PEER REVIEW HISTORY

ARTICLE DETAILS

Title (Provisional)

Aortic stiffness after living kidney donation: A Systematic Review and Meta-analysis

Authors

Rodriguez, Rosendo A.; McNeill, Kylie; Agharazii, Mohsen; Bugeja, Ann; Clark, Edward G; Burns, Kevin D.

VERSION 1 - REVIEW

Reviewer	**1**
Name	**Jha, Pranaw Kumar**
Affiliation	**Medanta The Medicity, Nephrology**
Date	**31-Dec-2023**
COI	**None**

Safety of a kidney donors are paramount. This becomes important as they are undergoing a surgery which is not necessary for their treatment. Hence the donor risk should be minimum. It is important to study various dimensions of the safety parameters (both short and long term). Authors have looked into this important aspect of aortic stiffness after living kidney donation. I have following comments to make:

• What percentage of the donors were marginal donors?

• How significant difference was there in the baseline characters of living kidney donors and healthy controls? Were the factors with significant difference adjusted for when finding out the difference in aortic stiffness?

• The study has predominantly Caucasians population (to the tune of 90%). The applicability to other ethnicity cannot be commented upon. This should be mentioned in conclusions.

• How was hypertension defined?

• 17% of cardiovascular disease looks pretty high in healthy controls and donors. What kind of cardiovascular disease were there in controls and living kidney donors. Please elaborate.

• Whether proportion of donors requiring antihypertensive increased post-donations? If yes then give the figures at different time points

Reviewer	**2**
Name	**Singer, Juilan**
Affiliation	**The University of Sydney, Kidney Node Laboratory**
Date	**07-Jan-2024**
COI	**No competing interests**

This is a well-planned, conducted, and written systematic review and meta-analysis that evaluates the effect of living kidney donation on aortic stiffness as assessed by cf-PWV.

The effect of living kidney donation on future cardiovascular risk is an important concern to donors, their recipients, and healthcare providers. Adequately assessing risk remains a challenge, and objective assessment tools are required to adequately inform donors of potential risks.

This manuscript evaluates the limited evidence available clearly and succinctly, identifies an evidence gap, and highlights the need for well-designed prospective research in this area.

I do not have any recommendations for the authors or editor.

Reviewer	**3**
Name	**Bentall, Andrew**
Affiliation Hypertension	**Mayo Clinic Rochester, Division of Nephrology and**
Date	**24-Jan-2024**
COI	**No competing interests**

Study Summary:

The Authors of this study have conducted a systematic review and Meta-analysis to determine the effect of living kidney donation on the Aortic stiffness and its differences versus non-nephrectomized healthy individuals comparators. Additionally, the authors also evaluated the effects of living kidney donation on glomerular filtration rate (GFR), systolic (SBP), diastolic blood pressure (DBP).

In this study the authors included 9 interventional studies (652 donors, 602 control) and 7 reference studies (8,436 individuals). Aortic stiffness was measured using carotid-femoral pulse wave velocity (cf-PWV) technique. The authors reported a cf-PWV increase at 1 year post-donation (p=0.03) and was 0.4 m/s (95% CI: 0.07; 0.60) higher than in healthy controls (p=0.01). These differences were nonsignificant 5 years post-nephrectomy (p=0.54). GFR

decreased after nephrectomy (p<0.001) and remained reduced compared to healthy controls (p<0.001), but SBP and DBP were not significantly different (p≥0.14). Yearly changes in cf-PWV post-nephrectomy were similar to age-adjusted reference values in healthy normotensive individuals (p=0.76).

Review Summary:

1- Aortic stiffness is a measure of vascular aging, and it has been demonstrated to be an independent risk factor for cardiovascular disease including coronary heart disease, hypertension and strokes. Aortic stiffness has been shown in previous studies to increase post kidney donation, however these studies have been small in size, of short follow-up periods and produced different results. However, in this study the included studies have also been small, calculated average study sample size is 86 individuals. Although the authors have already mentioned the study sample size as a potential source of confounding and bias, the results have to be carefully interpreted in that regard.

2- The authors have reported the female gender majority (calculated at 63% total of 9 studies) and the potential differences in aortic stiffness compared to male gender. However, the aortic stiffness gender differences have been documented by previous large sample size studies. Additionally, when compared to the included healthy comparator studies, the results are significantly overpowered for female gender. I suggest gender-adjusting the aortic stiffness in the statistical analysis to overcome this problem.

3- The authors have declared beforehand that all for-comparison used aortic stiffness values have not been adjusted to blood pressure (BP) and heart rate (HR), only 2 out of the 9 total studies have been adjusted. Earlier studies have shown that BP and HR have significant impact on measuring aortic stiffness through cPWV, therefore, the produced results and the reported differences have to be interpreted very carefully.

4- Previous studies reported the role of BMI, history of diabetes mellitus, racial and geographical region in affecting aortic stiffness, however the included studies did not provide these characteristics regarding the study subjects before and after kidney donation, which can confound the observed increase in aortic stiffness and the comparability of the obtained results. I highly recommend adjusting the observed increase in aortic stiffness by BMI, diabetes, race and geographical regions.

5- There are several methods to measure the aortic stiffness, in the included studies the carotid-femoral pulse wave velocity (cf-PWV) technique has been used, which is regarded as the gold standard in measuring vascular stiffness, however, several measuring devices have been reported to have inherent differences due to the PWV calculation algorithms. The included interventional study groups unanimously used the SphygmoCor device, however, the healthy comparator groups used different devices in addition to SphygmoCor including Complior and Vicorder. A previous study has evaluated the significant differences in calculating cf-PWV between SphygmoCor and Complior, the study recommended not to use these two devices interchangeably and the generated error when resulting values compared

between the two devices. I believe this will lead to a significant difficulty during analyzing and interpreting the overall results, and I recommend immediate removal of the Complior using study from the rest of the healthy comparator groups.

