PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Retinal microvasculature: Population epidemiology, concordance and reliability in 11-12 year old Australians and their parents
AUTHORS	Dascalu, Julian; Liu, Mengjiao; Lycett, kate; Grobler, Anneke; He, Mingguang; Burgner, David; Wong, Tien Yin; Wake, Melissa

VERSION 1 - REVIEW

REVIEWER	Pedro Romero-Aroca Hospital Universitario Sant Joan de Reus Institut d'Investigacions Sanitaries Pere Virgili (IISPV) Universitat Pere Virgili
REVIEW RETURNED	27-Feb-2018

GENERAL COMMENTS	Revision of the manuscript entitled: “Retinal microvasculature: Population epidemiology, concordance and reliability in 11-12-year-old Australians and their parents.” ID bmjopen-2018-022399 Declaration of interest: I am no conflicts of interest in the review of the present manuscript A. Summary In this manuscript authors studied the relationship of vascular level arteriolar and venular retina vessels diameters, among children aged between 11 and 12 years old and their parents. B. Strengths: In the literature there are many studies on the relationship between vascular diameters of retinal vascular diseases and various vessels, especially cardiovascular and cerebral vascular (particularly stroke), but as the authors say, there are few who compare the normal values of the retinal vessels especially between different ages. The present study is interesting since it compares the vascularization between parents and children, which allows to eliminate certain methodological defects of other studies, such as the problems derived from genetic particularities, which in this study can be partially eliminated. C. Commentaries. Weaknesses of this study are:

	1. Introduction is too long, the four paragraph that explain results of other studies can be displaced to discussion, that is too short, and few studies are described in which the present study is compared. 2. In methods the paragraph “Other sample characteristics” , it could be shortened by eliminating the description of how blood pressure is taken or the BMI is measured, since at the beginning of the paragraph reference is made to reference 24, where these methods are described 3. It is interesting that in the results the differences in the values of the standard deviation is less than the difference in absolute values, it would be interesting that the authors were extended in the discussion of these data. 4. In table 1 the mean value of systolic blood pressure in male is 238.4, I think this is a mistake, Can the value be 138.4 or similar? Resume. As authors said: “a need for reliable age-specific normative reference values across the lifecourse” for vessels diameters values should be necessary, present study is interesting, but a much longer cohort with all ages should be made in future to determine normal values in a population.

REVIEWER	Brian Stagg University of Michigan
REVIEW RETURNED	09-Mar-2018

GENERAL COMMENTS	This interesting article presents data regarding retinal microvasculature measurements for participants in the Child Health CheckPoint study in the Longitudinal Study of Australian Children. The authors looked at the mean values for children, the mean values for their parents, Pearson correlation for children with parents, and adjusted linear regression for correlation of children with parents. The article seems very suitable for publication, but I did identify a few issues: 1. Abstract, Participants: Please clearly state the inclusion and exclusion criteria for participants. 2. Abstract, Results: The authors state that the CRAE and CRVE were slightly larger in children than parents. A statistical test for this comparison would be useful for the reader to understand the difference. While the means were smaller, the difference seems small relative to the standard deviation and seems that it could have happened by chance alone. 3. Introduction, line 20: It would be helpful to describe the association of retinal vessel caliber with cardiovascular disease/risk factors for readers who are less familiar with this literature. Is larger or smaller vessel caliber associated with cardiovascular disease? How strong is the association? In line 27 the authors talk about retinal vasculature being a “robust biomarker” of disease, a concise explanation of this would be helpful. 4. Methods, Study Design and Participants: In this section please provide more clear description of inclusion and exclusion criteria for your study. Please make sure that each of these are clearly listed and are easy for readers to correlate with Figure 1. For example, I didn’t notice explanation in the methods regarding excluding non-biologic adult-child pairs and diabetic patients (though I may have just missed it).

