Circulation Research Peer Review Report for DOI: 10.1161/CIRCRESAHA.121.320546

Title: SIRPα Mediates IGF1 Receptor in Cardiomyopathy-Induced by Chronic Kidney Disease

In an effort to promote greater transparency in peer review, the authors and reviewers of this *Circulation Research* article have opted to post the original decision letter with reviewer comments to the authors and the authors' response to reviewers for each significant revision.

June 3, 2021

Dr. Sandhya S. Thomas
Baylor College of Medicine/ Michael E. DeBakey Veterans Affairs Medical Center
Medicine
One Baylor Plaza
Houston, Texas 77030

RE: CIRCRES/2021/319542: SIRPα Impairs IGF-1 Receptor Signaling in Cardiomyopathy Induced by Chronic Kidney Disease

Dear Dr. Thomas:

Your manuscript has been carefully evaluated by 3 external reviewers and the editors as a Regular Article. We regret to inform you that the paper is not acceptable for publication in Circulation Research.

As you will gather from the reviews, the referees identified a number of substantive conceptual and methodological problems. The editors concur. Major issues include inadequate sample size, lack of rigor in data presentation, and insufficient functional data to support the conclusions.

Given the nature of these concerns, which could not be adequately addressed without extensive new experimentation, the editors do not encourage revision. Nevertheless, if you feel that you can effectively address all of the reviewers' comments and are willing to perform the new experiments required, we would be willing to evaluate a resubmitted version on a de novo basis. The paper would be reviewed again, with no assurance of acceptance. Since the re-evaluation would be done de novo, the revised paper would be assigned a new number regardless of when it is resubmitted. One or more of the original reviewers would be re-consulted; the editors may also choose to obtain additional opinions from new reviewers. Please note that even after extensive modifications, we cannot guarantee that your manuscript will receive a priority sufficient for publication. Overall, fewer than 15% of all papers submitted to Circulation Research are eventually published.

As detailed in the reviewers' critiques, a responsive resubmission would require a substantial amount of new data. In particular, the editors feel that additional data would be necessary to increase the sample size in all the experiments suggested by reviewer #1, improve the quality of the western blot data, and provide some functional evidence to directly link SIRPα to changes in myocardial function in either patients or animals with chronic kidney disease.

To read the comments to authors from the reviewers, please see below.

If you choose to resubmit, please include a detailed response to each of the referees' and editors' comments, providing each comment verbatim in bold followed by your response and giving the exact page number(s), paragraph(s), and line number(s) where each change was made. If you make substantive changes to the manuscript, please provide a clear description of what you did and where. If you insert important sentences, paragraphs, or sections in response to the comments, please also include them in your response. Please indicate clearly any

deletions. Additionally, a marked up version of the resubmission with the changes highlighted or tracked should be uploaded as a supplemental file. If you do choose to resubmit, please do so online using the "Submit Resubmission" link available in your Author Tasks area or Post Decision Manuscripts folder.

Please ascertain that your resubmitted manuscript adheres to the Instructions to Authors as they appear online at https://www.ahajournals.org/res/author-instructions. Resubmission that do not conform to the current limits on numbers of words (8000 total) and display items (maximum of 8 tables and/or figures) will be returned to the authors for abbreviation. If you cannot reduce the overall word count, the editors may deem an extended print version appropriate; the authors should provide written assurance that they will cover the costs of the pages that are in excess of these limits. Note that paying for excess display items is not an option. Please refer to the Instructions to Authors for further details regarding our policy on page limits, articles with extended print versions, and related costs. No such limits apply to the online supplementary information, which can include supporting data and/or expanded text to offset the limits on the print version. Such online supplementary information can be cited in the print version as appropriate.

We know that you will be disappointed by this decision. Circulation Research currently receives approximately 2,000 manuscripts a year, of which fewer than 15% can be published; as a consequence, relative priorities must be considered in making the final decision.

Despite our decision, we wish to thank you for having submitted this manuscript to Circulation Research.

Sincerely,

Jane E. Freedman, MD
Editor-in-Chief
Circulation Research
An American Heart Association Journal

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Reviewer comments to the Authors:

Reviewer #1:
In the original study, Thomas et al investigated the mechanism by which chronic kidney disease promotes cardiomyopathy. It has become increasingly evident that various etiologies of cardiomyopathy can have distinct mechanisms of pathogenesis. The relationship between CKD and cardiomyopathy is complex and remains unclear. The authors hypothesize that CKD-mediated activation of SIRPalpha downregulates IGF-1 receptor signaling, therapy promoting adverse cardiac remodeling. They utilized a partial nephrectomy mouse model of CKD as well as whole body- and tissue-specific SIRPalpha knockout mice to demonstrate that SIRPalpha knockdown blocks deleterious cardiac remodeling. The experiments are well designed and mostly support the authors conclusions. Given the paucity of data in this field, this study presents interesting and novel findings for CKD-mediated cardiomyopathy.

Major points:
-please describe the severity of CKD caused by subtotal nephrectomy. Roughly how does it compare to human CKD (eg, stage III, IV, V, etc).
-Figure 1E: The number of samples in each group is small (n=4 each). One of the patients has diabetes which may affect the SIRPalpha/IGF-1 axis. Recommend analyzing a larger sample size.

-Figure 2E: Recommend removing the E/A ratio data. The differences are subtle and diastolic measurements are challenging to interpret in a mouse model. If diastolic measurements are included, would add tissue Doppler (E/e'), which would give more helpful information.
-Figure 3A: Please discuss why the SIRPalpha Mt sham animals have a greater heart weight/TL ratio than the WT sham animals. That would suggest that SIRPalpha knockdown can affect cardiac remodeling in the absence of CKD.
-Figure 3: Recommend WGA staining and measurement of cardiomyocyte size to differentiate between cardiac hypertrophy and increased number of cardiomyocytes.

Minor points:
-Figure 8: would change "uremic cardiomyopathy" to "CKD-mediated cardiomyopathy." This study does not delineate whether uremia or another aspect of CKD is the driver of adverse cardiac remodeling.

Reviewer #2:
Major:
1. This is a very exciting and potentially important observation. I commend the authors on their study.

2. My major concern is that the quality of the western blot data appears to be poor, and blots are submitted with extreme focus on the band of interest. Some blots appear saturated whereas others contain artifacts making quantification seem difficult. I think it would be very helpful if authors would provide original blots for review.

Minor

1. N values are stated as ranges rather than N for each experimental group.

2. Description of quantification methodology for fibrosis would be helpful (figure 4).

3. Echo data seem a little strange. This may be related to method of PNx, but I'm surprised that RWT and some other dimensions are not increased by PNx in WT animals. Also, MPI data are not shown. Variability in EF measurement seems high. Some statistics shown in figure 2 not shown in the supplementary table (e.g., statistical significance for EF changes).

Reviewer #3:
This paper examined whether chronic kidney disease (CKD) stimulates circulating signal regulatory protein alpha (SIRPα) to impair myocardial insulin/IGF-1 receptor signaling and adverse cardiac remodeling. SIRPα expression in mouse models and serum of patients with CKD were examined. In both mice and patients with CKD serum SIRPα expression was upregulated, while control mice with CKD displayed increased myocardial SIRPα with impaired insulin/IGF-1 receptor signaling, diastolic and systolic dysfunction and fibrosis. However, SIRPα KO mice with CKD displayed intact insulin/IGF-1 receptor signaling and myocardial function. Compared to control mice, KO mice maintained insulin/IGF-1 receptor signaling (PI3K, pAKT and pY-IGF1R) despite the presence of CKD. Recombinant SIRPα was able to re-establish a reduction in pAKT. A SIRPα and IGF-1R interactions is proposed to mediate these effects. It is concluded that upregulation of myokine SIRPα induces anti-insulin activities in cardiac muscle, disrupting protective insulin/IGF-1R signaling pathways. Circulating SIRPα constitutes an important readout of myocardial insulin resistance in CKD-induced cardiomyopathy.

