Author’s response to reviews

Title: Thyroid function, body mass index, and metabolic risk markers in euthyroid adults: a cohort study

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Author’s response to reviews:

Dear Editors:

Thank you for your kind letter on February 6, 2019 and for the reviewers’ comments concerning our manuscript entitled “Thyroid Function, Body Mass Index and Metabolic Risk Markers in a Cohort Study of Euthyroid Adult Population” (BEND-D-18-00355). Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We revised the manuscript in accordance with the reviewers’ comments. Revised portion are marked in red in the paper.

Here below is our description on revision according to the reviewers’ comments.

Responses to Editor
1) Language issues
   We have revised the manuscript by a language polishing service to improve readability.

2) Please ensure that Table 2 is properly formatted
   We have illustrated Table 2 in landscape view.

3) Please note that reviewer 3 has included an annotated version of the manuscript
   Thanks for reminding me. We have revised the manuscript according to the annotated version of reviewer 3 and marked them in red in the paper.
Responses to Reviewers

To Reviewer 1

1. Lack of novelty: The authors should make an effort to justify what this study adds to prior studies that have assessed the same parameters in other Chinese populations, as well as in other ethnic groups.

   Thank you for pointing this out. We acknowledge that the issues discussed in this study are not new. The association between thyroid stimulating hormone (TSH), free thyroxine (fT4) and body mass index (BMI) has already been evaluated in the past. However, on this topic, we think previous studies had some shortcomings and there are still some issues left undiscussed and reported. 1) Small numbers of patients, which increased error and makes the statistical results unconvincing. And we can find that the conclusions of these articles are different and contradictory. 2) Limited health screening programs and no comprehensive assessment was conducted. Most articles simply analyzed the relationship between a certain thyroid hormone (fT3 or fT4) or TSH and BMI, which could not evaluate the exact changes of hypothalamic-pituitary-thyroid axis (HPT axis) at different BMI levels, and the relationship between thyroid function and BMI cannot be analyzed in detail. 3) Most studies focused on overweight or obese, but not underweight. the changes of HPT axis in low body weight is the reverse process in overweight or obese or not also need evaluate. Understanding the changes of HPT axis during low body weight would be necessary for clarify the specific role of thyroid function in weight. For these reasons, we conducted this study with a large sample of 16,975 patients and conducted a comprehensive health examination using standard procedures. We divided the participants into low-weight, normal-weight, overweight, and obese groups, through the data statistics, obtained the relatively reliable results, and analyzed the relationship between weight and HPT axis in detail.

   Considering the reviewer’s suggestion, we have re-written the relevant part (Discussion section, line 268-271, page 12) to better highlight the importance and innovation of this study. The last, the reviewer proposed to evaluate the same parameters in other Chinese populations and other ethnic groups, which we thought was a great idea and could very much like to consider this direction of research in our future work.

2. A more novel approach would be to assess as part of the "metabolic risk factors" the presence/absence of NAFLD.

   Thanks for the proposal. We have carefully reviewed the literature mentioned by the reviewer and some other researches on NAFLD and thyroid function. It is very novel to assess the presence or absence of NAFLD as a metabolic risk factor. Unfortunately, NAFLD was not included in the health check-up. So, we have added this content to the discussion section (Discussion section, line 326-332, page 15) as a complement, so as to attract physicians’ attention and stimulate more relevant research.

3. Authors should rephrase their aim cause no causal relationship can be establish in cross-sectional studies.

   It is really true as reviewer suggested that this is a cross-sectional study, can only assess the presence of an association. The aim of the article ("elucidate whether and how thyroid hormones […] affect weight levels…"; "[…] to provide important statistical support for further exploration of the mechanism of the relationship […]") is misstated and we have made corresponding correction (Background section, line 125-128, page 5). Besides, we examined the same mistakes in the full text and made changes. (Conclusion section, line 341-347, page 16).
4. Table 2 is difficult to read because it is not properly formatted. The r numbers in Table 2 are all <0.25 which not show a strong correlation. Please rephrase conclusions accordingly. Table 2 has been illustrated in landscape view. We accept the comment of reviewer. The r numbers and corresponding P values show that the correlation is present, but not very strong. The statement of "correlation between thyroid hormones and metabolite components" in the "Result" section cannot quite illustrate the statistical results in Table 2. We have made modifications to the relevant content (Result section, line 205-206, page 9).