6- The included healthy comparator study groups have been characterized as healthy on the basis of normotensive, no history of cancer, cardiovascular, neurologic, inflammatory, or kidney disease. However, Framingham Heart Study (FHS) has previously demonstrated the strong relationship between family history of increased aortic stiffness and individual's aortic stiffness patterns (reported as 40% of increased aortic stiffness subjects have a positive family history), suggesting a genetic association. In this study, neither the interventional nor the healthy comparator groups have information regarding the study subject's family history of abnormal or increased aortic stiffness. It will be important to screen the donor's for abnormal aortic stiffness to discern the preexisting genetic patterns in the general population and differentiate the effect of donation on both normal and abnormal individual's vascular aging.

7- The reported interventional LKD group baseline aortic stiffness values were all above age adjusted expected values (>6.5 m/s) which can potentially lead to a selection bias, and can further emphasize the importance of reporting the donor relationships to the recipients and the genetic clustering of CVD and CKD.

8- Since the aortic stiffness is mathematically affected by the aortic vascular geometrical shape,luminal diameter and its smaller arterial contributories, It will be important to examine the effect of different types of juxta-aortic vascular surgeries on aortic stiffness, this will evaluate effect of surgical vascular bed disturbances versus kidney donation on vascular aging.

Detailed Study Review by Sections:

Introduction:

The scope of this study fits the biomedical research advancement interest of the Biomedcentral (BMC) journal, since it discusses the possible effect of living kidney donation on vascular aging and the associated implementations that are related to screening and donation consent discussion. This study is an attempt to understand vascular remodeling response inlight of kidney donation.

The purpose of this study was stated as " Aortic stiffness after living kidney donation: A Systematic Review and Meta-analysis " examining the effect of living kidney donation on vascular aging through measurement of aortic stiffness with carotid-femoral pulse wave velocity (cf-PWV) by systemic review and meta-analysis of 9 total prospective interventional studies.

The question of the study is stemming from the gap in data regarding the physiological effect of living kidney donation on age-related vascular aging. I believe this is a very valid question in order to understand the physiological short and long term effects of living kidney donation

and donor outcomes. Additionally, this study can provide valuable information to facilitate planning and evaluating living kidney donors for medical screening, consenting and future medical follow-ups.

The authors mentioned, however, very briefly, the theoretical development of the study concept, previous studies and results. I suggest adding additional and more detailed background theoretical development sections, explaining the utility of CF-PWV in assessing the vascular stiffness as an independent risk factor for CVD, this will strengthen the study argument and improve the reader's understanding.

Methodology:

The study manuscript did include a dedicated and separate methodology section to describe the study design, data extraction, sampling process, the demographic characteristics of the study samples were limited to gender, history of HTN and smoking. The study subjects other important baseline characteristics that the authors did not report include, BMI, diabetes, family history of CVD. The statistical analysis section was sufficiently detailed and explained, however, I recommend a professional statistical review by a more meta-analysis experienced statistician.

Results:

The study manuscript results section explained in detail the different study groups characteristics in well organized and easy to read tables, with appropriate table titles and descriptions. This section also included the follow-up periods and associated cf-PWV, GFR, SBP and DBP differences.

Discussion:

The study manuscript discussion section suggested the possible role of vascular remodeling in explaining the observed increase in aortic stiffness in the 1st year and the minimal effect at 5 years post donation.

A strength point of this study is that the authors dedicated a section for self criticism and mentioned comparison of his study results compared to the previous established results showing opposite observations in animal and human model, with a suggestion regarding very small sample size and follow-up duration in previous studies.

The author also suggested that additional future analytical studies required to examine these observed results differences in living kidney donors and normotensive healthy individuals.

Limited sample size of the study can be acceptable for now since this study is regarded as proof-of-concept study design. Future studies can address sample size to improve validity and statistical significance.

Study Tables and Figures:

The provided study tables and figures are very clearly explained and detailed, which has enhanced the understandability of the study material and data. I highly recommend considering all the study figures and tables for publication if the study is accepted for publication.

References:

1. Millasseau SC, Stewart AD, Patel SJ, Redwood SR, Chowienczyk PJ. Evaluation of Carotid-Femoral Pulse Wave Velocity. Hypertension 2005;45(2):222–6.

2. Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: 'establishing normal and reference values.' European Heart Journal 2010;31(19):2338–50.

3. Mitchell GF. Aortic stiffness, pressure and flow pulsatility, and target organ damage. Journal of Applied Physiology 2018;125(6):1871–80.

4. Ogola BO, Zimmerman MA, Clark GL, et al. New insights into arterial stiffening: does sex matter? American Journal of Physiology-Heart and Circulatory Physiology 2018;315(5):H1073–87.

5. Gómez-Sánchez M, Patino-Alonso MC, Gómez-Sánchez L, et al. Reference values of arterial stiffness parameters and their association with cardiovascular risk factors in the Spanish population. The EVA Study. Revista Española de Cardiología (English Edition) 2020;73(1):43–52.

6. Angoff R, Mosarla RC, Tsao CW. Aortic Stiffness: Epidemiology, Risk Factors, and Relevant Biomarkers. Frontiers in Cardiovascular Medicine 2021;8.

7. Lu Y, Kiechl SJ, Wang J, et al. Global distributions of age- and sex-related arterial stiffness: systematic review and meta-analysis of 167 studies with 509,743 participants. eBioMedicine 2023;92:104619.

Reviewer	**4**
Name	**Csontos, Judit**
Affiliation	**Cardiff University, School of Healthcare Sciences**
Date	**21-Feb-2024**

COI   **I have no competing interests. However, I would like to add that I am a systematic reviewer with a general knowledge with regards to meta-analysis and statistics. I am not a statistician, and comments provided are based on my systematic reviewing experience. This manuscript might also benefit from a review by a statistician or a data scientist.**

Title: Aortic stiffness after living kidney donation: A Systematic Review and Meta-analysis

Abstract:

- Data source: OVID is mentioned as an electronic database. It is a search platform that hosts databases, and not an electronic database in itself. Could the authors rectify this, please?
- Data extraction/synthesis: This is a minor issue, but GRADE is referred to as quality of evidence, while it should be referred to as the certainty in the evidence.

