CENTRO DE INVESTIGACIÓN Y DE ESTUDIOS AVANZADOS
DEL INSTITUTO POLITÉCNICO NACIONAL

Departamento de
Bioquímica

Dra. Angélica Rueda y Sánchez de la Vega

July 28, 2021

Timothy R. Koch, MD
Editor in Chief
World Journal of Diabetes

Dear Dr. Koch,

Please find enclosed the revised manuscript entitled "A tale of two kinases: PKA and CaMKII in pre-diabetic cardiomyopathy", for your consideration as a review article by invitation (ID 03920636) in *World Journal of Diabetes*. We are grateful for your willingness to evaluate this new version of our manuscript. We believe to have addressed all the concerns from the reviewers in the point-by-point response document (attached) which helped us to improve substantially our review.

All changes in the revised manuscript, including new references, are highlighted in yellow in the supplementary file 64251-Manuscript File_revised version_highlighted.doc.

We still believe that our revised manuscript is in line with the scope and mission of the *World Journal of Diabetes*. The manuscript has not been published, is not under consideration elsewhere, and has been approved by all the authors.

Yours faithfully,

Angélica Rueda, Ph.D.
Associate Professor

PEER-REVIEW REPORT

Name of journal: World Journal of Diabetes

Manuscript NO: 64251

Title: A tale of two kinases: PKA and CaMKII in prediabetic cardiomyopathy

Reviewer's code: 03649645

Position: Editorial Board

Academic degree: MD, PhD

Professional title: Professor

Reviewer's Country/Territory: China

Author's Country/Territory: Mexico

Manuscript submission date: 2021-02-25

Reviewer chosen by: Ya-Juan Ma

Reviewer accepted review: 2021-05-29 06:24

Reviewer performed review: 2021-05-31 05:16

Review time: 1 Day and 22 Hours

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [Y] Accept (General priority) [ ] Minor revision [ ] Major revision [ ] Rejection
Re-review	[ ] Yes [Y] No
Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

MetS is a serious public health problem with increased risk for cardiovascular disease and DM2, leading to cardiac dysfunction in the form of prediabetic cardiomyopathy. In this paper, the author first introduced the role of PKA and CaMKII in cardiomyocytes, and discussed the establishment of predictive cardiopathy model in animals with different diets, then analyzed the reasons β AR/AC/Camp/PKA axis in predictive cartography and CaMKII as a novel target in predictive cartography. This review has clear logic, sufficient theory, and a certain degree of innovation, would allow for laying the foundation for rational design of targeted therapies to treat or prevent the development of pre-diabetic cardiomyopathy. There are some deficiencies in this view, there are several kinds of ADR in cardiomyocytes, however, it is not known how prediabetic cardiomyopathy alters β1-AR expression and signaling, it is recommended to find relevant literature to confirm this point of view. Furthermore, the references are well cited in the recent 3-5 years.

Response:

We thank the reviewer for her/his positive comments and suggestions. In respect of the possible link between pre-diabetic cardiomyopathy and β-adrenoceptor expression and signaling, works addressing modifications in β-adrenoceptor (β-AR) expression induced by pre-diabetes are still scarce. Thus, in the revised version of our manuscript, (please see the supplementary file 64251-Manuscript File_revised version_highlighted_Adeptify-edits.doc, pages 12-13, lines 323-336) we discuss the controversial outcome of studies performed with animal models of diabetes, obesity or MetS on the expression of β-ARs, and our conclusion that further research is required to elucidate fully the link between MetS, β-AR expression and signaling alterations and cardiac dysfunction. For this purpose, we have included two studies not previously quoted.