5. What are the differences between the 2 p values in Table 2? We are sorry for not making clear the meaning of these two P values and caused confusion. The first P value represents the significance of the person correlation coefficient (r), and the second P value represents the significance of the standardized regression coefficients (β). We have already distinguished and annotated the two P in table 2. Thank you for your comments.

6. Results are difficult to interpret in Table 3 due to inconsistencies. For example:

1) the risk of obesity is higher with increasing TSH levels; however, this is not true for overweight in the highest quartile of TSH.

2) Increasing fT4 is associated with higher chances of being low-weight, but less so in the highest quartile of fT4.

3) While the risk of being overweight is lower in the highest quartiles of fT4, no changes are observed for obesity. It is really difficult to interpret the results showed in table 3. The manuscript did not discuss these questions in detail. Here are our views. The first and third inconsistencies can be explained by the opinion that the changes of HPT axis in obesity is not a further process of overweight, and low body weight is not just the reverse process of overweight or obese which we have mentioned in our manuscript (Discussion section, line 272-275, page 13; line 396-301, page 14). This opinion could be proved by previous studies. For example, in the research of Abdi et al, the Delta fT4 of overweight participants is greater than normal weight and obese. It is not the case that obese participants have bigger Delta fT4 than overweight, just as overweight participants have bigger Delta fT4 than normal weight.

The second contradiction is about the relationship between fT4 and low weight. From table 3, we can see that the OR for quartiles 2-4 is 2.526, 2.499 and 1.770, respectively. Quartiles 2-4 of fT4 had an increased risk of low weight compared to quartile 1, but it did not seem does dependent (OR not increasing through quartiles 2 to quartiles 4). So, it is not very suitable to say “Increasing fT4 is associated with higher chances of being low-weight”. We think it is safe to say “patients with the first quartile of fT4 had a decreased risk of being low weight as compared to the remaining patients”. We have modified the "Risk of low weight, overweight, obesity and dyslipidemia" in the results section (Result section, line 221-226, page 10). The discussion section was also corrected as described above (Discussion section, line 272-275, 277-278, page 13).
Reference:


7. Conclusions should be tempered based on the observations mentioned above.
   The conclusions have been adjusted accordingly (Conclusion section, line 341-344, page 16).

Special thanks to you for your good comments.

To Reviewer 2

1. Did the study exclude pregnancy and subjects with autoimmune thyroid disease?
   Yes, participants who have diseases that may affect thyroid hormone levels or metabolism, including pregnancy and autoimmune thyroid disease, have been excluded. We have revised the "Study participants" in the methods section to make it clearer(Methods section, line 141-143, page 6).

2. The statement of "correlation between thyroid hormones and metabolite components" in the "Result" section is confusing; for example, TSH did not significantly associated with BMI (Tab. 1); fT3/fT4 did not inversely associated with FBG; also homocysteine did not inversely associated with fT3 and fT3/fT4 (Tab. 2).
   Thanks for the review’s careful reading and pointing out these misstatements in the"Result" section. We have rechecked Table 1 and Table 2 and re-written "correlation between thyroid hormones and metabolite components" in the "Result" section according to the reviewer’s suggestion (Abstract section, line 39-40, line 43-44, line 47-48, page 2; Result section, line 206-216, page 10; Discussion section, line 272-275, page 13; line 320-321, page 15).

3. Please illustrate Tab. 2 in landscape view.
   Table 2 has been illustrated in landscape view.

4. Please list the range of the quartiles of TSH, fT3, fT4 as well as fT3/fT4 in Tabs. 3 and 4; please also illustrate the range of the quartiles of TSH, fT3, fT4 as well as fT3/fT4 in normal weight group in Tab. 3.
   We have added information about the range of the quartiles of TSH, fT3, fT4 as well as fT3/fT4 in tables 3 and 4 to make the tables more readable. The quartiles were calculated based on indicators for all participants, not a single group. So, we did not list the range of the quartiles in normal weight group in Tab. 3.

5. Please discuss the effect of thyroid hormone on glucose homeostasis, blood pressure and homocysteine Please in further detail.
   According to suggestions of reviewer, we have discussed the relationship between thyroid function
and glucose metabolism, blood pressure and homocysteine in the discussion section (Discussion section, line 313-325, page 14).

6. Please follow the author's instruction in the format of the manuscript (such as format of the references, tables and figures as well as their legends).
We re-read the author's instruction, and checked the format of references, tables and pictures as well as their legends, and made corresponding modifications.