Methods:

- Page 5: OVID is mentioned as an electronic database. It is a search platform that hosts databases, and not an electronic database in itself. Based on the search strategy presented In Appendix 1, EMBASE, MEDLINE, EBM Reviews were searched on OVID. Moreover, Cochrane register was searched via EBM, so the authors need to make this clear in the Methods section and not mention EBM and Cochrane as separate resources.
- Page 5: Authors mention grey literature. What grey literature was searched? Organisational websites, Overton, ProQuest, theses on Ethos… etc.? This needs more explanation. I can see reference to Grey Matters Light, did the authors use this resource to search grey literature. The text regarding searches will need to be edited to make it clear what databases were searched on what platforms, and what resources were searched for grey literature.
- Page 8: Description of meta-analysis seems sound, although I have a few doubts and questions, which will need more elaboration from the authors.
  - It is mentioned that where median or distribution was provided, the authors estimated means and SD using Luo's method. Median is often reported instead of mean, as it is a better measure of central tendency when data is skewed. Hence, it might be used in outcomes where data was skewed. While I can see that Luo's method provides valid mean calculations from skewed data, the authors would need to consider this, and detail whether the outcome median or mean could have come from skewed data, and how they dealt with this. Particularly, as based on Table 1 the largest sample size was 168, while the smallest was 21. Skewness could be a particular issue when sample sizes are small, and Cochrane have provided guidance for meta-analysis when data may be skewed.
    https://training.cochrane.org/handbook/current/chapter-10#section-10-5-3
  - The authors mention the issue of "double-counting" which can be a significant problem in meta-analyses. However, I am unfamiliar with the method of reducing study participants by 50%. A recent publication has highlighted the issue of double counting for observational studies, but taking 50% of participants have not been suggested as a solution.
    https://bmcpublichealth.biomedcentral.com/articles/10.1186/s12889-022-14213-6
    Moreover, the authors have not provided any reference to this method. What also needs some more explanation is how would reducing participant numbers affect the outcome means. The authors seem to have halved the participant numbers for the meta-analysis, but kept the same mean reported in the original studies for the whole sample. This could lead to bias, as I am unsure that half of the participants would have the same mean as the full sample. Could the authors elaborate on this issue and method, and use references as appropriate, please? This could aid transparency of the data analysis.

o The authors mention searching for reference studies (where they only had data from healthy individuals), and conducting an independent t-test to look at between-group differences. I think this section would benefit from being separated from the inverse variance analysis, as I do not think this has been conducted as part of the meta-analysis. Additionally, I am unsure whether Revman can produce t-tests by comparing means of different studies (as I understand the healthy population studies were separate studies). Could the authors provide more information regarding how and in what application the independent t-test was performed, please?

Additional comments:

- The authors seem to have used an edited version of the PRISMA flowchart, which is fine. I can see a clear indication of reasons for exclusion. However, as suggested by the PRISMA checklist too, and as stated in contemporary systematic review guidance (Cochrane, JBI, etc.), a list of studies excluded on full-text screening should be provided with citations. However, the authors only provide the reasons on the PRISMA flowchart, but not the list of excluded studies. This information should be included in the Appendix.

Reviewer's final decision: This seems like an interesting review, which follows generic systematic review procedures. The systematic review processes seem appropriate, with search strategy provided and all processes conducted by two independent reviewers. Risk of bias and GRADE assessment has also been conducted. However, this manuscript will need more work, because there is a lack of clarity in the bibliographic databases searched, how grey literature was identified, and how meta-analysis was conducted. I wonder whether an individual participant data (IPD) meta-analysis would have been a more appropriate way to synthesize findings. https://methods.cochrane.org/ipdma/about-ipd-meta-analyses

I cannot comment on the Nephrology content of the paper, as I am not an expert in this field but a systematic reviewer.

Reviewer	5
Name	Nenna, Antonio
Affiliation	Universita Campus Bio-Medico di Roma, cardiovascular surgery
Date	26-Apr-2024
COI	None.

Authors should be commended for their work. Statistical analysis is acceptable. Meta-regression analysis should be included or limitations should be stated.

VERSION 1 - AUTHOR RESPONSE

Reviewer: 1

Dr. Pranaw Kumar Jha, Medanta The Medicity

Safety of a kidney donors are paramount. This becomes important as they are undergoing a surgery which is not necessary for their treatment. Hence the donor risk should be minimum. It is important to study various dimensions of the safety parameters (both short and long term). Authors have looked into this important aspect of aortic stiffness after living kidney donation. I have following comments to make:

- Thank you for this positive feedback and for your helpful suggestions as to how we might improve the manuscript.

Comments to the Author:

1) What percentage of the donors were marginal donors?

- Eligible studies did not identify the proportion of participants under the definition of marginal donors. However, the incidence and nature of co-morbidities in donors at the time of donation can be assumed from the reports provided by 7 of the 9 studies (results section), as follows: "An average of 12.5% of donors (range: 0% to 32%) were considered hypertensives at the time of donation, 32.9% were smokers (range: 28% to 44%), 19.6% (range: 4.9% to 34% in 3 studies) had a history of cardiovascular disease and 1.6% (range: 0% to 5.9% in 4 studies) were considered to have diabetes mellitus." This information is reported on pages 13 and 14.

2) How significant difference was there in the baseline characters of living kidney donors and healthy controls? Were the factors with significant difference adjusted for when finding out the difference in aortic stiffness?

- Due to the small study sample size, we believe that any attempt to establish the statistical significance of the differences in the baseline characteristics between the 2 groups would have led to a statistical error and misleading conclusions. So, we opted for the use of descriptive statistics to report the rate of these differences (pages 13 and 14). An individual participant data (IPD) systematic review and meta-analysis would have been a more appropriate way to synthesize our data and adjust aortic stiffness according to the participants' baseline characteristics.