PEER-REVIEW REPORT

Name of journal: World Journal of Diabetes

Manuscript NO: 64251

Title: A tale of two kinases: PKA and CaMKII in prediabetic cardiomyopathy

Reviewer's code: 02541960

Position: Editorial Board

Academic degree: MD, PhD

Professional title: Doctor, Professor

Reviewer's Country/Territory: Japan

Author's Country/Territory: Mexico

Manuscript submission date: 2021-02-25

Reviewer chosen by: Ya-Juan Ma

Reviewer accepted review: 2021-05-25 07:45

Reviewer performed review: 2021-06-02 13:00

Review time: 8 Days and 5 Hours

Scientific quality	[ ] Grade A: Excellent [Y] Grade B: Very good [ ] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[Y] Grade A: Priority publishing [ ] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [Y] Minor revision [ ] Major revision [ ] Rejection
Re-review	[Y] Yes [ ] No
Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

This review summarizes the current knowledge of the pathophysiological consequences of enhanced and sustained β-adrenergic response in prediabetes, focusing on cardiac dysfunction. This review also outlines recent information on the role of PKA and CaMKII in abnormal Ca2+ handling by cardiomyocytes. This article is well written and of clinical interest. Major comments 1 I would like to know the difference between prediabetic and overt diabetic cardiomyopathies, although I understand the primary focus of the review for prediabetic cardiomyopathy. 2 In the mechanism shown in Figure 1, the contribution of insulin resistance or hyperinsulinemia is unclear. Please add it into or explain in the text. 3 Is it possible to provide human study for the alterations in PKA or studies corresponding for such animal study? 4 The difference between presence ☑ and increase in the Table 1. For instance, presence of insulin resistance usually means the increase of insulin resistance. Minor comments It may be better to change the symbol of × (no change) to → and ☑ (presence) to +.

Response

We thank the reviewer for her/his assertive comments and suggestions. Our specific responses are as follows (please refer to the supplementary file 64251-Manuscript File_revised version_highlighted_Adeptify-edits.doc):

Major comments:

1. I would like to know the difference between prediabetic and overt diabetic cardiomyopathies, although I understand the primary focus of the review for prediabetic cardiomyopathy.

Response

Because type 2 Diabetes Mellitus (DM2) is a well-recognized pathology, diabetic cardiomyopathy has been studied and characterized in depth and there exist compelling

reviews on this topic (for example, Jia et al., 2018; Pereira et al., 2014; Piché et al., 2020; Rotkvić et al., 2021).

For DM2 patients, the term diabetic cardiomyopathy refers to the presence of Ca^{2+} mishandling, cardiomyocyte hypertrophy, apoptosis and fibrosis, together with abnormal myocardial performance in the absence of hypertension, coronary artery disease, or valvular heart disease (Jia et al., 2018; Pereira et al., 2014). Although the clinical entity of pre-diabetic cardiomyopathy still lacks a universally accepted definition, studies have linked pre-diabetes to CVD. Each MetS component represents a risk factor for CVD; in combination, these components increase the rate and severity of CVD as it relates to several conditions including microvascular dysfunction, coronary atherosclerosis and calcification, and cardiac dysfunction, which lead to myocardial infarction and heart failure (HF) (Tune et al., 2017). In animal models of pre-diabetes, obesity, IR, and other components of MetS can lead to cardiac dysfunction associated with structural and functional abnormalities (see **Table 1**), implying cardiomyopathy mechanisms different from those of DM2.

Very few articles have considered MetS-associated cardiac alterations as pre-diabetic cardiomyopathy (Carvajal et al., 2014), most likely due to the lack of an accepted definition. Based on the graded effect of impaired glucose metabolism on diastolic function, it has been proposed that a morphological intermediate state between normal and diabetic states underlies pre-diabetic heart dysfunction (Stahrenberg et al., 2010). One feature that perhaps differentiates pre-diabetic from diabetic cardiomyopathy is the absence of overt structural changes in the heart in the former, although this interpretation is under discussion (see De Marco et al., 2011).

We have modified several paragraphs of the manuscript accordingly (please see the supplementary file 64251-Manuscript File_revised version_highlighted_Adeptify-edits.doc; page 5, lines 106-123 and lines 126-131; and page 10, lines 252-258).

2. In the mechanism shown in Figure 1, the contribution of insulin resistance or hyperinsulinemia is unclear. Please add it into or explain in the text.