	5. Methods, Measures: At the bottom of this section the numbers of images graded are listed. It seems strange to list this in the section of the manuscript describing the “Measures”. Perhaps this could go in the early results section? 6. Methods, Statistical Analyses: Why was the decision made to report the unweighted results rather than the weighted results? It seems that one of the strengths of this study is generalizability to the Australian population, but using the unweighted results seems like it would reduce this generalizability. 7. Methods, Statistical Analyses: At a few points in the paper the authors emphasize vessel calibers being smaller in the older (parent) population than the younger (child) population. And yet, I don’t see any explanation regarding how the vessel calibers would be statistically compared between the two groups. Is comparing the children to the adults one of the objectives of the paper? At the end of the introduction the authors state that their goals are to 1) assess the distribution and 2) look at parent-child correlation. They don’t mention comparing the means of the children to the means of the adults. If this is not one of their goals, then they don’t need to do this and explain it in their methods. However, if this isn’t one of their goals and they don’t evaluate the difference statistically, I think that they shouldn’t emphasize this difference throughout their manuscript. For example, the first conclusion in their Abstract is that vessel calibers were smaller in midlife than late childhood. I worry that this manuscript makes this assertion but doesn’t actually address this question. 8. Results, Sample characteristics: Again, please make sure that the inclusion/exclusion criteria are easy for the reader to correlate with the methods section and Figure 1. 9. Table 1: Systolic blood pressure for male adults is listed as 238.4. This seems really high. Can the authors please check this number? Also, please check other numbers in the table to make sure that they are accurate. 10. Table 1: The table mentions “Eye condition or glasses/contact lenses”. However, I didn’t notice mention of this variable in the methods section (though it may have been there and I just missed it). Can you please explain a bit more about this variable? Do we know what the eye conditions were? Do we know what their glasses prescription was? Why wasn’t this adjusted for in the model? It feels like these issues could affect the size measurements. 11. Results, Parent-child concordance: Reporting the confidence intervals for the correlations would be useful. 12. Results, Post-hoc analysis: Was the plan for this analysis discussed in the methods? Why is called a post-hoc analysis? It seems that it is a comparison with other published literature. It feels like this analysis is looking at a different question and seems out of place with the rest of the study. It seems that this is looking at the question, “how does vessel caliber vary with age?” though this issue wasn’t specifically addressed in the previous analyses (see comment #7 above). If the authors decide they want to include this analysis looking at other studies in the manuscript, it seems like the methods should be strengthened. A systematic method with defined inclusion/exclusion criteria for the papers should be used. It also seems that the statistical method used in the manuscript doesn’t account for the between-study variance and doesn’t account for study differences. 13. Conclusion, first paragraph: The first sentence talks about “demonstrating an age-related decrease in both mean CRAE and CRVE between mid-childhood and mid-life.” Again, this doesn’t

	seem like something that was evaluated in this study (see comment #7 above). 14. Conclusion, Limitations: In what would the correlations have changed for smoking status, sedentary lifestyle and diet? Why didn't you control for refractive errors? Why would you expect the effects from refractive error to be small? How about axial length? Does this influence your measurements?

VERSION 1 – AUTHOR RESPONSE

Editor/Reviewer Comments	Author's Response	Reference page & line numbers
Reviewer 1 : Pedro Romero-Aroca, Hospital Universitario Sant Joan de Reus, Institut d'Investigacions Sanitaries Pere Virgili (IISPV), Universitat Pere Virgili		
R.1.1 Introduction is too long, the four paragraph that explain results of other studies can be displaced to discussion, that is too short, and few studies are described in which the present study is compared.	We understand that opinions differ as to the purpose of an Introduction in peer-reviewed articles. Our view is that it establishes the context of a topic, the current state of knowledge regarding a very specific question, the gap to be addressed in the paper, and the justification and opportunity to do so. At just over 600 words we don't think this is a long Introduction. The Discussion (800 words) then examines how our data move the field forward, in light of but not repeating the Introduction. We hope this is satisfactory, particularly since it aligns with the other papers in this Special Issue.	N/A
R.1.2. In methods the paragraph "Other sample characteristics" , it could be shortened by eliminating the description of how blood pressure is taken or BMI is measured, with reference to reference 24, where these methods are described.	Thank you, we have substantially shortened this section, referring readers to the detailed protocol in the cited reference 24.	Page 8
R.1.3 It is interesting that in the results the differences in the values of the standard deviation is less than the difference in absolute values, it would be interesting that the authors were extended in the discussion of these data.	We are not quite sure what the reviewer is implying here. We showed a larger standard deviation in the parent than the child group, indicating a greater spread of variation in retinal calibre with age. This is in keeping with most of the other measures in this Special Issue and most likely reflects decades of response to differing exposures with physiologic dysregulation in some. We have added a comment to this effect in the	Page 16