General Comments:

This paper provides some intriguing data to suggest that SIRPα increases in chronic kidney disease (CKD) and can impair myocardial insulin signaling via interacting with the insulin/IGF-1 receptor. However, there are a number of issues related to the interpretation of the experimental data, as well as a lack of data directly linking SIRPa to

changes in myocardial function in either patients or animals with (CKD). Very little data is provided as to what effect modifying SIRPα, or SIRPα interaction with the insulin/IGD-1 receptor, has on actual cardiac function in the mouse model. That data provided in Figure 2B suggesting that deletion of SIRPα prevents cardiac dysfunction in CKD is not convincing, as there is no difference in %EF between wild type CKD mice and SIRPα Mt mice subjected to CKD. Also, no functional data in other models of tissue specific SIRPα deletion is provided. This is a major limitation to the conclusion that increased SIRPα may adversely influence myocardial function.

Specific Comments:

1) How does adding extracellular SIRPα modulate insulin signaling? SIRPα is a transmembrane glycoprotein which contains three extracellular immunoglobulin-like domains and a cytoplasmic region containing src homology-2 (SH-2) binding motifs. SIRPα is a docking protein for tyrosine phosphatases (i.e. SHP1-2), promoting insulin resistance. The authors do not address what the relationship is between adding extracellular SIRPα to cells versus being an integral membrane protein already existing in the muscle.
2) Figure 3C,D, E and F should have the individual values indicated in the figures.
3) Figure 3C: Why was heart weight/TL increased in the SIRPα Mt mice?
4) Figure 3D: SIRPα Mt mice did not express fetal gene program, but were hypertrophied (Figure 3A). What is happening here?
5) Figure 3C: Systolic BP is decreased in SIRPα Mt CKD and csSIRPa-/- CKD mice compared to WT and floxed CKD? Why?
6) The authors should be using the Cre controls, not the flox controls.
7) Figure 4A: Fibronectin is already increased in the SIRPa Mt mice in the absence of CKD. Why? It is proposed that SIRPα prevents cardiac fibrosis in response to CKD because there is a rise in fibronectin in WT vs WT CKD, but this is not seen in the SIRPα Mt vs SIRTα Mt CKD. However, fibronectin is already dramatically elevated in the SIRPα Mt sham even in the absence of CKD. The authors don't explain this.
8) Figure 5A: in mSIRPα-/- there was a dramatic decrease in pY-IGF1R in the absence of adding back rSIRPα. What was responsible for this?
9) Figure 5B: It suggested that addition of rSIRPα to C2C12 cells reduced pY-IGF1R. However, the data in Figure 5B does not actually quantify the blots. Furthermore, in Figure 5C, the addition of the SIRPa plasmid does not actually result in a reduction in pY-IGF1R. Therefore, the conclusion that exogenous and endogenous SIRPα influence impaired insulin/IGF-1 receptor signaling is not justified.
10) It is suggested that a link between interaction between SIRPα and the IGF-1R receptor to mediates cardioprotection in diabetes and myocardial infarction. This has not been established in this study.
11) Figure 7: This data should be quantified.

Baylor College of Medicine

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November 23, 2021

Dear Dr. Freedman,

We are re-submitting the enclosed manuscript entitled "SIRPα Mediates IGF1 Receptor Signaling in Cardiomyopathy-Induced by Chronic Kidney Disease " for your consideration in *Circulation Research*. We sincerely appreciate the thorough review of our manuscript by the Editorial Board and the Reviewers. We have comprehensively addressed the Editors' and Reviewers' comments:

Editor's Comments:

As detailed in the reviewers' critiques, a responsive resubmission would require a substantial amount of new data. In particular, the editors feel that additional data would be necessary to increase the sample size in all the experiments suggested by reviewer #1, improve the quality of the western blot data, and provide some functional evidence to directly link SIRPα to changes in myocardial function in either patients or animals with chronic kidney disease.

Answer: We sincerely thank the Editors for their comments and the opportunity to resubmit a revised manuscript. In short, we have increased the sample sizes for the experiments suggested by Reviewer #1 including the human evaluations. Additionally, we have improved the Western blot data presentations and provide evidence for a functional link between suppression of SIRPα impacting cardiac function, despite the presence of CKD. In fact, the original submission revealed statistically significant differences in cardiac function (e.g. ejection fraction/ fractional shortening %) with higher EF/FS % in SIRPα Mt mice with CKD when compared to WT mice with CKD. We have improved the presentation to reveal these differences more clearly in Figure 2, as well as Supplemental Table 2. Additionally, based on comments by Reviewer #3, we have provided new echo data revealing cardio-protection in cardiac muscle specific SIRPα KO (csSIRPα$^{-/-}$) mice, despite the presence of CKD.
See pg. 18, Figure 2.

Reviewer comments to the Authors:
Reviewer #1:

In the original study, Thomas et al investigated the mechanism by which chronic kidney disease promotes cardiomyopathy. It has become increasingly evident that various etiologies of cardiomyopathy can have distinct mechanisms of pathogenesis. The relationship between CKD and cardiomyopathy is complex and remains unclear. The authors hypothesize that CKD-mediated activation of SIRPalpha downregulates IGF-1 receptor signaling, thereby promoting adverse cardiac remodeling. They utilized a partial nephrectomy mouse model of CKD as well as whole body- and tissue-specific SIRPalpha knockout mice to demonstrate that SIRPalpha knockdown blocks deleterious cardiac remodeling. The

experiments are well designed and mostly support the authors conclusions. Given the paucity of data in this field, this study presents interesting and novel findings for CKD-mediated cardiomyopathy.

Answer: Thank you for the encouraging comments.

Major points:

-please describe the severity of CKD caused by subtotal nephrectomy. Roughly how does it compare to human CKD (e.g., stage III, IV, V, etc.).

Answer: We have been investigating the metabolic abnormalities including elevated BUN or uremia that occur in kidney disease. Creatinine levels were 2-3-fold higher than sham control mice which corresponds to advanced CKD. We have included this point in our discussion: " After subtotal nephrectomy, WT and SIRPα Mt mice had similar serum creatinine and blood urea nitrogen (BUN) levels (2-3-fold higher; **Figure 1A**). Additionally, mice subjected to subtotal nephrectomy exhibit metabolic acidosis, increased parathyroid hormone levels [1, 2], and muscle wasting, all of which typically occurs in patients with advanced CKD [3] ." **See pg: 10, line 13-16.**

-Figure 1E: The number of samples in each group is small (n=4 each). One of the patients has diabetes which may affect the SIRPalpha/IGF-1 axis. Recommend analyzing a larger sample size.

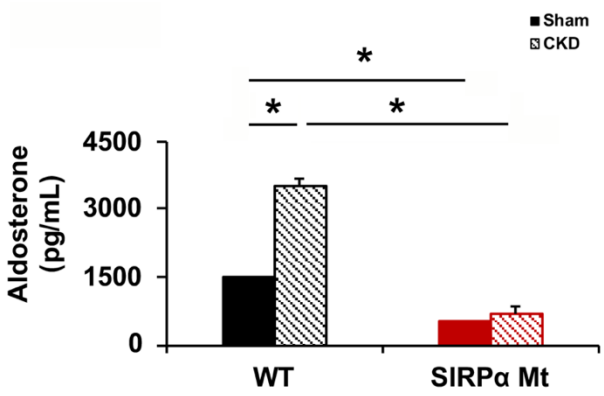
Answer: We have increased the sample size in our immunoblot evaluations for human serum SIRPα to n=20 in each group. **Pg. 17, Figure 1E.**

-Figure 2E: Recommend removing the E/A ratio data. The differences are subtle and diastolic measurements are challenging to interpret in a mouse model. If diastolic measurements are included, would add tissue Doppler (E/e'), which would give more helpful information.

Answer: We have removed the comments associated with diastolic dysfunction and provided E/a and E/e' in Table S2 as suggested by Reviewer #1. **See pg:31, Table S2.**

-Figure 3A: Please discuss why the SIRPalpha Mt sham animals have a greater heart weight/TL ratio than the WT sham animals. That would suggest that SIRPalpha knockdown can affect cardiac remodeling in the absence of CKD.

