December 22, 2014

Dear Editor,

Please find enclosed the edited manuscript in word format (14018-review.doc).

Title: Serum Hepcidin Concentrations and Type 2 Diabetes

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Name of Journal: World Journal of Diabetes

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

The format has been updated to meet the requirements of a minireview

We thank the editor-in-chief, co-editor and the reviewers for their constructive and helpful comments and criticism. Revision has now been made according to the suggestions of the reviewers

Reviewer 1 (2446516)

This review summarized the studies involved the relationship between hepcidin and T2DM. However, it can't make a conclusion that hepcidin plays role in the development of T2DM. More basal and clinical studies are needed. The subtitle "Role of hepcidin in the aetiopathogenesis of T2D" is not appropriate. All the evidence only showed the relationship between hepcidin and T2D, could not suggested any aetiopathogenesis. Please change the subtitle.

REPLY: We agree with the reviewer that a role cannot be convincingly deduced yet for hepcidin in the aetiopathogenesis of T2D from the existing evidence, thus, we also concluded in our review by making a call for more data in this study question. We have now changed the subtitle in the manuscript as suggested to "Mechanisms linking hepcidin and T2D". Also we have rephrased the CONCLUSION part of the manuscript to address this concern: "Thus, more experimental and clinical studies are needed to confirm or refute the claim that hepcidin has a role inT2D".

Reviewer 2 (1404215)

The manuscript by Aregbesola et al. is a brief review of the relationship between Type 2 diabetes and serum hepcidin concentrations. Only a few articles have been published on this relationship, and consequently the authors are not justified in deducing that hepcidin increases the risk of developing T2D.

REPLY: We reported on a study which showed a decreased risk in T2D with iron-lowering variants of TMPRSS6. We are also not drawing such conclusion from this brief review. We were only strengthening the point for TMPRSS6 as one of the possible links between hepcidin

and T2D. We have now rephrased some parts of the manuscript to address this concern. In ABSTRACT: "This review briefly reports the existing evidence on the possible links between hepcidin and T2D and concludes that more data are needed to confirm or refute hepcidin's role in the development of T2D". In the MAIN TEXT: "To further strengthen the association between hepcidin and T2D". We have also changed the subtitle in the MAIN TEXT from "Role of hepcidin in the aetiopathogenesis of T2D" to "Mechanisms linking hepcidin and T2D". In the CONCLUSION: "Thus, more experimental and clinical studies are needed to confirm or refute the claim that hepcidin has a role in T2D".

Moreover, it is not clear whether the increase in serum iron concentrations promotes T2D in patients through the action of hepcidin.

REPLY: Our claim from this brief review is to highlight other emerging mechanistic links between hepcidin and T2D. To address this question, we have now added to the CONCLUSION part of the manuscript: "Although the causative role of body iron in insulin resistance and T2D has been documented in both observational [41] and interventional [42] studies, the role of hepcidin in this process is still uncertain. However, in addition to the regulatory role in body iron stores, serum hepcidin concentrations have been linked to pro-inflammatory cytokines, STAT3, and TMPRSS6, all of which have been associated with T2D."

My feeling is that many more articles on the topic will need to be published before a Review can clarify whether hepcidin can induce insulin resistance or sensitivity in patients.

REPLY: One of our objectives was to stimulate readers and investigators of urgent need for more studies to clarify if hepcidin has a role in the aetiopathogenesis of T2D or if it is just mere speculations. We have now added to the manuscript under the subtitle Studies linking hepcidin and T2D: "suggesting an association between prohepcidin and body iron in insulin sensitivity" We also reported in the manuscript under the subtitle Mechanisms linking hepcidin and T2D, that: "It is at least plausible to report that lower hepcidin concentration exacerbates insulin resistance seen in T2D, if causal relationship is yet to be fully established" and concluded that more studies are needed to confirm or refute this claim.

Reviewer 3 (2584208)

This review briefly reports the existing evidence on the possible role of hepcidin in the development of T2D.Authors concluded thta furher studies are necessary to fully elucidated this item. This review is cleraly written and the topic of interest. Previously published papers regarding this topic are few and with contradictory results. I only suggest to describe more in detail these papers and try to speculate a possible pathogenetic mechanism.