3) The study has predominantly Caucasians population (to the tune of 90%). The applicability to other ethnicities cannot be commented upon. This should be mentioned in conclusions.

- We thank the reviewer for this suggestion and this limitation is now included in page 27, paragraph 2, as follows:

"In particular, over-representation of the Caucasian population in these studies, prevents the applicability of our conclusions to other ethnicity groups."

4) How was hypertension defined?

- We have revised the definition of hypertension and our findings are as follows:

"Only 2 studies [9,30] reported a detailed definition of hypertension characterized as SBP >140 mm Hg and/or DBP >90 mm Hg; or by the use of antihypertensive therapy due to previously diagnosed hypertension."
This is now included in the results section (page 13, paragraph 1).

5) 17% of cardiovascular disease looks pretty high in healthy controls and donors. What kind of cardiovascular disease were there in controls and living kidney donors. Please elaborate.

- Unfortunately, there was no report on cardiovascular risk assessment or type of cardiovascular disease (page 15, paragraph 1) in the eligible studies. So, we have added the following statement in page 15, paragraph 1):

"Additional baseline characteristics were either part of the exclusion criteria or were not sufficiently reported."

6) Whether proportion of donors requiring antihypertensive increased post-donations? If yes, then give the figures at different time points.

- We thank the reviewer for this suggestion and so, we have revised the rates of hypertension in kidney donors at different time points post-donation and our findings are now reported in the results section (page 13, paragraph 1) as follows:

"In 7 of the 9 studies, an average of 12.5% (range: 0% to 32%) of LKD were hypertensive at the time of donation and this rate increased to an average of 17.2 .% (range: 4% to 32%; 4 studies) and 12.8% (range: 5.4% to 18.8%; 4 studies) at 12 months and 5-to-9 years post-donation respectively."

Reviewer: 2
Dr. Juilan Singer, The University of Sydney, Royal Prince Alfred Hospital
Comments to the Author:
This is a well-planned, conducted, and written systematic review and meta-analysis that evaluates the effect of living kidney donation on aortic stiffness as assessed by cf-PWV.

The effect of living kidney donation on future cardiovascular risk is an important concern to donors, their recipients, and healthcare providers. Adequately assessing risk remains a challenge, and objective assessment tools are required to adequately inform donors of potential risks.

This manuscript evaluates the limited evidence available clearly and succinctly, identifies an evidence gap, and highlights the need for well-designed prospective research in this area.
- I do not have any recommendations for the authors or editor.

We thank the reviewer for his positive comments.

Reviewer: 3

Dr. Andrew Bentall, Mayo Clinic Rochester

Study Summary:

The Authors of this study have conducted a systematic review and Meta-analysis to determine the effect of living kidney donation on the Aortic stiffness and its differences versus non-nephrectomized healthy individuals comparators. Additionally, the authors also evaluated the effects of living kidney donation on glomerular filtration rate (GFR), systolic (SBP), diastolic blood pressure (DBP).

In this study the authors included 9 interventional studies (652 donors, 602 control) and 7 reference studies (8,436 individuals). Aortic stiffness was measured using carotid-femoral pulse wave velocity (cf-PWV) technique. The authors reported a cf-PWV increase at 1 year post-donation (p=0.03) and was 0.4 m/s (95% CI: 0.07; 0.60) higher than in healthy controls (p=0.01). These differences were nonsignificant 5 years post-nephrectomy (p=0.54). GFR decreased after nephrectomy (p<0.001) and remained reduced compared to healthy controls (p<0.001), but SBP and DBP were not significantly different (p≥0.14). Yearly changes in cf-PWV post-nephrectomy were similar to age-adjusted reference values in healthy normotensive individuals (p=0.76).

Comments to the Author:

Review Summary:

1- Aortic stiffness is a measure of vascular aging, and it has been demonstrated to be an independent risk factor for cardiovascular disease including coronary heart disease, hypertension and strokes. Aortic stiffness has been shown in previous studies to increase post kidney donation; however, these studies have been small in size, of short follow-up periods and produced different results. However, in this study the included studies have also been small, calculated average study sample size is 86 individuals. Although the authors have already mentioned the study sample size as a potential source of confounding and bias, the results have to be carefully interpreted in that regard.

- *We agree with the reviewer's comments and the weaknesses of our study including the study sample size are discussed as part of the limitations (page 25, paragraph 2).*

2- The authors have reported the female gender majority (calculated at 63% total of 9 studies) and the potential differences in aortic stiffness compared to male gender. However, the aortic stiffness gender differences have been documented by previous large sample size studies. Additionally, when compared to the included healthy comparator studies, the results are significantly overpowered for female gender. I suggest gender-adjusting the aortic stiffness in the statistical analysis to overcome this problem.

- As indicated by the reviewer, females tend to have lower arterial stiffness than age-matched males from puberty to menopause (Weng et al. 2013). In general, adults of

both sexes experience an increase in arterial stiffness with aging (AlGhatrif et al 2013), but the increase seems to be steeper in males than females. Although we agree that it would be desirable to adjust aortic stiffness by female gender, this may be problematic due to the small sample size of our study and the absence of individual participant values. We believe that an individual participant data meta-regression would have been a more appropriate way to synthesize our data and adjust aortic stiffness according to modifiable and non-modifiable risk factors.

The issue of gender differences has been clarified in the limitations section (page 28, paragraph 1) as follows:

"Moreover, the confounding effects of anti-hypertensive therapy on the control of BP after donation and the limitations for adjusting the effects of gender and age [72,73] in our analysis cannot be ignored. Age in particular, may have a differential effect on arterial stiffness for males and females.[72] Although both sexes experience an increase in arterial stiffness with aging, the increase seems to be steeper in males than females.[72,73]We believe that an individual participant data meta-analysis would have been a more appropriate way to synthesize our data and adjust aortic stiffness according to the different risk factors."