7. Please use 118,540, 3,908, 114,632…etc.
The correction has been made according to the reviewer’s comment (Abstract section, line 36, page 2; Methods, line 150, page 6; Result section, line 186-187, page 8; Discussion section, line 269, page 12).

8. Please give the full name terminology before the abbreviation such as shown in lines 126-128.
This error has been corrected(Result section, line 191-193, page 8)

9. Please improve the English writings by a native English speaker.
We have sent the manuscript to a native English speaker for grammar editing.

Once again, thank you very much for your valuable comments.

To Reviewer 3
1. My major comment relates to the methodology which should be described in more details. I do not quite understand how standardized measurements were ensured in such a large scale setting, where patients were selected post-hoc based on whether normal thyroid samples were available or not. I might have misunderstood the design; please add details.
We are sorry for not making the method part clear. In 2017, total 118,540 citizens received comprehensive health check-up at the health manage center of Tongji Hospital (Wuhan, China). They underwent a routine medical examination, took anthropometric measurements, and provided overnight fasting blood samples and information on medical history, as these were basic items of a health check-up. The hospital has standardized rules and procedures to ensure that every patient receives a standardized health check-up. Participants who met our inclusion criteria and exclusion criteria were selected from these people.
We have rewritten the "Methods" of the manuscript to make it clear (Methods, line 134-137, page 6).

2. Table 2 is illisible in its current form, and I suggest that it is deleted and data given in an alternative way
We have illustrated Table 2 in landscape view.

3. Other comments in the annotated version of the manuscript
   1) "fT4 was associated with diastolic blood pressure (DBP), FBG, hemoglobin A1c (HbA1c), TG and high-density lipoprotein cholesterol (HDL-C). fT3/ fT4 was associated with FBG, HbA1c, TG and HDL-C. " How were they associated?
Our description of the results is somewhat vague and the manuscript has been corrected accordingly (Abstract section, line 42-48, page 2).
   2) Wrong format of reference
It has been corrected (Background, line 119-120, page 5 ).
3) How was standardized procedures ensured in more than 16000 patients included based on thyroid hormone assessment? Was the antropometric assessment performed at time of biochemical assessment or post hoc?
I may misunderstood the protocol. Did the patients meet for reexamination after the initial sampling to ensure standardized procedures?
In 2017, total 118,540 citizens underwent a routine medical examination, took anthropometric measurements, and provided overnight fasting blood samples and information on medical history, as these were basic items of a health check-up. Participants who met our inclusion criteria and exclusion criteria were enrolled into this study. We have rewritten the "Methods " of the manuscript to make it clear (Methods, line 134-137, page 6).

4) Were the logistic regression analyses of the risk of dyslipidemia adjusted for age, gender, BMI or other?
Thank you for the suggestion. Logistic regression model was used to predict the risk of dyslipidemia and adjusted for age and gender (no BMI), as described in the "Methods " section of the "Statistical analysis". We added this information in table 4 which was ignored before.

5) From table 3 Q2-4 had an increased risk of low weight, but it did not seem does dependent (OR not increasing through Q2 to Q4). Thus, it seems that those patients with Q1 fT4 was a decreased risk of being low weight as compared to the remaining patients. the OR for Q2-4 of fT4 is 2.526, 2.499 and 1.770 (not increasing through Q2 to Q4). So, it is safer to say “patients in the first quartile of fT4 had a lower risk of being underweight than the other participants” than to say “fT4 of higher quartiles was observed to increase the risk of low body weight”. We have revised it in the manuscript (Discussion section, line 277-278, page 13).

6) " To sum up, overweight or obese participants had high levels of TSH, fT3, fT3/ fT4 and low levels of fT4. " The patients did not have ‘high’ levels, but they seemed to have higher levels compared to low weight.
Thanks to correct me. We have revised the content as “ Compared with normal weight individuals, overweight individuals are more likely to have high serum concentrations of fT3 and low concentrations of fT4; obese individuals are more likely to have high concentrations of TSH and fT3/fT4; and underweight individuals are more likely to have high concentrations of fT4 and low concentrations of fT3.” (Conclusion section, line 341-344, page 16).

Special thanks to the review for suggesting how to improve our paper.

We appreciate for editors and reviewers’ warm work earnestly. The reviewer’s comments are quite helpful, and we revised the manuscript point-by-point. Thank you and the reviewer again for your help!

Sincerely,

Qingquan Liu