in the same analysis required, and results need to explain what proportion of patients had DKA vs DK. Do the finding of this paper hold across DKA and DK patients, or only with DKA? The results need to explore this. If this is explored the inclusion of DK patients could become a strength to this paper rather than a weakness.

Response: Thank you so much for highlighting this gap. In the current study, a total of 194 children qualified for the final analysis. Among them, 88 were adjudicated to the euthyroid group
including 19 cases of DKA and 69 cases of DK, and 106 to the ESS group including 79 cases of DKA and 27 cases of DK. All the analyses were conducted without discrimination between DKA and DK for the main focus of the study was the difference between ESS and euthyroidism groups. The full name of DK has been given in revised manuscript (Page 5, Line 8 and Page 6, line 1).

3. The way ESS is diagnosed here is important. Cutoffs for what were normal or abnormal levels should be given.
Response: Thank you so much for the suggestion. We have defined the threshold for ESS in revised manuscript (Page 6, Line 3-6).

4. The authors cite a review by Warner. This review indicates that FT4 and FT3 are frequently erroneously measured in the context of ESS. It is not clear if the methodology used in this study is prone to this error. Some discussion about this is required. Even if it is prone to error, the observation may still be valid as the method appears to one commonly used in clinical practice, but the limitations could be discussed.
Response: The reviewer’s comment has been considered carefully. For the limitations on the detecting techniques, we did not apply equilibrium dialysis or ultrafiltration methods to measure the free thyroid hormones in serum of patients which might underestimate the concentration of FT3 or FT4. Although the setting of euthyroidism control compensated the weakness partly, the impact of methodology on the outcome might be inevitable. We have specified the limitation in revised manuscript (Page 16, Lines 7-11).

Results:
1. There needs to be a clear description of how many children had DKA vs DK. If DK is uncommon in this sample, I would suggest considering excluding them from the analysis. If DK is quite common, then it may be worth looking at whether the associations identified hold in both patient groups or only DKA. If DK is very common making up more than half of the patients then the study has been mischaracterised as being focussed in DKA. This is important and there must be some revision/explanation here.
Response: Thank you so much for highlighting this gap. In the current study, a total of 194 children qualified for the final analysis. Among them, 88 were adjudicated to the euthyroid group including 19 cases of DKA and 69 cases of DK, and 106 to the ESS group including 79 cases of DKA and 27 cases of DK. All the analyses were conducted without discrimination between DKA and DK for the main focus of the study was the difference between ESS and euthyroidism groups. We have revised DKA to DKA/DK throughout the manuscript.

2. The use of abbreviations that are never defined is inappropriate. On my first review of this manuscript I could not find the glucose data, I believe that PG may represent plasma glucose, but I could be wrong about this.
Response: Thank you so much for pointing out the issue. In revised manuscript, we have added full name to all the abbreviations when they first appeared (Page 8, Lines 5-8).

3. It may be worth presenting the corrected sodium in data tables in addition to the measured sodium, as the low sodiums likely represent pseudohyponatraemia.
Response: Thank you so much for pointing out the issue. The serum sodium was lower in ESS patients in the current study, but when corrected by using blood glucose concentration it showed
no significant difference between the ESS and euthyroidism groups. So this could be a pseudohyponatremia due to the water being osmotically drawn into the vascular space in hyperglycemia. Although the low serum sodium concentration has been shown indicative of poor outcome or cerebral edema in DKA, the present study did not reveal the relationship of it with ESS. We have provided the corrected serum data to the manuscript (all the tables) and made a discussion on it (Page 15, Lines 17-22).

4. In the final sentence of the final paragraph the phrase "significantly affected" implies causation that this study is not capable of determining. "Significantly associated with" or similar is more appropriate.
Response: Thank you so much for the suggestion. We have revised the text as “serum HCO3-levels were the only factor that independently and significantly associated with thyroid function group adjudication” (Page 12, Lines 6-7).

Discussion
1. para 1: Triglyceride levels are not a measure of glycaemic control.
Response: Thank you so much for pointing out the issue. We have removed the triglyceride level as the measure of glycaemic control in revised manuscript (Page 13, Lines 5-6).