Answer: SIRPα Mt sham mice show evidence of physiologic hypertrophy with statistically significant increases in cardiomyocyte size (new data), cardiac output, and a trend towards an increased EF/FS % when compared to WT sham mice. There is no evidence of fibrosis, collagen deposition based on sirius red staining, elevations of αSMA, or systolic blood pressure with suppression of aldosterone levels in SIRPα Mt mice (new data shown here). In fact, aldosterone levels were lower in SIRPα Mt control mice when compared to WT control mice (n=3 mice/sham group, n=6 mice/CKD group). Since SIRPα Mt mice are global KO mice, we created cardiac muscle-specific SIRPα KO mice and compared these mice to littermate flox control mice in order to determine the muscle-specific effect on cardiac remodeling. We did not see any differences in heart weight between sham csSIRPα$^{-/-}$ vs. littermate flox sham control mice (Figure 3B) new data. We have included this point in our discussion. **See pg:11, line 6-8, pg: 13, line 39-42.**



-Figure 3: Recommend WGA staining and measurement of cardiomyocyte size to differentiate between cardiac hypertrophy and increased number of cardiomyocytes.

Answer: We have included cardiomyocyte size in **Figure 3B** which indicates that WT mice with CKD hypertrophied, while cardiomyocytes in SIRPα Mt with CKD did not hypertrophy in response to CKD when compared to their sham control mice. We did note that similar to heart weights, cardiomyocyte size in SIRPα Mt sham animals was larger. Our explanation for this is similar to that was previously stated above. **See pg:19 Figure 3B.**

Minor points:
-Figure 8: would change "uremic cardiomyopathy" to "CKD-mediated cardiomyopathy." This study does not delineate whether uremia or another aspect of CKD is the driver of adverse cardiac remodeling.

Answer: We have changed the labeling for Figure 8 from uremic cardiomyopathy to CKD-induced cardiomyopathy.

Reviewer #2:

Major:

1. This is a very exciting and potentially important observation. I commend the authors on their study.

Thank you.

2. My major concern is that the quality of the western blot data appears to be poor, and blots are submitted with extreme focus on the band of interest. Some blots appear saturated whereas others contain artifacts making quantification seem difficult. I think it would be very helpful if authors would provide original blots for review.

Answer: We have improved the quality of Western blots and compiled all original blots. Please see the quality of all new immunoblots.

Minor

1. N values are stated as ranges rather than N for each experimental group.

Answer: We have now included each N for each experimental group. Thank you for your suggestion.

2. Description of quantification methodology for fibrosis would be helpful (figure 4).

Answer: We included a description of quantification methods for collagen deposition based on sirius red staining in the methods section. See the been highlighted lines. **See pg. 9, line 41-45**.

3. Echo data seem a little strange. This may be related to method of PNx, but I'm surprised that RWT and some other dimensions are not increased by PNx in WT animals. Also, MPI data are not shown. Variability in EF measurement seems high. Some statistics shown in figure 2 not shown in the supplementary table (e.g., statistical significance for EF changes).

Answer: Thank you for the comment. RWT did not reveal a statistically significant difference between the groups which may be related to no significant difference in systolic blood pressure between WT sham vs. CKD mice. MPI data was included in **Table S2**. We have corrected the statistical significance in **Table S2** which was inadvertently omitted. Both fractional shortening and ejection fraction were statistically different, as indicated correctly in **Figure 2**. We have correctly included those differences in Table S2 and have improved graphically the statistical differences in **Figure 2** to show these differences more clearly. **See pg. 18, Figure 2 and pg. 31, Table S2**.

Reviewer #3:

This paper examined whether chronic kidney disease (CKD) stimulates circulating signal regulatory protein alpha (SIRPα) to impair myocardial insulin/IGF-1 receptor signaling and adverse cardiac remodeling. SIRPα expression in mouse models and serum of patients with CKD were examined. In both mice and patients with CKD serum SIRPα expression was upregulated, while control mice with CKD displayed increased myocardial SIRPα with impaired insulin/IGF-1 receptor signaling, diastolic and systolic dysfunction and fibrosis. However, SIRPα KO mice with CKD displayed intact insulin/IGF-1 receptor signaling and myocardial function. Compared to control mice, KO mice maintained insulin/IGF-1 receptor signaling (PI3K, pAKT and pY-IGF1R) despite the presence of CKD. Recombinant SIRPα was able to re-establish a reduction in pAKT. A SIRPα and IGF-1R interactions is proposed to mediate these effects. It is concluded that upregulation of myokine SIRPα induces anti-insulin activities in cardiac muscle, disrupting protective insulin/IGF-1R signaling pathways. Circulating SIRPα constitutes an important readout of myocardial insulin resistance in CKD-induced cardiomyopathy.

General Comments:

This paper provides some intriguing data to suggest that SIRPα increases in chronic kidney disease (CKD) and can impair myocardial insulin signaling via interacting with the insulin/IGF-1 receptor. However, there are a number of issues related to the interpretation of the experimental data, as well as a lack of data directly linking SIRPa to changes in myocardial function in either patients or animals with (CKD). Very little data is provided as to what effect modifying SIRPα, or SIRPα interaction with the insulin/IGF-1 receptor, has on actual cardiac function in the mouse model. That data provided in Figure 2B suggesting that deletion of SIRPα prevents cardiac dysfunction in CKD is not convincing, as there is no difference in %EF between wild type CKD mice and SIRPα Mt mice subjected to CKD. Also, no functional data in other models of tissue specific SIRPα deletion is provided. This is a major limitation to the conclusion that increased SIRPα may adversely influence myocardial function.

Answer: To address the major concerns of Reviewer #3, we offer the following. There was a statistically significant difference between WT with CKD vs. SIRPα Mt mice with CKD. Specifically, SIRPα Mt mice displayed improved EF%, FS%, and cardiac output. We have improved the figure presentation to better illustrate those differences in **Figure 2** and **Table S2, See pg: 18 Figure 2 A-D, pg: 31Table S2**. Additionally, as suggested by Reviewer #3, we have performed additional experiments to include echo data in cardiac muscle specific (cs) SIRPα KO mice with CKD which were compared to flox mice with CKD and determined that cardiac muscle specific SIRPα KO were protected from cardiac dysfunction despite the presence of CKD when compared to flox control mice with CKD. **See pg: 18, Figure 2 (E-H).**

Specific Comments:

1) How does adding extracellular SIRPα modulate insulin signaling? SIRPα is a transmembrane glycoprotein which contains three extracellular immunoglobulin-like domains and a cytoplasmic region containing src homology-2 (SH-2) binding motifs. SIRPα is a docking protein for tyrosine phosphatases (i.e. SHP1-2), promoting insulin resistance. ***The authors do not address what the relationship is between adding extracellular SIRPα to cells versus being an integral membrane protein already existing in the muscle.***

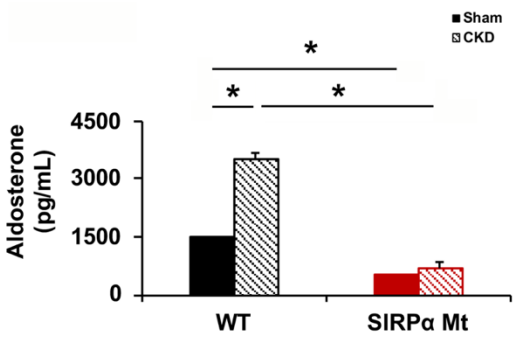
Answer: This is a very important point. Thank you. We have included a discussion of how extracellular SIRPα impacts insulin/IGF-1 signaling. We believe further work will be required to delineate the relationship between extracellular SIRPα, but this is beyond the scope of this investigation. However, SIRPα is not normally expressed in muscle cells but in response to NF-kB activation or CKD [1]. SIRPα is upregulated in both cardiac and skeletal muscles after CKD exposure. Furthermore, Umemori et al. has identified that the extracellular domain of membrane bound SIRPα is cleaved and released by skeletal muscle cells. While Londino et al. determined that inflammation stimulates myeloid SIRPα extracellular domain cleavage. We have now included this point in our discussion. Thank you again. **See pg:14, line 45-46 and pg:15, line 1-3.**

2) Figure 3 C,D, E and F should have the individual values indicated in the figures.