	Discussion, but not gone deeper as this was not one of our Aims. We do hope these findings will stimulate further research, including from this dataset. We inserted this text in the Limitations section of the Discussion: “We showed a larger standard deviation in the parent than the child group, indicating a greater spread of variation in retinal calibre with age. This would be in keeping with greater physiologic dysregulation for some inidividuals with age in response to genetic and risk exposure (eg higher blood pressure, obesity etc) over multiple decades.”	
R.1.4. In table 1 the mean value of systolic blood pressure in male is 238.4, I think this is a mistake?	Thank you sincerely for noting this error! – we have corrected this to read 128.4 mmHg. We have reviewed all of the tables to ensure correct data is displayed. (Thanks also to Reviewer 2 for picking up this same point – see 2.9, below).	Page 11
Reviewer 2: Brian Stagg, University of Michigan		
R.2.1. Abstract, Participants: Please clearly state the inclusion and exclusion criteria for participants.	The abstract now states, within the 300 word limit: • "Design: Cross-sectional study based on the Child Health CheckPoint study, between Waves 6 and 7 of the national population-based Longitudinal Study of Australian Children (LSAC)." and • "Diabetic participants and non-biological pairs were excluded from concordance analyses." More detail as to both is provided in the main Methods, our updated Figure 1, and references to other papers and technical resources with fuller study descriptions.	Page 3
R.2.2. Abstract, Results: The authors state that the CRAE and CRVE were slightly larger in children than parents. A statistical test for this comparison would be useful for the reader to understand the difference.	The first Aim of the paper was to describe separately the distribution in children and parents. Since we did not state a hypothesis regarding differences in child and adult distributions, we have not reported a statistical test in the paper. However, we now note that the group differences are around 0.6 standard deviation for both the arteriolar and venular measures.	Page 3

	Two-tailed t-tests comparing parents with children on mean (SD) arteriolar and venular calibres both in fact show p-values <0.001. If you feel strongly that these p-values should be inserted we are willing to do so, but prefer not to for the reason above.	
R.2.3. Introduction, line 20: It would be helpful to describe the association of retinal vessel caliber with cardiovascular disease/risk factors for readers who are less familiar with this literature. Is larger or smaller vessel caliber associated with cardiovascular disease? How strong is the association? In line 27 the authors talk about retinal vasculature being a "robust biomarker" of disease, a concise explanation would be helpful.	We have amended the second paragraph of the introduction. It now reads: "Meta-analyses have demonstrated statistically significant correlations of smaller retinal arteriolar and wider retinal venular vessel calibre with subsequent cardiovascular disease, including stroke, obesity and coronary heart disease.[11-14] Cardiovascular risk factors (such as hypertension, diabetes mellitus and obesity) have been similarly associated with smaller retinal arterioles and larger retinal venules via both shared and unique underlying pathophysiology.[6, 15, 16]"	Page 5
R.2.4. Methods, Study Design and Participants: In this section please provide more clear description of inclusion and exclusion criteria for your study. Please make sure that each of these are clearly listed and are easy for readers to correlate with Figure 1. For example, I didn't notice explanation in the methods regarding excluding non-biologic adult-child pairs and diabetic patients (though I may have just missed it).	We have aimed for a balance such that each article in this Special Issue stands alone, while as little information as possible is repeated about study design in every paper; in general we have provided the greatest shared detail in the overarching Cohort Profile to which this paper refers). However, we have added some detail here to the Methods: "Study Design and Participants: ….using a 2-stage clustered design. First, 10% of Australian postcodes (stratified by state and urban/rural locations) were randomly selected, then in-age children (born between March 2003 and February 2004) within those enrolled in the Medicare Australia database (Australia's universal healthcare system, into which 98% of children are enrolled by their first birthday) were selected." There were no exclusion criteria for the descriptive Aim 1 analyses. We excluded non-biologic pairs from the concordance (Aim 2) analyses only, as we were assessing intergenerational concordance between maternal-child or	Page 6 Figure 1