Response

The Figure 1 depicts the abnormal β_1-AR signaling pathway activation in cardiomyocytes in the presence of several MetS components (obesity, increased triglyceride levels, decreased high-density lipoprotein cholesterol and hypertension), including insulin resistance. Although we indeed think insulin resistance to be a critical factor underlying several MetS-associated pathologies, we cannot emphasize it as the unique trigger of MetS pathogenesis in cardiac cells. Following the reviewer's suggestion, we have added the aforementioned biochemical alterations as text in Figure 1 to make it more explicit.

3. Is it possible to provide human study for the alterations in PKA or studies corresponding for such animal study?

Response

We thank the reviewer to bring to our attention the importance of evaluating alterations not only in PKA but also in CaMKII expression/activity in the human heart undergoing pre-diabetes.

To date, we found no data on PKA alterations in diabetic or pre-diabetic patients; clearly studies addressing this issue would provide valuable information on the pathophysiology of MetS- and diabetes-induced cardiomyopathy, as stated in the revised manuscript (supplementary file 64251-Manuscript File_revised version_highlighted_Adeptify-edits.doc; page 14, lines 371-373).

Notably, post-translational modifications (specifically, oxidation, O-glycosylation and

phosphorylation) of CaMKII are increased in heart samples of diabetic patients (Daniels et al., 2018; Erickson et al., 2013; Luo et al., 2013), suggesting altered kinase activity. As for PKA, research on the possible role of CaMKII alterations in diabetic or pre-diabetic patients is required to increase our understanding of the pathophysiology of MetS- and diabetes-induced cardiomyopathy.

We have included this information in the revised manuscript (page 16, lines 414-418).

4. The difference between presence ☑ and increase in the Table 1. For instance, presence of insulin resistance usually means the increase of insulin resistance.

Response

Most studies quoted in Table 1 refer to insulin resistance evaluated by different criteria, such as HOMA-IR index or the glucose tolerance test, but not all of them quantify the increase in blood insulin levels. Therefore, we prefer to indicate the presence of insulin resistance with the symbol ✓as a qualitative feature. We hope the reviewer finds this explanation convincing.

Minor comments

It may be better to change the symbol of × (no change) to → and ☑ (presence) to +.

Response

We thank the reviewer for this valuable observation. We agree on the use of the symbol × being potentially confusing to indicate the lack of change; therefore, it has been substituted by the symbol ↔.

PEER-REVIEW REPORT

Name of journal: World Journal of Diabetes

Manuscript NO: 64251

Title: A tale of two kinases: PKA and CaMKII in prediabetic cardiomyopathy

Reviewer's code: 03701805

Position: Editorial Board

Academic degree: MD, PhD

Professional title: Associate Professor

Reviewer's Country/Territory: China

Author's Country/Territory: Mexico

Manuscript submission date: 2021-02-25

Reviewer chosen by: Ya-Juan Ma

Reviewer accepted review: 2021-05-26 08:29

Reviewer performed review: 2021-06-04 03:35

Review time: 8 Days and 19 Hours

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [Y] Minor revision [ ] Major revision [ ] Rejection
Re-review	[Y] Yes [ ] No
Peer-reviewer statements	Peer-Review: [ ] Anonymous [Y] Onymous Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

The interesting review by Gaitán-González et al summarized the current knowledge of the pathophysiological consequences of enhanced and sustained beta-adrenergic response in prediabetes, focusing on protein kinase A (PKA) and Ca2+/calmodulin-dependent protein kinase II (CamKII). The manuscript is well written, the figure and tables presented the most important message well. I have some suggestions: 1. Introduction: to emphasize the importance for recognizing the pathophysiological consequences of prediabetes, it is important to note that prediabetes, although still with controversy, observational studies and large sample meta-analyses had shown that prediabetes is associated with increased risk of cardiovascular disease and all-cause mortality in general population, as well as in those in baseline CVD (PMID: 27881363; PMID: 32669282) 2. Is prediabetic cardiomyopathy really exist or with clinical importance? I think it is true. Recently study also reported that prediabetes is associated with increased risk of heart failure. Such data further support the important term "prediabetic cardiomyopathy" proposed in this review. I suggest to cite these clinical findings (PMID: 33769672). 3. It should be note that the clinical definition of prediabetes including those with impaired fast blood glucose (IFG) and impaired glucose tolerance (IGT, 2 hour plasma glucose concentration 7.8-11.0 mmol/L during an oral glucose tolerance test). These two are reflecting different pathophysiological mechanisms in blood glucose regulation. However, not optimal animal models are suitable for distinguishing IFG or IGT models. 4. Keep consistent for the spelling of "pre-diabetic" with "prediabetic".