Answer: We have indicated individual numbers with each experimental group **pg. 19**.

3) Figure 3C: Why was heart weight/TL increased in the SIRPα Mt mice?
Answer:

SIRPα Mt sham mice have evidence of physiologic hypertrophy with statistically significant increases in cardiomyocyte size (new data), cardiac output, and a trend towards an increased EF/FS % when compared to WT sham mice. There is no evidence of collagen deposition based on sirius red staining, elevation of αSMA, or systolic blood pressure plus suppression of aldosterone levels in SIRPα Mt mice (new data, below). In fact, aldosterone levels were lower in SIRPα Mt control mice when compared to WT control mice. Since SIRPα Mt mice are global KO mice we utilized cardiac muscle-specific SIRPα KO mice and compared these mice to littermate flox control mice in order to determine the muscle-specific effect on cardiac remodeling. We did not see any differences in heart weight between sham csSIRPα$^{-/-}$ vs. littermate flox control mice, see (Figure 3B). We have now included this point in our discussion. **See pg:13, line 39-44.**



4) Figure 3D: SIRPα Mt mice did not express fetal gene program, but were hypertrophied (Figure 3A). What is happening here?

Answer:

Thank you for asking this important question. Your questions are similar to the ones raised by Reviewer #1: SIRPα KO sham mice have evidence of physiologic hypertrophy, including increased CO, and a trend of increased EF or FS % when compared to WT sham mice, but no evidence of collagen deposition based on sirius red staining or elevations in systolic blood pressure or aldosterone (which is new data see above). In fact, aldosterone levels were lower in SIRPα KO sham mice (above) when compared to WT sham mice. Additionally, the fetal gene program was in fact upregulated in SIRPα Mt control mice (as indicated in **Figure 3E**), when compared to WT control mice.

5) Figure 3C: Systolic BP is decreased in SIRPα Mt CKD and csSIRPa-/- CKD mice compared to WT and floxed CKD? Why?

Answer: Aldosterone levels were lower in SIRPα Mt when compared to WT mice (as above). Further investigation is required to evaluate how SIRPα suppression mitigates RAAS activation in response to CKD, and therefore systolic BP. We have included this point in our discussion. **See pg:15, line 12-13.**

6) The authors should be using the Cre controls, not the flox controls.

Answer: Thank you for the suggestion. Myocardial SIRPα suppression contributed to the cardio-protection in csSIRPα KO mice when compared to flox mice subjected to CKD (**Pg 18, Figure 2 E-H**).

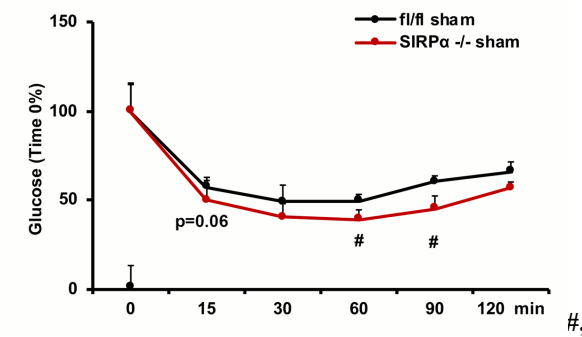
csSIRPα KO mice display improved EF%, FS% and cardiac output despite the presence of CKD. In fact, previous reports suggest that α*MyHC-Cre* mice display evidence of cardiac dysfunction and toxicity by greater than 3 months of age when compared to WT littermate controls [4], however these csSIRPα KO mice with CKD were protected, in spite of the same Cre driver and impaired renal function. We thank the Reviewer for his comments and have included this in our discussion. **See pg:13, line 44-46, and pg:14, line 1-2.**

7) Figure 4A: Fibronectin is already increased in the SIRPα Mt mice in the absence of CKD. Why? It is proposed that SIRPα prevents cardiac fibrosis in response to CKD because there is a rise in fibronectin in WT vs WT CKD, but this is not seen in the SIRPα Mt vs SIRRα Mt CKD. However, fibronectin is already dramatically elevated in the SIRPα Mt sham even in the absence of CKD. The authors don't explain this.

Answer: SIRPα Mt mice are global KO mice. Therefore, since we did see an increase in fibronectin in SIRPα Mt mice baseline without any changes in cardiac function. Additionally, we did not see increased collagen deposition by picrosirius staining in SIRPα Mt sham mice. Therefore, we obtained muscle plus cardiac muscle-specific KO mice to determine the organ-specific effects. We did not see these baseline elevations in fibronectin in mSIRPα$^{-/-}$ mice when compared to their flox littermate controls (**Figure 4C**). We thank Reviewer #3 for identifying this point. We have added it to our discussion. **See pg: 13, line 39-42.**

8) Figure 5A: in mSIRPα-/- there was a dramatic decrease in pY-IGF1R in the absence of adding back rSIRPα. What was responsible for this?

Answer: In the data presented in **Figure 5A** of the original submission, we added insulin prior to the harvest. Since mSIRPα$^{-/-}$ mice are more insulin sensitive when compared to flox control based on insulin tolerance tests (see below), the responses to insulin stimulation are different between these 2 groups. When mice hearts were not exposed to insulin prior to harvest there were no significant differences in yP-IGF-1R which was normalized to total IGF-1R between flox and mSIRPα$^{-/-}$ control mice treated with the diluent (see new **Figure 6A, pg. 22).**



9) Figure 5B: It suggested that addition of rSIRPα to C2C12 cells reduced pY-IGF1R. However, the data in Figure 5B does not actually quantify the blots. Furthermore, in Figure 5C, the addition of the SIRPa plasmid does not actually result in a reduction in pY-IGF1R. Therefore, the conclusion that exogenous and endogenous SIRPα influence impaired insulin/IGF-1 receptor signaling is not justified.

Answer: We have included quantification for the original **Figure 5B** and updated the figure. Previously after transfection we had placed transfected cells in high glucose media (4.5g/L, 25mM) which affected GFP or basal tyrosine phosphorylation of IGF-1R therefore, making it difficult to detect differences after SIRPα plasmid transfection. Therefore, we placed newly transfected cells in low glucose media (5mM) instead in order to determine differences in myotubes after SIRPα transfection. Therefore, overexpression of SIRPα downregulated pY-IGF1R relative to total IGF1R in myoblasts. Additionally, we added new data, HL-1 cardiomyocytes were transfected with SIRPα plasmid which additionally downregulated pY-IGF1R relative to total IGF1R. **See new Figure 6 C-D, pg 22.**

10) It is suggested that a link between interaction between SIRPα and the IGF-1R receptor to mediates cardio-protection in diabetes and myocardial infarction. This has not been established in this study.

Answer: We have modified the discussion as suggested by Reviewer #3 and removed the implication that SIRPα and IGFR1 interactions may mediate cardio-protection in diabetes and myocardial infarction since this has not been established in this study.

11) Figure 7: This data should be quantified.

Answer: We have quantified all of Figure 7 immunoblots and updated the figure. See **Figure 7, pg: 24**. Thank you.

Thank you for all your insightful suggestions. We hope we have comprehensively addressed all of the Editors' and Reviewers' concerns.