	paternal-child pairs. Similarly, we excluded diabetic patients from these Aim 2 analyses because diabetes directly affects the retinal vasculature. We now note this more clearly in the Methods: “ Twenty nine diabetic participants, and 10 non-biological child-parent pairs were excluded from concordance analyses.” We have also updated Figure 1 and its footnotes to include mention of the diabetic participants excluded from the concordance analysis.	
R.2.5. Methods, Measures: At the bottom of this section the numbers of images graded are listed. It seems strange to list this in the section of the manuscript describing the “Measures”. Perhaps this could go in the early results section?	We felt it important to report both the number of participants: • for whom images were available and scored (1307 children and 1317 parents, reported in Methods) • included in the analyses (1288 children and 1264 parents, reported in Results) once exclusion criteria were applied and missing data removed. Figure 1 shows the reasons for excluding participants. We think that reporting both sets of numbers aids understanding of why data are missing. If preferred we are happy to remove this information from Methods, or put the two sets of information side-by-side in Results – however, this would differ from the other papers in this Special Issue. For clarity, we have slightly modified the Methods text to now read: "In total, 2624 images were graded, including from 1307 children and 1317 parents (87% and 92% from the right eye for children and parents, respectively). 19 child and 53 parent images did not meet the quality criteria for use in analyses." The Results text reads: "A total of 2552 participants (1288 children and 1264 adults) were included in the descriptive Aim 1 analyses (figure 1). This represents 95% of the 1356 pairs who attended CheckPoint assessment centres with retinal photography (where retinal photography was offered). "	Page 7 Page 10

R.2.6. Methods, Statistical Analyses: Why was the decision made to report the unweighted results rather than the weighted results? It seems that one of the strengths of this study is generalizability to the Australian population, but using the unweighted results seems like it would reduce this generalizability.	For Aim 1 (table 1, the epidemiology amongst all children and all parents) we used weighted results to estimate the likely distribution in the Australian populations of 11-12 year olds, and mid-life adult parents. For Aim 2, we present unweighted results because the value of applying weightings is less clear for associational analyses, and because the weighted and unweighted results were similar (so don't change the interpretations of our results). This approach is in keeping with all the papers in this Special Issue. However, we have amended the Analyses text to clarify this: Aim 1: Continuous descriptive variables were summarised using weighted means and standard deviations (SD); categorical variables were summarised by number and weighted percentage for children and adults separately and by gender Aim 2: … The Pearson's correlation and linear regression analyses were repeated using weighted multi-level survey analyses; as these yielded similar results, unweighted results are displayed. For the reviewer's information, Table a (not included in the paper) at the end of this document shows these weighted and unweighted results for Aim 2. We would be happy to add this to the Supplementary Tables, although we don't think it necessary.	Page 9
R.2.7 Methods, Statistical Analyses: At a few points in the paper the authors emphasize vessel calibers being smaller in the older (parent) population than the younger (child) population….. Is comparing the children to the adults one of the objectives of the paper? ….For example, the first conclusion in their Abstract is that vessel calibers were smaller in midlife than late childhood. I	See response to 2.2 above. The reviewer is correct in that the first Aim of our study was to describe the epidemiology of vessel caliber in children and parents separately. To meet this aim we need to state the values, but not to statistically compare the distributions, in the two age groups. We have reviewed the abstract and manuscript to be sure it doesn't imply that we formally compared the two age groups.	Pages 3, 15, 16