2. "Children with ESS were also more malnourished as evidenced by a lower level of serum albumin and prealbumin" - This statement is without basis. The difference in albumin between groups was small, so that even if this analysis was done on children who were well, it would not be reasonable to conclude that the children with ESS were malnourished. Further, these children were acutely unwell many (although you didn't report how many), had DKA a situation of significant metabolic derangement, catabolism and fluid shifts. Thus lower albumin does solely represent nutrition, it is directly related to how unwell the children were.
Response: The reviewer’s comment has been considered carefully. In revised manuscript, the explanation on the low serum albumin or pre-albumin in DK/DKA with ESS patients has been corrected (Page 14, Lines 19-22 and Page 15, lines 1-8). We agreed that the malnutrition might not be a key underlying mechanism, for there was no significant difference in BMI between ESS and euthyroidism groups. The albumin synthesis in hepatocytes depends on the sufficient insulin secretion. It has been demonstrated that the insulin deficiency led to diminished liver albumin production while the insulin infusion in diabetic patients enhanced live albumin synthesis. So the absolute lack of insulin in T1DM could account for the reduction of serum albumin and prealbumin in the present study. Moreover, as the albumin deficiency arises with critical ill condition while the DKA/DK children patients tend to be in severe status, the diminished albumin in ESS as compared to euthyroidism might indicate the former are in an inferior situation and subject to more intensive treatment.

3. "These proteins also serve as carrier proteins for thyroid hormones and hence could thus affect occurrence of more deranged thyroid function tests during DKA episodes thus signifying a poor reserve of thyroid hormones in the blood". There are problems with this statement. First, if there was insufficient T4 and T3 in the blood for the requirement (without changes in hypothalamic-pituitary function), this would lead to an elevation of TSH. This is not what is seen in ESS, so is probably not an important effect. Further, a loss of transport proteins may lead to an increase in
free hormone (which is what was measured in the study), not a decrease as was seen here (e.g. in polycystic ovarian syndrome there is suppression of sex hormone binding globulin which leads to an increase in free testosterone, not a decrease). I would note that the excellent review you cited by Warner stated "Thus, the concentration of total T3 and total T4 in plasma is heavily dependent on the concentration of these binding proteins, whilst the free hormone concentrations should be largely independent of binding protein concentrations." - suggesting binding proteins are probably not very important in effecting the levels of FT3 and FT4.

Response: Thank you so much for giving us the constructive suggestion. We agreed with the reviewer on the comments and have removed the sentence in revised manuscript.

4. "Children in the ESS group had a higher level of WBC signifying either a greater acute phase response or more degree of systemic infection". It is not clear exactly what is meant by a greater degree of systemic infection, do you mean bacteraemia? What evidence do you have of this? Do you mean inflammation rather than infection?

Response: Thank you so much for pointing out the issue. Children in the ESS group had a higher level of WBC and was negatively correlated with levels of FT3, FT4, and TSH in the current work. Elevated WBC has also been shown significantly correlated with severity of DKA and DK in other studies. However, this phenomenon is most likely a leukemoid like reaction instead of a systemic inflammatory response as no fever or bacteria or virus infection evidence was present. Also, in both adult and pediatric DKA patients, the response to milieu interne changes including deranged hormones, cytokines and mediators tend to actuate the elevation of white blood cell count. We have added the explanation to discussion in revised manuscript (Page 15, Line 9-16).

5. Para 2. Greater albumin was associated with greater FT4 (weakly). You suggest this is because it is a binding protein, but if that was the case you might expect a positive association between albumin and bound T4, but a negative association with FT4 if anything and I would draw your attention again to the Warner review you cited that implied minimal effects would be expected with changes in binding proteins. I think you are simply wrong with the conclusion you are making. It appears more likely that degree of illness is associated with ESS, and albumin being low is a marker of more severe illness? Further, to really support investigating the effects of binding proteins it would have been necessary to measure and report Thyroglobulin - the major binding protein for thyroid hormone.

Response: Thank you so much for giving us the constructive suggestion. We agreed with the reviewer on the comments and have removed the relevant information in revised manuscript.

6. Your own findings show that the only factor independently associated with ESS vs not ESS was bicarbonate level. This suggests the albumin is not directly important. I would suggest that in children with DKA bicarbonate is the most direct measure of the degree of illness, and that is why it correlates best with a disorder associated with sickness. The correlation of all other factors in the study is probably simple because they also associated with the degree of sickness. Your study cannot determine whether this is a direct effect of pH or a more general effect of severity of DKA illness.

Response: Thank you so much for the good suggestion. We agreed with the reviewer on the comments and have revised the relevant content in revised manuscript (Page 13, Lines 21-22 and Page 14, Lines 1-9).
7. 5th Para of Discussion:
It is not clear what the point of the first sentences is here.
6th Para. This very long sentence is unfounded and inappropriate. Multiple steps of causation are implied that your study does not support.
Response: Thank you so much for pointing out the issue. We have revised the discussion thoroughly to raise the logic, relevance and accuracy of manuscript.