Sincerely,

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1. Thomas SS, Dong Y, Zhang L and Mitch WE. Signal regulatory protein-alpha interacts with the insulin receptor contributing to muscle wasting in chronic kidney disease. *Kidney Int*. 2013;84:308-16.
2. Wu J, Dong J, Verzola D, Hruska K, Garibotto G, Hu Z, Mitch WE and Thomas SS. Signal regulatory protein alpha initiates cachexia through muscle to adipose tissue crosstalk. *J Cachexia Sarcopenia Muscle*. 2019;10:1210-1227.
3. Sharma D, Hawkins M and Abramowitz MK. Association of sarcopenia with eGFR and misclassification of obesity in adults with CKD in the United States. *Clin J Am Soc Nephrol*. 2014;9:2079-88.
4. Pugach EK, Richmond PA, Azofeifa JG, Dowell RD and Leinwand LA. Prolonged Cre expression driven by the alpha-myosin heavy chain promoter can be cardiotoxic. *J Mol Cell Cardiol*. 2015;86:54-61.

Circulation Research Peer Review Report for DOI: 10.1161/CIRCRESAHA.121.320546

January 5, 2022

Dr. Sandhya S. Thomas
Baylor College of Medicine/ Michael E. DeBakey Veterans Affairs Medical Center
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RE: CIRCRES/2021/320546D: SIRPα Mediates IGF1 Receptor Signaling in Cardiomyopathy-Induced by Chronic Kidney Disease

Dear Dr. Thomas:

Your manuscript has been carefully evaluated by 5 external reviewers and the editors as a Regular Article. We regret to inform you that the paper is not acceptable for publication in its present form.

As you will gather from the reviews, the referees identified a number of conceptual and methodological problems. The editors concur. Major issues include, the experimental numbers are still low in some experiments, which negatively impacts rigor. There are also several statistical and technical issues.

Despite these concerns, the editors see this paper as potentially important and wish to encourage revision. If you would like to revise the manuscript in accordance with the suggestions of the reviewers and editors, we would be willing to evaluate a new version. The manuscript would be reviewed again, with no assurance of acceptance.

The Editors strongly encourage you to adhere to the journal's Statistical Reporting Recommendations in your revision, which can be found here: https://www.ahajournals.org/statistical-recommendations.

Among the concerns cited by the reviewers, the editors feel that the most important issue that need to be addressed is to increase the n value in all experiments per recommendation by reviewer #2, respond to questions and suggestions raised by reviewer #1, and it will also be necessary to respond to all the statistical and technical reviewers' concerns.

Upon revision, authors of manuscripts that contain cropped gels/blots will be required to submit a separate PDF file that contains the entire unedited gel for all representative cropped gels in the manuscript. Authors should label each gel as "Full unedited gel for Figure _" and highlight which lanes of the unedited gel correspond to those shown in the cropped images within the manuscript. For more information, please go to https://www.ahajournals.org/res/manuscript-preparation.

All research materials listed in the Methods should be included in the Major Resources Table file, which will be posted online as PDF with the article Supplemental Materials if the manuscript is accepted. A template Major Resources Table file (.docx) is available for download here: AHAJournals_MajorResourcesTable_2019.docx. Authors are required to upload the Table at the revision stage. Authors should reference the PDF in their Methods as follows: "Please see the Major Resources Table in the Supplemental Materials."

To read the comments to authors from the reviewers, please see below.

Please note that revised and resubmitted manuscripts are not assured of publication, and that fewer than 15% of all papers submitted to Circulation Research are eventually published.

Our current guidelines allow authors 90 days to complete the revision. If the manuscript is resubmitted within 90 days, one or more of the original reviewers will be re-consulted; the editors may also choose to obtain additional opinions from new reviewers. If you need more than 90 days to submit a revised paper, please notify the editorial office. In general, extensions over the revision time limit will not be granted except under special circumstances at the editors' discretion.

If you choose to revise, please include a detailed response to each of the referees' and editors' comments, providing each comment verbatim in bold followed by your response and giving the exact page number(s), paragraph(s), and line number(s) where each revision was made. If you make substantive changes to the manuscript, please provide a clear description of what you did and where. If you insert important sentences, paragraphs, or sections in response to the comments, please also include them in your response. Please indicate clearly any deletions. Additionally, a marked up version of the revision with the changes highlighted or tracked should be uploaded as a supplemental file. Number each page in the top right corner, using your manuscript number followed by /R1 to denote a first revision.

NEW: We are piloting an integration with SciScore (https://www.sciscore.com) to provide authors automatically generated reports during revision submission containing a reproducibility score and tables on rigor adherence and key resources such as antibodies, experimental models, recombinant DNA, and software. You are also welcome to start the revision submission process at any time to receive your report. We strongly encourage you to use the provided report while revising your manuscript to improve the study's reproducibility and reporting quality.

Please ascertain that your revised manuscript adheres to the Instructions to Authors as they appear online at https://www.ahajournals.org/res/author-instructions. Revisions that do not conform to the current limits on numbers of words (8000 total) may be returned to the authors for abbreviation. If you cannot reduce the overall word count, the editors may deem an extended print version appropriate; the authors should provide written assurance that they will cover the costs of the pages that are in excess of these limits. Note that paying for excess display items is not an option. Please refer to the Instructions to Authors for further details regarding our policy on page limits, articles with extended print versions, and related costs. No such limits apply to the online supplementary information, which can include supporting data and/or expanded text to offset the limits on the print version. Such online supplementary information can be cited in the print version as appropriate.

All corresponding authors of articles accepted to AHA Journals are required to link an ORCID iD to their profile in the AHA Journal submission system. To avoid potential processing delays in future, we recommend that you link an ORCID iD to your profile when you submit your revision. To register with ORCID or link your profile, please go to "Modify Profile/Password" on the submission site homepage, and click the link in the "ORCID" section.

We wish to thank you for having submitted this manuscript to Circulation Research.

Sincerely,

Jane E. Freedman, MD
Editor-in-Chief
Circulation Research
An American Heart Association Journal

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Reviewer comments to the Authors:

Reviewer #1:
In this de novo resubmission, Thomas et al studied the mechanistic link between CKD and cardiomyopathy, an important but underexplored area. The authors hypothesized that CKD leads to increased SIRPalpha expression causing impaired IGF-1 receptor signaling and subsequent pathologic cardiac remodeling. They used a CKD mouse model (partial nephrectomy) combined with SIRPalpha whole body and tissue specific knockouts. They found that SIRPalpha knockout preserved IGF-1 signaling and was protective against CKD-mediated cardiomyopathy. Moreover, these KO mice showed signs of physiologic hypertrophy. Administration of exogenous SIRPalpha to the KO mice restored the pathologic phenotype. This resubmission is significantly improved in a couple of respects: 1. increased sample sizes for the mouse and human experiments; 2. More detailed echo and histologic data demonstrating that SIRPalpha KO sham mice develop physiologic rather than pathologic hypertrophy. Overall, this study is well designed and provides novel findings for a mechanism for CKD-mediated cardiomyopathy.

Comments:
-Figure 2 C and G: why is the FS significantly worse in the WT CKD vs fl/fl CKD mice and the SIRPalpha CKD mice vs csSIRPalpha mice?
-Figure 3: Why did CKD lead to increase SBP in the WT but not the SIRPalpha MT mice?
-Discussion: consider commenting in more detail on the potential implications of your SIRPalpha/IGF-1 findings on understanding the pathogenesis of diabetic cardiomyopathy.

Reviewer #2:

I complement the authors on a nice piece of work, and I appreciate their thorough consideration of my previous comments. I do note that although N values are now clearly stated and are generally acceptable, there are still experiments with very low N values per group. I would defer to Circ Res statistical folks, but I would think a minimum N of 5 or 6 for all experimental measurements per group would be appropriate. This is not meant to minimize the tremendous amount of work that obviously went into this manuscript, but I am concerned that the small N in some experiments might detract from the validity of these exciting studies.

Reviewer #3:
No further comments.

Statistical Reviewer:

Please provide basic demographic information for healthy controls as well as CKD cases. Please also note race/ethnicity of participants.

Please provide precise p-values with two significant digits (rather than P<0.0x). Scientific notation is strongly encouraged. These can be provided with other additional statistical details (eg normalization procedures, tests establishing normality, sample sizes, named statistical tests, named post hoc correction, raw/corrected pvalues) in a supplemental table if that is more convenient.