References:
73. Weng et al. Am J Med Sci 2013;346:289-294.
74. AlGhatrif M, Strait JB, Morrell CH, . Hypertension 2013;62:934-941

3- The authors have declared beforehand that all for-comparison used aortic stiffness values have not been adjusted to blood pressure (BP) and heart rate (HR), only 2 out of the 9 total studies have been adjusted. Earlier studies have shown that BP and HR have significant impact on measuring aortic stiffness through cfPWV, therefore, the produced results and the reported differences have to be interpreted very carefully.

- We agree with the reviewer and this has been reported accordingly in page 25 paragraph 2 as follows:

"Varying study designs, small sample sizes, short-term follow-ups and differences between BP-adjusted and non-adjusted cf-PWV values may have contributed to the heterogeneity in the results."

4- Previous studies reported the role of BMI, history of diabetes mellitus, racial and geographical region in affecting aortic stiffness, however the included studies did not provide these characteristics regarding the study subjects before and after kidney donation, which can confound the observed increase in aortic stiffness and the comparability of the obtained results. I highly recommend adjusting the observed increase in aortic stiffness by BMI, diabetes, race and geographical regions.

- We recognize that co-morbidities, ethnicity, geographical location, gender and other risk factors may have a confounding effect on aortic stiffness and adjusting the outcomes for all these factors would be desirable. However, our study did not have enough power to adjust for these confounding effects and in the absence of individual data this may have led to misleading conclusions. An individual participant data meta-analysis and meta-regression would have been a better model of analysis to adjust aortic stiffness according to the different risk factors.

5- There are several methods to measure the aortic stiffness, in the included studies the carotid-femoral pulse wave velocity (cf-PWV) technique has been used, which is regarded as the gold standard in measuring vascular stiffness, however, several measuring devices have been reported to have inherent differences due to the PWV calculation algorithms. The included interventional study groups unanimously used the SphygmoCor device, however, the healthy comparator groups used different devices in addition to SphygmoCor including Complior and Vicorder. A previous study has evaluated the significant differences in calculating cf-PWV between SphygmoCor and Complior, the study recommended not to use these two devices interchangeably and the generated error when resulting values compared between the two devices. I believe this will lead to significant difficulty during analyzing and interpreting the overall results, and I recommend immediate removal of the Complior using study from the rest of the healthy comparator groups.

- We thank the reviewer for his comments and agree that the algorithms for calculating arterial stiffness between SphygmoCor and Complior are different. Therefore, their level of agreement between these 2 devices is debatable. So, based on this recommendation, we have removed the study by Baldo et al. (2018) from Table S-1. In consequence, averages, standard deviations and statistical analyses for the comparisons have been re-calculated and revised. The conclusions for the between-groups comparisons did not change. Additionally, in our limitations section, we have recognized that the comparability between different medical devices and operator techniques may represent an issue in the interpretation of our results (page 27, paragraph 2). Our correction is as follows:

"Since cf-PWV is an operator-dependent technique,[67] important issues in the interpretation of these results are the comparability between medical devices,[68] the variation due to the different calculating algorithms[68,69] and the technical reproducibility of these measurements.[67]"

References:

Milan et al. Current assessment of pulse wave velocity: comprehensive review of validation studies. Journal of Hypertension 2019; 37(8):1547-1557.

Millasseau SC, Stewart AD, Patel SJ, Redwood SR, Chowienczyk PJ. Evaluation of Carotid-Femoral Pulse Wave Velocity. Hypertension 2005;45(2):222–6.

Davies et al. Pulse Wave velocity and non-invasive methods used to asses it: complior, sphymoCor, Arteriograph and Vicorder. Vascular 2012;20(6):342-349.

6- The included healthy comparator study groups have been characterized as healthy on the basis of normotensive, no history of cancer, cardiovascular, neurologic, inflammatory, or kidney disease. However, Framingham Heart Study (FHS) has previously demonstrated the strong relationship between family history of increased aortic stiffness and individual's aortic stiffness patterns (reported as 40% of increased aortic stiffness subjects have a positive family history), suggesting a genetic association. In this study, neither the interventional nor the healthy comparator groups have information regarding the study subject's family history of abnormal or increased aortic stiffness. It will be important to screen the donors for abnormal aortic stiffness to discern the preexisting genetic patterns in the general population and differentiate the effect of donation on both normal and abnormal individual's vascular aging.

- We thank the reviewer for his comments and agree on the importance of identifying the subject's family history as a potential risk factor for developing abnormal patterns of aortic stiffness. Regrettably, studies did not perform detailed cardiovascular risk assessment and did not establish a clear relationship between donors and recipients. The potential association between genetic patterns and aortic stiffness are discussed in the discussion section.

  7- The reported interventional LKD group baseline aortic stiffness values were all above age adjusted expected values (>6.5 m/s) which can potentially lead to a selection bias, and can further emphasize the importance of reporting the donor relationships to the recipients and the genetic clustering of CVD and CKD.

- We thank the reviewer for suggesting the addition of selection bias as a source of heterogeneity. This has been included as part of our limitations (page 28, paragraph 1).
"Finally, the risk of publication and selection bias cannot be entirely ruled out. "

8- Since the aortic stiffness is mathematically affected by the aortic vascular geometrical shape, luminal diameter and its smaller arterial contributories, It will be important to examine the effect of different types of juxta-aortic vascular surgeries on aortic stiffness, this will evaluate effect of surgical vascular bed disturbances versus kidney donation on vascular aging.

- We agree with the reviewer in the importance of the vascular network on aortic stiffnes and the potential effect of the different types of juxta-aortic vascular surgeries. None of the existing studies reported specific details on the surgical procedures. We have added this comment in our discussion section (page 26, paragraph 3, page 27 paragraph 1) as follows:
"Beyond biological effects of reduced kidney function, nephrectomy may also result in alterations of the arterial network that are associated with changes in hemodynamics and functional stiffness of the arterial tree including those associated with the effect of the different types of yuxta-aortic vascular surgeries.[61]"

Detailed Study Review by Sections:

A. Introduction:

The scope of this study fits the biomedical research advancement interest of the Biomedcentral (BMC) journal, since it discusses the possible effect of living kidney donation on vascular aging and the associated implementations that are related to screening and donation consent discussion. This study is an attempt to understand vascular remodeling response in light of kidney donation.

