July 19, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: Zhou_(R1).doc).

Title: Vitamin paradox in obesity: deficiency or excess?

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The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated

2 Revision has been made according to the suggestions of the reviewers (in **blue**)

Reviewer 1

It is generally thought that the low vitamin status in obesity is due to vitamin deficiency. However, this article through a phenomenon: the obese individuals show lower fasting serum folate concentrations, but their red blood cell (RBC) folate concentrations are significantly higher, compared with non-obese individuals, combined with their previous research, they think obese individuals may involve vitamin excess rather than deficiency.

The topic is novel, but there are a few questions need to be considered.

1. In different organs, the level of Folic acid is different, the increase of RBC folate concentrations historically has been considered the best indicator of tissue (liver) body stores (Tinker SC, et al. Birth Defects Res A Clin Mol Teratol. 2012;94(10):749-55. Subramanian VS, et al. J Cell Physiol. 2003;196(2):403-8.). How to reasonably assess the overall level of vitamins (folic acid), through the serum folate or the RBC folate?

   This comment, although interesting, is beyond the scope of our MS. Moreover, it is impossible to study the distribution of folic acid in human tissues. However, the effect of folic acid supplementation on RBC folate content has been studied (e.g., Pietrzik K, et al. Calculation of red blood cell folate steady state conditions and elimination kinetics after daily supplementation with various folate forms

and doses in women of childbearing age. Am J Clin Nutr 2007;86:1414 –9).

2. In vivo and in vitro experiments demonstrated that folate deficiency leads to a significant up-regulation in folate uptake (Said HM, et al. Am J Physiol Cell Physiol. 2000 Dec;279(6):C1889-95.), it provides a possible that low serum folate may stimulate folate uptake by red blood cells in obesity. This issue might be taken consideration.

   It is not known whether increased RBC folate contents may involve an increase in folate uptake. The term "increased folate storage" used in our MS does not exclude the possible involvement of uptake mechanism.

3. Obesity is related to diabetes and non-alcoholic fatty liver disease (NAFLD), in diabetes and NAFLD patients, the serum level of vitamins also present low level (Valdes-Ramos R, et al.Endocr Metab Immune Disord Drug Targets. 2015; 15(1): 54-63. Kwok RM, et al. Hepatology. 2013;58(3):1166-74. Pacana T, S et al. Curr Opin Clin Nutr Metab Care. 2012;15(6):641-8.). Although antioxidant vitamins at large doses may have pro-oxidant effects, a series of clinical studies have shown that vitamins can obviously improve diabetes and NAFLD (Sato K, et al. Nutrition.2015; 31:923-30. Iqbal S, et al. Nutrition.2015;31:901-7.), which indicated the obesity may not be vitamin excess.

   As discussed in the MS, serum vitamin level is not a reliable marker of vitamin deficiency. There is lack of larger, multicenter, longer-term trials on the effect of antioxidant vitamins on diabetes and NAFLD. Moreover, many large-scale antioxidant-vitamin trials show that high–dose vitamin E supplementation may increase cardiovascular events and all-cause mortality (relevant references have been added to the revised version), and so does vitamin A supplementation (Bjelakovic G, Nikolova D, Gluud C. Meta-regression analyses, meta-analyses, and trial sequential analyses of the effects of supplementation with beta-carotene, vitamin A, and vitamin E singly or in different combinations on all-cause mortality: do we have evidence for lack of harm? PLoS One 2013;8:e74558). There is also a meta analysis showing that antioxidant supplements may increase liver enzyme activity (Bjelakovic G, et al. Antioxidant supplements for liver diseases. Cochrane Database Syst Rev. 2011 Mar 16;(3):CD007749). Overall, most existing evidence supports the hypothesis of vitamin excess.

Reviewer 2

The authors described vitamin paradox in obesity. Overall, the review is well-written. The authors focused on vitamin homeostasis, oxidative stress and folate status. It would be better to add the relationship each vitamins such as vitamin D and obesity in greater detail.

Indeed, low vitamin D status is associated with various diseases on the one hand, and on the other hand, most, it not all, vitamin D supplementation trials show a negative outcome. We have discussed this phenomenon in this revised version.

Reviewer 3

The manuscript entitled "Vitamin paradox in obesity: deficiency or excess? "is an interesting, nicely written and covers most updated studies on the intake of vitamins, its metabolisms and effects on obesity and diabetes. I have a few minor comments.

Minor comments:

1. Please tweak the 2nd statement in bold on page 7.
   Revised

2. Although several studies on an update of vitamins intake and their association with obesity/diabetes have been documented in this manuscript, it will be great if authors could compile data on vitamins intake and their relationship with obesity in other major countries of Europe and Asia.

   While this issue has been discussed in our previous publication (World J Diabetes 2014), we have briefly mentioned this in the revised Introduction.

I would like to thank you and the reviewers for the very valuable comments that will help us improving the manuscript.

Sincerely yours,

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