Some tests (eg t-tests, ANOVA) used assume normality, however it is not clear how normality was established. **Note that common tests of normality are not powered to detect departures from normality when n is small (eg n<6) and in these cases normality should be support by external information (eg from larger samples sizes in the literature) or non-parametric tests should be used.**

The variance of the samples in fig 1B WTsham look odd (identical values for 4 samples, and two samples with ~equal distance above and below the rest). Please verify that the data here are correct.

How were representative images/figures chosen? Please note the approach used to select representative images in the main text.

Please give exact sample sizes (these are sometimes given as ranges, see fig 3).

Please show data points in the manuscript and supplement (eg see Fig 3, S Fig 2).

It's not always clear what statistical tests were used to derive presented p values (eg what tests were run in fig 3 e, f, g, or fig 4 b, c, fig 6, S fig 2, etc). Please give the details of the statistical testing in the figure legends, including whether across-group tests were run (as in fig 5a), or only within group.

Consider whether a repeated measures test should be applied in fig 7C,D? If the same specimen is being tested repeatedly over time, this may be an appropriate test.

Please add columns to tables s2, s3 to include statistical values.

Technical Reviewer:

Comments to Authors on Rigor Checklists:
The current study was carefully evaluated for inclusion of guideline items present in the Circulation Research checklists for rigor, transparency, and reproducibility. The reviewer has identified a number of items that were either omitted or not adequately addressed in the text. Please see below for details:

In vitro checklist items:
• Please provide uncropped western blots for review.
• Please provide a full description of antibodies with clones, can be included in the Major Resources table.

In vivo checklist items:
• Please provide some detail with regards to the subtotal nephrectomy method. This can be included in supplemental methods instead of main text if the authors prefer or there are space limitations.

Other:
• Per the journal's requirements, please complete and submit a "Major Resources Table". Please refer to the website for formatting instructions.

Baylor College of Medicine

DEPARTMENT OF MEDICINE
Section of Nephrology
One Baylor Plaza, BCM 395
Houston, Texas 77030

April 5, 2022

Re: CIRCRES/2021/320546DR1: SIRPα Mediates IGF1 Receptor Signaling in Cardiomyopathy-Induced by Chronic Kidney Disease

Dear Editorial Board,

We are re-submitting the enclosed manuscript entitled "SIRPα Mediates IGF1 Receptor Signaling in Cardiomyopathy-Induced by Chronic Kidney Disease" for your consideration in *Circulation Research*. We sincerely appreciate the thorough review of our manuscript by the Editorial Board and the Reviewers. We have comprehensively addressed the Editors' and Reviewers' comments as follows:

Editor's Comments:

As you will gather from the reviews, the referees identified a number of conceptual and methodological problems. The editors concur. Major issues include, the experimental numbers are still low in some experiments, which negatively impacts rigor. There are also several statistical and technical issues.

Answer: We sincerely thank the Editors for their comments and the opportunity to resubmit a revised manuscript. In short, we have increased the sample sizes for each evaluation to greater than 6 and if samples were less than 6 we have included our evaluations of non-parametric testing, as suggested by Reviewers. Additionally, we have included both parametric and nonparametric testing to each evaluation. We have provided all the information requested by the Reviewers, including those made by the Statistical and Technical Reviewers. I hope we have addressed all their concerns sufficiently.

Reviewer comments to the Authors:

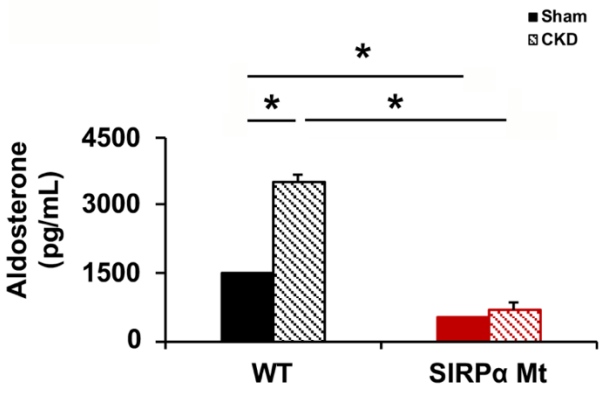
Reviewer #1:

In the original study, Thomas et al investigated the mechanism by which chronic kidney disease promotes cardiomyopathy. It has become increasingly evident that various etiologies of cardiomyopathy can have distinct mechanisms of pathogenesis. The relationship between CKD and cardiomyopathy is complex and remains unclear. The authors hypothesize that CKD-mediated activation of SIR alpha downregulates IGF1 receptor signaling, thereby promoting adverse cardiac remodeling. They utilized a partial nephrectomy mouse model of CKD as well as whole body- and tissue-specific SIRPalpha knockout mice to demonstrate that SIRPalpha knockdown blocks deleterious cardiac remodeling. The experiments are well designed and mostly support the authors conclusions. Given the paucity of data in this field, this study presents interesting and novel findings for CKD-mediated cardiomyopathy.

Figure 2 C and G: why is the FS significantly worse in the WT CKD vs fl/fl CKD mice and the SIRPalpha CKD mice vs csSIRPalpha mice?

Answer: We thank the Reviewer for the insightful comments. The flox vs. csSIRPα KO with CKD had lower FS secondary to the fact that these mice were fed a high protein diet while the WT vs. SIRPα Mt mice with CKD were on a normal chow diet. We have noted these differences in diet in the methods and in the figure legend (Pg. 19, line 6).

Figure 3: Why did CKD lead to increase SBP in the WT but not the SIRPalpha MT mice?
Answer: Aldosterone levels were lower in SIRPα Mt control mice when compared to WT control mice (n=3 mice/sham group, n=6 mice/CKD group), see data shown below. Future studies are required to determine SIRPα effects on RAAS activation. Since these mice were global KO we created csSIRPα KO mice which did not reveal these differences.



Discussion: consider commenting in more detail on the potential implications of your SIRPalpha/IGF-1 findings on understanding the pathogenesis of diabetic cardiomyopathy.

Answer: We have included a discussion of the implications of SIRPα/IGF1R interactions in the pathogenesis of diabetic cardiomyopathy (Pg. 16, line 4-6).

Reviewer #2:

I complement the authors on a nice piece of work, and I appreciate their thorough consideration of my previous comments. I do note that although N values are now clearly stated and are generally acceptable, there are still experiments with very low N values per group. I would defer to Circ Res statistical folks, but I would think a minimum N of 5 or 6 for all experimental measurements per group would be appropriate. This is not meant to minimize the tremendous amount of work that obviously went into this manuscript, but I am concerned that the small N in some experiments might detract from the validity of these exciting studies.

Answer: Thank you for the encouraging comments. As stated, we have increased the sample sizes for each evaluation to greater than 5-6. We have also provided non-parametric testing, as suggested by the Statistical Reviewer.

Reviewer #3:

No further comments.

Statistical Reviewer:

Please provide basic demographic information for healthy controls as well as CKD cases. Please also note race/ethnicity of participants.

Answer: We have added race/ethnicity of participants with CKD however healthy controls were not available as these were anonymous donors, see **Table S1**.

Please provide precise p-values with two significant digits (rather than P<0.0x). Scientific notation is strongly encouraged. These can be provided with other additional statistical details (eg normalization procedures, tests establishing normality, sample sizes, named statistical tests, named post hoc correction, raw/corrected pvalues) in a supplemental table if that is more convenient.

Answer: We have added precise p-values in the supplemental table labelled Statistical Analysis.

*Some tests (eg t-tests, ANOVA) used assume normality, however it is not clear how normality was established. **Note that common tests of normality are not powered to detect departures from normality when n is small (eg n<6) and in these cases normality should be support by external information (eg from larger samples sizes in the literature) or non-parametric tests should be used.***

Answer: We have increased the sample sizes for each evaluation to greater than 6. If samples were less than 6 we have included our evaluations of non-parametric testing, as suggested by the Statistical Reviewer.

The variance of the samples in fig 1B WT sham look odd (identical values for 4 samples, and two samples with ~equal distance above and below the rest). Please verify that the data here are correct.