The question of the study is stemming from the gap in data regarding the physiological effect of living kidney donation on age-related vascular aging. I believe this is a very valid question in order to understand the physiological short- and long-term effects of living kidney donation and donor outcomes. Additionally, this study can provide valuable information to facilitate planning and evaluating living kidney donors for medical screening, consenting and future medical follow-ups.

The authors mentioned, however, very briefly, the theoretical development of the study concept, previous studies and results. I suggest adding additional and more detailed background theoretical development sections, explaining the utility of CF-PWV in assessing the vascular stiffness as an independent risk factor for CVD, this will strengthen the study argument and improve the reader's understanding.

- We thank the reviewer for his comments and so, we have amended the introduction section (page 4, paragraph 2) as follows:

"Carotid-femoral Pulse wave velocity (cf-PWV) is a surrogate of the intrinsic stiffness of the arterial wall and has been reported highly predictive of cardiovascular events in high risk populations.[6,7] The prognostic value of cf-PWV has been associated to the integrated measure of the impact of cardiovascular risk factors on the arterial wall and to the adverse hemodynamic effect of aortic stiffness.[6-8]"

References:

- Laurent S, Katsahian S, Fassot C, Tropeano AI, Laloux B, Boutouyrie P. Aortic stiffness is an independent predictor of fatal stroke in essential hypertension. *Stroke.* 2003; *34*: 1203–1206. Crossref. PubMed.
- Boutouyrie P, Tropeano AI, Asmar R, Gautier I, Benetos A, Lacolley P, Laurent S. Aortic stiffness is an independent predictor of primary coronary events in hypertensive patients: a longitudinal study. *Hypertension.* 2002; *39*: 10–15. Crossref. PubMed.
- Cruickshank K, Riste L, Anderson SG, Wright JS, Dunn G, Gosling RG. Aortic pulse-wave velocity and its relationship to mortality in diabetes and glucose intolerance: an integrated index of vascular function? *Circulation.* 2002; *106*: 2085–2090. Crossref. PubMed.

B. Methodology:
The study manuscript did include a dedicated and separate methodology section to describe the study design, data extraction, sampling process, the demographic characteristics of the study samples were limited to gender, history of HTN and smoking. The study subjects other important baseline characteristics that the authors did not report include, BMI, diabetes, family history of CVD. The statistical analysis section was sufficiently detailed and explained, however, I recommend a professional statistical review by a more meta-analysis experienced statistician.

- Although our original case report form included more than 15 baseline characteristics, only those that were consistently reported such as hypertension, medications, history of cardiovascular disease and diabetes mellitus were included in our report. Other baseline characteristics such as BMI, obesity, cancer, history of renal disease, alcohol abuse was either part of the exclusion criteria or were not reported. We have added in our results section (page 15, paragraph 1) the following:

"Additional baseline characteristics were either part of the exclusion criteria or were not sufficiently reported."

C. Results:

The study manuscript results section explained in detail the different study groups characteristics in well organized and easy to read tables, with appropriate table titles and descriptions. This section also included the follow-up periods and associated cf-PWV, GFR, SBP and DBP differences.

- We thank the reviewer for his comments.

D. Discussion:

The study manuscript discussion section suggested the possible role of vascular remodeling in explaining the observed increase in aortic stiffness in the 1st year and the minimal effect at 5 years post donation.

A strength point of this study is that the authors dedicated a section for self-criticism and mentioned comparison of his study results compared to the previous established results showing opposite observations in animal and human model, with a suggestion regarding very small sample size and follow-up duration in previous studies.

The author also suggested that additional future analytical studies required to examine these observed results differences in living kidney donors and normotensive healthy individuals.

Limited sample size of the study can be acceptable for now since this study is regarded as proof-of-concept study design. Future studies can address sample size to improve validity and statistical significance.

- We thank the reviewer for his comments.

E. Study Tables and Figures:

The provided study tables and figures are very clearly explained and detailed, which has enhanced the understandability of the study material and data. I highly recommend considering all the study figures and tables for publication if the study is accepted for publication.

- We thank the reviewer for his comments; however, we have limited the number of figures in the text to comply with the Journal requirements.

F. References:

1. Millasseau SC, Stewart AD, Patel SJ, Redwood SR, Chowienczyk PJ. Evaluation of Carotid-Femoral Pulse Wave Velocity. Hypertension 2005;45(2):222–6.
2. Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: 'establishing normal and reference values.' European Heart Journal 2010;31(19):2338–50.
3. Mitchell GF. Aortic stiffness, pressure and flow pulsatility, and target organ damage. Journal of Applied Physiology 2018;125(6):1871–80.
4. Ogola BO, Zimmerman MA, Clark GL, et al. New insights into arterial stiffening: does sex matter? American Journal of Physiology-Heart and Circulatory Physiology 2018;315(5):H1073–87.

5. Gómez-Sánchez M, Patino-Alonso MC, Gómez-Sánchez L, et al. Reference values of arterial stiffness parameters and their association with cardiovascular risk factors in the Spanish population. The EVA Study. Revista Española de Cardiología (English Edition) 2020;73(1):43–52.
6. Angoff R, Mosarla RC, Tsao CW. Aortic Stiffness: Epidemiology, Risk Factors, and Relevant Biomarkers. Frontiers in Cardiovascular Medicine 2021;8.
7. Lu Y, Kiechl SJ, Wang J, et al. Global distributions of age- and sex-related arterial stiffness: systematic review and meta-analysis of 167 studies with 509,743 participants. eBioMedicine 2023;92:104619.

- We thank the reviewer for providing us with additional references.

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Reviewer: 4

Miss Judit Csontos, Cardiff University
Please also see attached document.