REPLY: We have now described the studies further: "In Sam et al. study [15], the authors measured serum hepcidin and serum hepcidin:ferritin ratio, which has been suggested as a marker of adequate hepcidin production for a particular iron dosage [36]. Aso et al. [29] also measured serum ferritin, prohepcidin and adiponectin, and both studies showed decrease in serum hepcidin/prohepcidin in T2D subjects when compared with the healthy control [15, 29].

Because of the confounding effect of obesity and renal status in serum hepcidin measurement, Sam et al. matched their control subjects for body mass index (BMI) and serum creatinine [15]. Aso et al. assessed the correlation between adiponectin and prohepcidin in T2D subjects on the basis that adiponectin has a beneficial role in glucose homeostasis [37]; hence, in glucose dysregulation observed in T2D, adiponectin and prohepcidin were expected to be low. In keeping with their hypothesis, they found low concentrations of adiponectin and prohepcidin and a positive correlation between them in T2D subjects. The other two case-control studies [14, 16] showed increased hepcidin/prohepcidin in T2D subjects when compared with the control group. In Jiang et al. study [14], T2D subjects had higher BMI and creatinine than the controls, thus suggesting obesity and renal impairment as possible reasons for the elevated serum hepcidin. Further, inflammatory markers, i.e. IL-6, C - reactive protein and white cell counts, were elevated in T2D subjects compared to the controls, speculating inflammatory signals as the cause of the elevated hepcidin." We have now added to the concluding part of the subtitle Mechanisms linking hepcidin and T2D: " It is therefore enticing to speculate from the available evidence that hepcidin has a role in insulin resistance, the hallmark of T2D, through iron regulation from interrelated signals of STAT3, pro-inflammatory cytokines and the TMPRSS6 enzyme."

Furthermore, some of these papers are related to hepcidin and others to pro-hepcidin. Can you comment this point? At least for chronic renal patients major differences can occur between hepcidin and pro-hepcidin concentrations.

REPLY: We believe that the lack of an accurate assay to evaluate serum hepcidin in the past may have influenced the choice of prohepcidin by some investigators which is thought to be easier to measure due to its higher immunogenicity. Although some studies have shown that prohepcidin does not accurately reflect iron status and iron absorption [38], while others have claimed that it is an indicator of endogenous hepcidin levels [39] in healthy subjects. This could be the reason why some papers were related to hepcidin and others to prohepcidin. Further, increased serum prohepcidin concentration has been observed in subjects having renal insufficiency (Taes et al. 2004), hence, the poor iron absorption seen in chronic kidney disease patients. Because biologically active hepcidin-25 is a relatively small peptide, it is difficult to raise antibodies against it, and particularly in CKD patients in end-stage renal failure with elevated hepcidin of different types. Thus, there is cross reactivity in serum hepcidin-25 measurement with other hepcidins, i.e. hepcidin-20 and hepcidin-22 in CKD patients [40]. Prohepcidin appears reliable to assay more so that studies have found prohehcidin to be elevated and correlated negatively with glomerular filtration rate in CKD patients (Taes et al 2004, Malyszko J et al 2006). We have now added to the manuscript under the subtitle Studies linking hepcidin and T2D: "One factor that could be responsible for the contradictory findings in the hepcidin-T2D association study is the wide range of assays with varying degrees of limitation that were used in evaluating serum hepcidin and serum prohepcidin. The lack of an accurate assay to evaluate serum hepcidin in the past may have influenced some investigators to choose prohepcidin, which is thought to be easier to measure due to its higher immunogenicity. Although some studies have shown that prohepcidin does not accurately reflect iron status and iron absorption [38], others have claimed that it is an

indicator of endogenous hepcidin levels [39] in healthy subjects. This could be the reason why some studies used hepcidin while others used prohepcidin in examining the association between hepcidin and T2D. This is of particular interest in chronic renal disease patients in end-stage renal failure, where there is cross-reactivity in serum hepcidin-25 measurement with that of other hepcidins, i.e. hepcidin-20 and hepcidin-22 [40].”

References were carefully checked and corrected. All the corrections and inclusions are highlighted in red in the revised manuscript.

Thank you again for publishing our manuscript in the World Journal of Diabetes.

Sincerely yours,

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