Answer: We have added more samples to **Figure 1B** and reanalyzed the data to verify the results.

How were representative images/figures chosen? Please note the approach used to select representative images in the main text.

Answer: We have included a statement concerning representative images chosen in the figures to include, for example, "representative immunoblots of averaged data are shown."

Please give exact sample sizes (these are sometimes given as ranges, see fig 3).

Answer: We have provided exact sample sizes for each and all figures. Thank you.

Please show data points in the manuscript and supplement (eg see Fig 3, S Fig 2).

Answer: We have added all data points in each figure now. Thank you.

It's not always clear what statistical tests were used to derive presented p values (eg what tests were run in fig 3 e, f, g, or fig 4 b, c, fig 6, S fig 2, etc). Please give the details of the statistical testing in the figure legends, including whether across-group tests were run (as in fig 5a), or only within group.

Answer: We have provided each statistical evaluation in the figure legends and we have included across-group evaluations.

Consider whether a repeated measures test should be applied in fig 7C,D? If the same specimen is being tested

repeatedly over time, this may be an appropriate test.

Answer: In **Figures 7C, D** each time point was completed as individual cell experiments and compared to time zero. For example, in a six well plate of cells each time point has individual cells in each well which were treated as indicated for the time listed. We have added additional data, indicating different experiments to the figure and included the exact statistical evaluations in the figure legend. We hope we have addressed the Reviewers concerns.

Please add columns to tables s2, s3 to include statistical values.

Answer: We have added all exact statistical values for **Tables S2, S3**. Thank you.

Technical Reviewer:

In vitro checklist items:
• Please provide uncropped western blots for review.

Answer: We have provided uncropped Western blots for your review.

• Please provide a full description of antibodies with clones, can be included in the Major Resources table.

Answer: We have provided a full description of antibodies with their clones in the Major Resource Table.

In vivo checklist items:
• Please provide some detail with regards to the subtotal nephrectomy method. This can be included in supplemental methods instead of main text if the authors prefer or there are space limitations.

Answer: In our updated Methods section we have included a full description of the subtotal nephrectomy model (Pg. 6, line 25-27).

Other:
• Per the journal's requirements, please complete and submit a "Major Resources Table". Please refer to the website for formatting instructions.

Answer: We have added a "Major Resources Table". Thank you for your suggestion

Additionally, we have added new data in cardiomyocytes that suggest that exposure to uremic toxins stimulates SIRPα in cardiomyocytes and in cultured media (Figure S5F-G). Thank you for all your insightful suggestions. We hope we have comprehensively addressed all of the Editors' and Reviewers' concerns.

Sincerely,

Sandhya S. Thomas, MD, FASN
Assistant Professor of Medicine-Nephrology
One Baylor Plaza | BCM 395 | Houston, Texas 77030
Telephone: 713-798-2402 | FAX: 713-798-5010

April 27, 2022

Dr. Sandhya S. Thomas
Baylor College of Medicine/ Michael E. DeBakey Veterans Affairs Medical Center
Medicine
One Baylor Plaza
Houston, Texas 77030

RE: CIRCRES/2021/320546DR1: SIRPα Mediates IGF1 Receptor Signaling in Cardiomyopathy-Induced by Chronic Kidney Disease

Dear Dr. Thomas:

Your manuscript has been carefully evaluated by 4 external reviewers and the editors as a Regular Article. While we are interested in your paper, further minor revision is required before we can accept the manuscript for publication in Circulation Research.

Please read the entire content of this letter and carefully address all of the comments in the reviewers' critiques and all of the formatting concerns described below. Please note that the paper cannot be accepted until you have addressed both the reviewers' critiques and all of the formatting issues. Please submit your revision at your earliest convenience.

All corresponding authors of articles accepted to AHA Journals are required to link an ORCID iD to their profile in the AHA Journal submission system. To register with ORCID or link your profile to your ORCID iD, please go to "Modify Profile/Password" on the submission site homepage (insert journal homepage link), and click the link in the "ORCID" section. Please note that upon resubmission, the corresponding author will be required to have their ORCID iD linked to their profile; processing of the revision will be held until the ORCID link is complete.

The Editors strongly encourage you to adhere to the journal's Statistical Reporting Recommendations in your revision, which can be found here: https://www.ahajournals.org/statistical-recommendations.

1. Reviewers' Critiques:

To read the comments to authors from the reviewers, please see below.

If you wish to respond to these suggestions, please include a detailed response to each of the referees' and editors' comments, providing each comment verbatim in bold followed by your response and giving the exact page number(s), paragraph(s), and line number(s) where each revision was made. If you make substantive changes to the manuscript, please provide a clear description of what you did and where. Additionally, a marked-up version of the revision with the changes highlighted or tracked should be uploaded as a supplemental file.

2. Formatting Issues:

Please ascertain that your revised manuscript adheres to the Instructions to Authors as they appear online at https://www.ahajournals.org/res/author-instructions. Accepted manuscripts are published online ahead of print. Therefore, when submitting the final files of the manuscript and figures, please ensure you have made any essential changes or corrections to content, grammar, and formatting. Please also ensure that author information provided in the online submission system is correct, including author order, proper names, and institutions. Once published ahead of print, you will be unable to make any revisions to the manuscript until you receive your author proofs from

the publisher and any changes made to proofs will be reflected in the final print and online journal version of your article.

As your article may be published online upon acceptance, neither the Editorial Office nor the AHA will be responsible for any consequences with regard to intellectual property rights. To safeguard their intellectual property, authors should ensure that appropriate reports of invention and patent applications have been filed before the manuscript is accepted. If you should need to delay publication of your article for any reason, please let the Editorial Office know as soon as possible.

Please provide/address the following areas:

Manuscript Text:
- Please be sure to provide your revised manuscript text in an editable Word Doc file containing all sections of the manuscript, including tables and figure legends. Tables should be embedded within the body of the text as they are mentioned to ensure proper ordering of references.
-Please move the Novelty and Significance section to the very end of your Word file. Instructions for the Novelty and Significance section can be found at
https://www.ahajournals.org/res/revised-accepted-manuscripts.
- We request that all authors adhere to the 8,000 word limit. PLEASE NOTE: Word limit includes all sections of the manuscript (Title Page, Abstract, Text, Acknowledgment and COI Sections, References, Figure Legends, and Tables.) Online Supplements and the list of non-standard abbreviations and non-standard acronyms are excluded from the word limit.
Open Access Publication: We request that manuscripts published open access still adhere to the 8,000 word limit as much as possible. However, no excess charges will be rendered above the flat fee for open access publication.
Non Open Access Publication: Options for publishing a manuscript that is above 8,000 words may be found at:
https://www.ahajournals.org/res/revised-accepted-manuscripts under the 'Costs to Authors' subheading. You may wish to move supplemental material to an online supplement, which can include supporting data and/or expanded text to offset the limits on the print version. Such online supplementary information can be cited in the print version as appropriate.
- Please ensure that the title is no more than 80 characters in length, including spaces.
- NEW: Please organize the Abstract into four sections: Background, Methods, Results, and Conclusions.
- Authors are encouraged to provide a detailed, expanded Methods section as an online data supplement, especially if word limit constraints do not allow you to provide a detailed Methods section in the main manuscript. Methods sections should be detailed enough to enable readers to replicate the experiments without consulting previous articles.
- Please create a Sources of Funding section and cite the source of research support for the article.

Figures:
- Provide one full set of publication-quality figures as electronic files. Please ensure that electronic figure files are in tiff format and RGB color scale. Color and half-tone figures must have at least 600 dpi resolution; line drawings must have a 1200 dpi resolution or their original file format.
Online figures should be provided only in PDF format as part of the online supplement file.
- Color figure charges are a flat per page rate of $653 per color page. There are no color figure charges for open access publication beyond the flat fee.
- Please note that color figures cannot be changed to black and white after the manuscript is accepted. Please make any color changes to your figures during the final revision.