Comments to the Author:

A. Abstract:

⇒ Data source: OVID is mentioned as an electronic database. It is a search platform that hosts databases, and not an electronic database in itself. Could the authors rectify this, please?

Response: This has been amended in page 1, paragraph 3, as follows:

"Electronic databases (MEDLINE, EMBASE, Cochrane Central databases, Cochrane register of controlled trials, Cochrane Methodology Register, Health Technology Database, Technologies in Health, EBM Reviews and "Grey Matters Light")."

⇒ Data extraction/synthesis: This is a minor issue, but GRADE is referred to as quality of evidence, while it should be referred to as the certainty in the evidence.

Response: This has been corrected.

B. Methods:

⇒ Page 5: OVID is mentioned as an electronic database. It is a search platform that hosts databases, and not an electronic database in itself. Based on the search strategy presented In Appendix 1, EMBASE, MEDLINE, EBM Reviews were searched on OVID. Moreover, Cochrane register was searched via EBM, so the authors need to make this clear in the Methods section and not mention EBM and Cochrane as separate resources.

Response: This has been amended in page 6, paragraph 1, as follows:

"The search was applied to several electronic databases including MEDLINE, EMBASE, Cochrane Central databases, Cochrane Register of controlled trials, Cochrane Methodology Register, Health Technology Database, Technologies in Health, and EBM Reviews. EMBASE, MEDLINE, EBM reviews were searched through the OVID platform and Cochrane register searched via EBM."

⇒ Page 5: Authors mention grey literature. What grey literature was searched? Organisational websites, Overton, ProQuest, theses on Ethos… etc.? This needs more explanation. I can see reference to Grey Matters Light, did the authors use this resource to search grey literature. The text regarding searches will need to be edited to make it clear what databases were searched on what platforms, and what resources were searched for grey literature.

Response: This has been clarified in the methods section (page 6, paragraph 1) as follows:

"We searched for grey literature through the "Grey Matters Light" platform from the Canadian Agency for Drugs and Technology in Health (CADTH) and the ProQuest website for dissertations and theses."

⇒ Page 8: Description of meta-analysis seems sound, although I have a few doubts and questions, which will need more elaboration from the authors.
  o It is mentioned that where median or distribution was provided, the authors estimated means and SD using Luo's method. Median is often reported instead of mean, as it is a better measure of central tendency when data is skewed. Hence, it might be used in outcomes where data was skewed. While I can see that Luo's method provides valid mean calculations from skewed data, the authors would need to consider this, and detail whether the outcome median or mean could have come from skewed data, and how they dealt with this. Particularly, as based on Table 1 the largest sample size was 168, while the smallest was 21. Skewness could be a particular issue when sample sizes are small, and Cochrane has provided guidance for meta-analysis when data may be skewed. https://training.cochrane.org/handbook/current/chapter-10#section-10-5-3

Response: We thank the reviewer for her comments. Deviations from the normal distribution were handled according to what is recommended by the Cochrane handbook and this has been included in the methods section (page 9, paragraph 1) as follows:

"To determine the level of skewness in small sample size studies (n <35), we subtracted the extreme value of the reported range or quartile distribution from the estimated means calculated by the Luo et al's method[26] and divided by the estimated standard deviation according to Altman and Bland.[27] Only cases with a ratio less than 1 (suggesting severe skewness) were log transformed."

⇒ The authors mention the issue of "double counting" which can be a significant problem in meta-analyses. However, I am unfamiliar with the method of reducing study participants by 50%. A recent publication has highlighted the issue of double counting for observational studies, but taking 50% of participants has not been suggested as a solution. https://bmcpublichealth.biomedcentral.com/articles/10.1186/s12889-022-14213-6

⇒ Moreover, the authors have not provided any reference to this method. What also needs some more explanation is how would reducing participant numbers affect the outcome means. The authors seem to have halved the participant numbers for the meta-analysis, but kept the same mean reported in the original studies for the whole sample. This could lead to bias, as I am unsure that half of the participants would have the same mean as

the full sample. Could the authors elaborate on this issue and method, and use references as appropriate, please? This could aid transparency of the data analysis.

Response: Double counting in meta-analysis is a significant problem, particularly when double counting occurs in overlapping situations like in the case of before-and-after studies (Cuijpers et al. 2017). To improve transparency, we have provided the rationale for making this adjustment and one reference. Also, we have presented supplementary analyses to determine the strength of this approach including a new Table S-6 and new Figures S-5, S-6, S-7, S-8. A description of this analysis is described in the "Methods" and "Sensitivity analysis" sections as follows:

- Cuijpers P, Witz E, Cristea LA, Twisk. Pre-post effect sizes should be avoided in meta-analysis. Epidemiology and Psychiatric Sciences 2017;26:364-368.

Amendments:
In the methods section (Page 9, paragraph 1):

"To minimize the risk of artificially increasing the precision on the effect estimates due to counting the same patient twice in before-and-after studies ("double counting" error),[28] we reduced by 50% the number of study participants for each measurement.[29] To determine the strength of this approach, a sensitivity analysis between the models with and without adjustment was performed."

28. Senn SJ. Overstating the evidence – double counting in meta-analysis and related problems. BMC Medical Research Methodology 2009; 9:10.

29. Cheung MWL. A guide to conducting a meta-analysis with non-independent effect sizes. Neuropsychology Review 2019; 29:387-396.

In the sensitivity analysis section (page 22, paragraph 3), we have added the following statement:

"We evaluated the impact of adjusting our model for "double counting" errors on the effect estimates in studies with before-and-after design [11,12,14,15,30-33] by investigating the differences in the model with and without adjustment. The Forest plots for the non-adjusted analyses (primary and secondary outcomes) are illustrated in Figure S-5 (panels A, B, C, D). The mean differences and statistical heterogeneity for the model with and without adjustment are summarized in Table S-5. The pooled mean differences and their precision were not significantly different between the two quantitative models. Although the standard error in the non-adjusted model increased only by 3% (quartiles: -5.1% to 7.4%), its statistical heterogeneity (I^2 value) notably increased by 35% (range: 22% to 47%) compared with the adjusted model."