1. Introduction: to emphasize the importance for recognizing the pathophysiological consequences of prediabetes, it is important to note that prediabetes, although still with controversy, observational studies and large sample meta-analyses had shown that prediabetes is associated with increased risk of cardiovascular disease and all-cause mortality in general population, as well as in those in baseline CVD (PMID: 27881363; PMID: 32669282).

2. Is prediabetic cardiomyopathy really exist or with clinical importance? I think it is true. Recently study also reported that prediabetes is associated with increased risk of heart failure. Such data further support the important term "prediabetic cardiomyopathy" proposed in this review. I suggest to cite these clinical findings (PMID: 33769672).

Response

We think points 1 and 2 are closely related, and we have answered to them jointly.

We thank the reviewer for her/his suggestions. Articles supporting the link of pre-diabetes to increased risk of cardiovascular disease (CVD), heart failure (HF), myocardial infarction and stroke have been quoted in the revised manuscript (please see the supplementary file 64251-Manuscript File_revised version_highlighted_Adeptify-edits.doc; page 5, lines 109-123) as follows:

'Although the clinical entity of pre-diabetic cardiomyopathy still lacks a universally accepted definition, studies have linked pre-diabetes to CVD. Each MetS component represents a risk factor for CVD; in combination, these components increase the rate and severity of CVD as it relates to several conditions including microvascular dysfunction, coronary atherosclerosis and calcification, and cardiac dysfunction, which lead to myocardial infarction and heart failure (HF) (Tune et al., 2017). In animal models of pre-diabetes, obesity, IR, and other components of MetS can lead to cardiac dysfunction associated with structural and functional abnormalities (see **Table 1**), implying cardiomyopathy mechanisms different from those of DM2. Furthermore, observational studies and large sample meta-analyses show that pre-diabetes, defined as impaired glucose tolerance, impaired FBG, or raised glycated hemoglobin, was associated with increased risk of CVD (Cai et al., 2020; Huang et al., 2016) and HF (Mai et al., 2021). Moreover, meta-analysis of longitudinal studies indicates that MetS is linked to

increased risk of myocardial infarction, stroke, and CVD, with the risk estimate being higher than that corresponding to its individual components (Gami et al., 2007; Mottillo et al., 2010).'

3. It should be note that the clinical definition of prediabetes including those with impaired fast blood glucose (IFG) and impaired glucose tolerance (IGT, 2 hour plasma glucose concentration 7.8-11.0 mmol/L during an oral glucose tolerance test). These two are reflecting different pathophysiological mechanisms in blood glucose regulation. However, not optimal animal models are suitable for distinguishing IFG or IGT models.

Response

We agree on the lack of optimal animal models suitable to differentiate impaired fast blood glucose (IFG) from impaired glucose tolerance (IGT), and the underlying pathophysiological mechanisms. We think that this vision is reflected in our manuscript (supplementary file 64251-Manuscript File_revised version_highlighted_Adeptify-edits.doc; page 11, lines 274-277), which states that although diet-based MetS animal models bear relevance for pre-diabetic cardiomyopathy research, their utility is hampered by discrepancies in biochemical and corporal parameters, along with dissimilar outcomes that might be associated with the type and length of the diet.

4. Keep consistent for the spelling of "pre-diabetic" with "prediabetic".

Response

The manuscript has been checked and corrected for consistency (pre-diabetes) as indicated.