Online Supplement:
- Upload the online data supplement as one complete PDF labeled "Supplemental Material" at the top of the first page.
- Rename the supplemental figures and/or table using S before the figure # (i.e., Figure S1, Table S1, Figure S2, Table S2, etc.). Ensure that this change is made on the display item itself, in the legend, and throughout the text.
- In the manuscript text, following the Acknowledgments, Sources of Funding, & Disclosures section, please include a list of the supplemental materials with a callout to any references that are in the Supplemental Material only.
For example:
Supplemental Materials
Expanded Materials & Methods
Online Figures S1-S5
Online Video 1
Data Set
References 34-39

Other Items:
- All persons acknowledged by name in the manuscript must send an email to CircRes@circresearch.org citing their permission to be acknowledged.
- A Graphical Abstract (to be uploaded as a separate supplemental file): The intent of the graphical abstract is to provide readers with a succinct summary of the study in a form that facilitates its dissemination in presentations. It should emphasize the new findings in the paper. Do not include data items; all content should be graphical. The graphical abstract should conform to the following format: A single figure panel, no more than a 15 cm square (15 cm x 15 cm); Font: prefer a san serif font that is no less than 12 point. Please upload as a graphic abstract file in JPG file format.
- A supplement containing a short tweet that can be used to promote the article and a 1-2 line 'lay sentence' similar to those provided for NIH grants.
- Recent studies have shown that active engagement in social media is beneficial in advancing your science. Circulation Research encourages all authors to provide their twitter handles, if possible.

The Editors strongly encourage you to submit potential cover images. Appropriate figures should be both aesthetically beautiful and scientifically exciting. Potential cover images should be associated with the general topic of the paper, or may be altered/enhanced versions of an original figure within the manuscript. Potential cover figures should have a single panel, with no labels or text of any kind. The figure file should be supplied at exactly 8 1/8" width by 10 7/8" height. Please submit figure initially as a low-resolution PDF. Include a figure legend with the figure. If your figure is chosen, we will request a high-resolution version (minimum of 600 DPI, RGB color format, and TIF or EPS file format).

We look forward to receiving the final revised version of your manuscript as soon as possible. Thank you for contributing to Circulation Research.

Sincerely,

Francesco Violi, MD
Guest Editor
Circulation Research
An American Heart Association Journal

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REVIEWER COMMENTS FOR THE AUTHORS:

Reviewer #1:

In this resubmission, Thomas et al investigated the mechanisms of CKD-related cardiomyopathy. The authors hypothesized that CKD leads to increased SIRPalpha expression causing impaired IGF-1 receptor signaling and subsequent pathologic cardiac remodeling. They used a CKD mouse model (partial nephrectomy) combined with SIRPalpha whole body and tissue specific knockouts. They found that SIRPalpha knockout preserved IGF-1 signaling and was protective against CKD-mediated cardiomyopathy. Moreover, these KO mice showed signs of physiologic hypertrophy. Administration of exogenous SIRPalpha to the KO mice restored the pathologic phenotype. Moreover, the author demonstrated that exposure to hygerglycemia or uremic toxins led to increased SIRPalpha secretion from cardiomyocytes.

This resubmission is significantly improved for the following reasons: 1. Greater numbers for the groups in the animal experiments; 2. More mechanistic experiments (eg, hyperglycemia and uremic toxin in vitro studies).

Major Comments:
None

Minor Comment:
-Discussion: consider briefly discussing how SGLT2i and GLP1 agonists could potentially affect the CKD/SIRPalpha/IGF-1/cardiomyopathy axis.

Reviewer #2:

All of my comments on the previous submission were addressed in a thoughtful and complete manner. I have no additional criticisms or comments.

Statistical Reviewer:

Range p values are still being reported, eg P<0.0001. Precise p values can be obtained in GraphPad by going to the settings section of the preferences menu and changing the number of significant figures. Scientific notation is strongly encouraged. If you would prefer to not present exact p-values, you are welcome to provide effect sizes and confidence intervals, as an interested reader can then derive the p-value for themselves from this information. If you prefer to not report exact p-values or effect sizes, please provide the individual level raw data that was used in the calculation so that an interested reader can derive whatever test statistic is needed for reproducibility.

Technical Reviewer:

Comments to Authors on Rigor Checklists:
No further comments.

Baylor College of Medicine

DEPARTMENT OF MEDICINE
Section of Nephrology
One Baylor Plaza, BCM 395
Houston, Texas 77030

May 10, 2022

Re: CIRCRES/2021/320546DR1: SIRPα Mediates IGF1 Receptor in Cardiomyopathy-Induced by Chronic Kidney Disease

Dear Editorial Board,

We are re-submitting the enclosed manuscript entitled "SIRPα Mediates IGF1 Receptor in Cardiomyopathy-Induced by Chronic Kidney Disease" for your consideration in *Circulation Research*. We sincerely appreciate the thorough review of our manuscript by the Editorial Board and the Reviewers. We hope we have comprehensively addressed the Editors' and Reviewers' comments as follows:

Editor's Comments:

The Editors strongly encourage you to adhere to the journal's Statistical Reporting Recommendations in your revision, which can be found here: https://www.ahajournals.org/statistical-recommendations.

Answer: We sincerely thank the Editors for their comments and the opportunity to resubmit a revised manuscript. In short, we have adhered to the journal's requirements for statistical reporting and addressed all reviewers comments and concerns.

Reviewer comments to the Authors:

Reviewer #1:

In this resubmission, Thomas et al investigated the mechanisms of CKD-related cardiomyopathy. The authors hypothesized that CKD leads to increased SIRPalpha expression causing impaired IGF-1 receptor signaling and subsequent pathologic cardiac remodeling. They used a CKD mouse model (partial nephrectomy) combined with SIRPalpha whole body and tissue specific knockouts. They found that SIRPalpha knockout preserved IGF-1 signaling and was protective against CKD-mediated cardiomyopathy. Moreover, these KO mice showed signs of physiologic hypertrophy. Administration of exogenous SIRPalpha to the KO mice restored the pathologic phenotype. Moreover, the author demonstrated that exposure to hygerglycemia or uremic toxins led to increased SIRPalpha secretion from cardiomyocytes.

This resubmission is significantly improved for the following reasons: 1. Greater numbers for the groups in the animal experiments; 2. More mechanistic experiments (eg, hyperglycemia and uremic toxin in vitro studies).

Major Comments:
None

Minor Comment:

-Discussion: consider briefly discussing how SGLT2i and GLP1 agonists could potentially affect the CKD/SIRPalpha/IGF-1/cardiomyopathy axis.

Answer: We thank the Reviewer for their favorable and insightful comments. We have addressed the minor comment and added a discussion regarding how SGLT2i and GLP receptor agonist could impact the SIRPα/IGF1R axis in response to CKD. See **pg. 11, line 43-46**.

Reviewer #2:

All of my comments on the previous submission were addressed in a thoughtful and complete manner. I have no additional comments.

Answer: We thank the Reviewer.

Statistical Reviewer:

Range p values are still being reported, eg P<0.0001. Precise p values can be obtained in GraphPad by going to the settings section of the preferences menu and changing the number of significant figures. Scientific notation is strongly encouraged. If you would prefer to not present exact p-values, you are welcome to provide effect sizes and confidence intervals, as an interested reader can then derive the p-value for themselves from this information. If you prefer to not report exact p-values or effect sizes, please provide the individual level raw data that was used in the calculation so that an interested reader can derive whatever test statistic is needed for reproducibility.

Answer: We have included all exact p-values, please see complete table of all statistical information.

Technical Reviewer:

No further comments.

Thank you for all your insightful suggestions. We have formatted the manuscript to fit the requirements as listed. We hope we have comprehensively addressed all of the Editors' and Reviewers' concerns. We look forward to hearing from you.

Sincerely,

Sandhya S. Thomas, MD, FASN
Assistant Professor of Medicine-Nephrology
One Baylor Plaza | BCM 395 | Houston, Texas 77030
Telephone: 713-798-2402 | FAX: 713-798-5010