⟹ The authors mention searching for reference studies (where they only had data from healthy individuals) and conducting an independent t-test to look at between-group differences. I think this section would benefit from being separated from the inverse variance analysis, as I do not think this has been conducted as part of the meta-analysis. Additionally, I am unsure whether Revman can produce t-tests by comparing means of different studies as I understand the healthy population studies were separate studies).

Could the authors provide more information regarding how and in what application the independent t-test was performed, please?

Response: In response to the reviewer's suggestion, we have created a new section titled: "Reference studies" (page 10, paragraph 2). This section has been separated from the "Meta-analysis" section and amended as follows:

"The significance of between-group comparisons was assessed by independent t-tests (2-tailed) ($p<0.05$). The differences in cf-PWV are reported as the means and their 95% CI (or their SD, if noted), while for absolute cf-PWV values, medians and quartiles are described. Quantitative analyses utilized IBM SPSS statistics, version 29 (Armonk, NY, USA)."

$\Rightarrow$ Additional comments:
• The authors seem to have used an edited version of the PRISMA flowchart, which is fine. I can see a clear indication of reasons for exclusion. However, as suggested by the PRISMA checklist too, and as stated in contemporary systematic review guidance (Cochrane, JBI, etc.), a list of studies excluded on full-text screening should be provided with citations. However, the authors only provide the reasons on the PRISMA flowchart, but not the list of excluded studies. This information should be included in the Appendix.

Response: We have included the list of the excluded studies as suggested at the end of the appendix section (appendix 5).

$\Rightarrow$

Reviewer's final decision: This seems like an interesting review, which follows generic systematic review procedures. The systematic review processes seem appropriate, with search strategy provided and all processes conducted by two independent reviewers. Risk of bias and GRADE assessment has also been conducted. However, this manuscript will need more work, because there is a lack of clarity in the bibliographic databases searched, how grey literature was identified, and how meta-analysis was conducted. I also wonder whether an individual participant data (IPD) meta-analysis would have been a more appropriate way to synthesize findings. https://methods.cochrane.org/ipdma/about-ipd-meta-analyses
I cannot comment on the Nephrology content of the paper, as I am not an expert in this field but a systematic reviewer.

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Reviewer: 5
Dr. Antonio Nenna, Universita Campus Bio-Medico di Roma
Comments to the Author:
Authors should be commended for their work. Statistical analysis is acceptable. Meta-regression analysis should be included, or limitations should be stated.

Response: Limitations on the meta-regression were discussed in previous paragraphs of this letter and included in our limitations section.
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Reviewer: 1
Competing interests of Reviewer: None

Reviewer: 2
Competing interests of Reviewer: No competing interests

Reviewer: 3
Competing interests of Reviewer: No competing interests
===

Reviewer: 4

Competing interests of Reviewer: I have no competing interests. However, I would like to add that I am a systematic reviewer with a general knowledge with regards to meta-analysis and statistics. I am not a statistician, and comments provided are based on my systematic reviewing experience. This manuscript might also benefit from a review by a statistician or a data scientist.

Response: We recognize the reviewer's concerns, but we have provided the best possible analyses according to the nature and quality of data.
===
Reviewer: 5
Competing interests of Reviewer: None.

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Additional corrections undertaken by the authors:

- References were revised, amended and new citations added.
- New Figures S-5, S-6, S-7 and S-8 have been included in the Supplemental File, and a new Table S-6 has been added.

Suggestions from Editorial Office:

=== 1) Please ensure that author affiliations (including departments) are identical in both the Title Page and submission system. Any secondary affiliations must also be listed in both.

- We have ensured that the submission system and the manuscript match the authors' affiliations.

=== 2) Please combine Figures with sub-parts into one to have a single file figure and make sure that they have a resolution of at least 300 dpi and at least 90mm x 90mm of width. Figures in document, excel and powerpoint format are not acceptable.
Note: If you cannot convert your figure into one, kindly renumber the figure legends into Figure 1, Figure 2, etc.

- To improve Figure resolution, we have created individual figures instead of Figures with sub-parts as suggested by the Editorial Office. In the revision, the new main document includes 5 Figures and those remaining have been allocated to the Supplementary File. In consequence, to comply with the Journal requirements (max 5 Fig/Tables), all Tables have been included in the Supplementary File.

=== 3) Your tables are too large to be included in the manuscript, specifically tables 1, 2, and 3. Please either reduce their size (maximum 2 pages / 8 columns) or upload them as supplemental material.

- All Tables have been uploaded as supplemental material. No Tables are included in the main document.

==== 4) Please ensure that all authors are included in your author contributor statement and that it is identical in the manuscript and submission system. For further information please see https://authors.bmj.com/policies/bmj-policy-on-authorship/

- We have copied the "author contributor statement" from the manuscript to the submission system including the name of the guarantor of this work. So, the 2 documents look identical.

==

VERSION 2 - REVIEW

Reviewer	**4**
Name	**Csontos, Judit**
Affiliation	**Cardiff University, School of Healthcare Sciences**
Date	**09-Oct-2024**
COI	

The authors have responded to all my comments and made sufficient edits to increase clarity in the reporting of the methods.

I only have two minor comments, but these could be fixed during copyediting.

1. In the Abstract under data sources ProQuest is not mentioned. However, it is mentioned in the Methods section. Could ProQuest be added to the abstract too, please?

2. In the Methods section under the Certainty of Evidence heading (Page 8 in the clean version) the sentence starts as: "Quality of the certainty of the evidence was evaluated...etc." Could "quality" be removed from the sentence. I think the sentence should be: "The certainty of the evidence was evaluated...etc.

Reviewer	**5**
Name	**Nenna, Antonio**
Affiliation surgery	**Universita Campus Bio-Medico di Roma, cardiovascular**
Date	**01-Oct-2024**
COI	

thank you for the revised version of the manuscript.