Answers to the reviewers' comment

1) Reviewer id (00503228)

Comment: Very good study. Congratulations!

Answer: We sincerely thank the reviewer for his/her valuable time and effort to review our manuscript and appreciate our work.

2) Reviewer id (00503199)

Comment: The review is nice. Some minor editing comments: "of glucose from the glomeruler ultrafiltrate" glomerular "In 2012, study from DeFronzo lab" a study from or In 2012 DeFronzo et al elucidated….. "may regulate gluconeogeneis through luminal substrate uptake" gluconeogenesis "may elevate gluconeogenesis" may increase.

Answer: We sincerely appreciate the reviewer's valuable inputs to improve the quality of our manuscript. We apologize for the oversight of typographical errors. All the typographical errors have been corrected and highlighted in the revised manuscript.

3) Reviewer id (03475636)

Comment: This review is original written. No significant plagiarism is detected. However, there are many incorrect grammar uses. The manuscript would benefit from copy edits. Will need certificate for English edits. To mention as a few: Misspelled words as listed below. RE: "Dysregulaion" should be "Dysregulation" RE: "pleitropic" should be "pleiotropic function" RE: "glomeruler" should be "glomerular" RE: "gluconeogeneis" should be "gluconeogenesis" RE: "inhibiton" should be "inhibition" RE: "beside" should be "besides" RE: "clatherin-mediated endocytosis" should be "clathrin-mediated endocytosis".

Answer: We are thankful for the reviewer's valuable comments to improve the quality of our manuscript. We apologize for the oversight of typographical and grammatical errors. We have

made necessary corrections, and all the edits are highlighted in the revised manuscript. Our revised manuscript has been proofread by Dr. Carolyn M. Ecelbarger (Associate Professor, Division of Endocrinology and Metabolism, Georgetown University, Washington D.C., USA), who is an expert in the field. We have acknowledged her contribution in the revised manuscript.

4) Reviewer id (00502999)

Comment: The topic of the manuscript is interesting, but there are no personal inputs by the authors. It looks like a monograph. Major concerns: I would change the title to INSULIN AND ITS RECEPTORS IN HEALTH AND DISEASE Avoid IR abbreviation for Insulin receptors, as it may be mistaken by readers for insulin resistance. Why do authors do not talk about insulin receptor dysfunction and the mesangium?. What are the interactions between angiotensin II and insulin on mesangial cells secretion of TGF-beta1 and matrix expansion?. The paper could be improved if the impact of the different receptors dysfunctions are detailed stga eby stage of CKD. Are the tubular segments with the sodium handling affected the same way in diabetes compared to glomerulopathies, intersttial nephritis or primary hypertension? What is the impact of insulin receptor dysfunction on proteijn handling by the interstitium, i.e: IFTA? It is not cubulin, it s cublilin. It is not clatherin, it is clathrin. What does ORAI stand for? It does need a great deal of English language polishing.

Answer: We are thankful for the reviewer's valuable comments to improve the quality of our manuscript.

A) We appreciate the reviewer's suggestion to change the title; however, we have chosen the title **"Insulin receptors in the kidneys in health and disease"** because this review specifically highlights the role of the insulin receptor (not the insulin-like growth factor (IGF-I) receptor and the insulin receptor-related receptor) in the kidney, which could affect renal as well as systemic metabolism in health and diseased condition.

B) Since the term "insulin receptor" is used repeatedly, '**IR**' is used to abbreviate the insulin receptor, which we have mentioned in the manuscript. We have not used any abbreviation for insulin resistance to avoid confusion.

C) Mesangial cells are crucial for renal function because these cells regulate glomerular hemodynamics. In mesangial cells, the insulin receptors (IRs) are present in small number and the IGF-1R is the dominant receptor for insulin's action[1]. Therefore, we did not focus on the insulin receptor dysfunction in mesangial cells.

Angiotensin II is reported to induce mesangial cell expansion and fibrosis via upregulation of TGF-β[2]. Moreover, insulin is shown to have an additive effect on Angiotensin II-mediated induction of TGF-β and extracellular matrix production[3]. However, Anderson *et al.* reported that additive effect of insulin is not because of modulation at the receptor level, but due to insulin-mediated activation of MAP kinase[3].

D) We agree with the reviewer's comment that it is important to comprehend the effect of different insulin receptors (the IRs, the IGF-1R, and the IRRs) in the pathogenesis of CKD. However, in the present manuscript, our main focus was to accentuate the role of the insulin receptors (IRs) in the kidney. Therefore, we excluded other insulin receptors in this review. Those studies can be reviewed in future manuscripts.

E) Are the tubular segments with the sodium handling affected the same way in diabetes compared to glomerulopathies, intersttial nephritis or primary hypertension?

In diabetes, sodium reabsorption in the proximal tubule is increased due to hyperglycemia-induced upregulation of Na^+-glucose cotransporters (SGLT1 and SGLT2) and tubular growth[4]. In interstitial nephritis, interstitial inflammation causes tubulointerstitial injury, which may lead to reduced or altered tubular sodium retention[5, 6].

Dietary sodium intake is reported to play a crucial role in primary hypertension. High sodium intake results in enhanced sodium reabsorption from proximal tubules, which contributes to increased fluid volume and resultant hypertension[7]. Moreover, lysine deficient protein kinases (WNKs) stimulate sodium retention in the distal nephron and are accountable for familial hypertension. Studies have shown that activity of WNKs is partly regulated by insulin[7].

F) Interstitial fibrosis and tubular atrophy (IFTA) is central to the progression of chronic kidney disease. Activation of different cell types and molecular mechanisms such as inflammation, myofibroblast differentiation, activation of TGF-β and bone morphogenic protein (BMP), excess deposition of ECM proteins lead to IFTA[8].

One of the key mechanisms involved in the etiology of IFTA is proteinuria/albuminuria that provokes tubulointerstitial injury and increases the susceptibility of the kidney towards disease conditions. Excess amount of protein induces apoptosis, pro-inflammatory, and fibrogenic responses in renal interstitium and tubule cells, which result in various pathological alterations and tubulointerstitial damage[9, 10]. Since dysfunction of the insulin receptors in renal tubules is linked with increased albuminuria (which we have discussed in the manuscript), it is possible that the insulin receptor dysfunction might play a causal role in the development of IFTA. However, further investigation is needed to elucidate this interaction.

G) We apologize for the oversight of typographical errors. ORAI stands for **Calcium release-activated calcium channel proteins** encoded by ORAI gene. We have made necessary corrections, and all the edits are highlighted in the revised manuscript. Our revised manuscript has been proofread by Dr. Carolyn M. Ecelbarger (Associate Professor, Division of Endocrinology and Metabolism, Georgetown University, Washington D.C., USA), who is an expert in the field. We have acknowledged her contribution in the revised manuscript.

References

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