worry that this manuscript makes this assertion but doesn't actually address this question.		
R.2.8 Results, Sample characteristics: Again, please make sure that the inclusion/exclusion criteria are easy for the reader to correlate with the methods section and Figure 1.	Thank you, see 2.4 above. We have updated the Results text to give a complete description of all inclusion/exclusion criteria for each Aim's analysis, which we hope is now clear and consistent with the Methods section. The Results text now reads: "Sample characteristics: A total of 2552 participants (1288 children and 1264 adults) were included in the descriptive Aim 1 analyses (figure 1). This represents 95% of the 1356 pairs who attended CheckPoint assessment centres (where retinal photography was offered). Reasons for participants not having retinal photography images and data are attendence at smaller assessment centres without a retinal camera or home visit (n=518 participants), participant refusal (n=160 participants), and an image not able to be taken (eg the camera required repair) or the quality was too poor (n=5 participants, figure 1). A total of 1186 parent-child pairs were included in Aim 2 analyses; 10 non-biologic adult-child pairs and 29 diabetic participants were excluded from the concordance assessments (figure 1). " Figure 1 and its footnote now note the exclusion of diabetic participants from the parent-child correlations.	Page 10 and Figure 1
R.2.9 Table 1: Systolic blood pressure for male adults is listed as 238.4.	Thank you, amended, and values in this and all other tables double-checked to ensure correct data are displayed – as per 1.4 above.	Page 11
R.2.10. Table 1: The table mentions "Eye condition or glasses/contact lenses". However, I didn't notice mention of this variable in the methods section (though it may have been there and I just missed it). Can you please explain a bit more about this variable? Do we know what the eye conditions were? Do we	In Table 1, we report the percentage of the participants who reported eye conditions (including refractive error), but in this large population study we were not able to accurately measure refractive errors (which is not the same as visual acuity, which we did measure). We have added the following text to the Methods: "Child and parent participants who attended CheckPoint assessment centres also completed a visual acuity	Page 8

know what their glasses prescription was? Why wasn't this adjusted for in the model? It feels like these issues could affect the size measurements.	assessment (not conducted in home visits). As part of this assessment, they were asked if they "ususally wear glasses or contact lenses". Staff members recorded their response as yes or no; the strength of prescription was not captured. " We therefore could not adjust for refractive error but, as the literature shows small and inconsistent effects on measurement of retinal vessel caliber, we doubt that it would have greatly affected our results.	
R.2.11. Results, Parent-child concordance: Reporting the confidence intervals for the correlations would be useful.	The confidence intervals of the correlations are provided in Table 3.	
R.2.12. Results, Post-hoc analysis: Was the plan for this analysis discussed in the methods? Why is called a post-hoc analysis? It seems that it is a comparison with other published literature. It feels like this analysis is looking at a different question and seems out of place with the rest of the study. It seems that this is looking at the question, "how does vessel caliber vary with age?" though this issue wasn't specifically addressed in the previous analyses (see comment #7 above). If the authors decide they want to include this analysis looking at other studies in the manuscript, it seems like the methods should be strengthened. A systematic method with defined inclusion/exclusion criteria for the papers should be used. It also seems that the statistical method used in the manuscript doesn't account for the between-study variance and doesn't account for study differences.	Mean parent arteriolar caliber was markedly different from what we expected to find, at around 0.6 standard deviations smaller (rather than larger as expected from previous reviews) than those of the children. We felt that is sufficiently surprising to explore further - what it mainly shows is the dearth of mid-life literature. However, because this wasn't a planned analysis, we don't think it should be described in Methods. Rather than removing, we have added following text to more clearly indicate that (a) these were unplanned analyses in response to a surprising findings, and (b) that they should be considered exploratory only: "As noted above, both CRAE and CRVE were around 0.6 standard deviations smaller in the mid-life parents than the 11-12 year olds. This contrasts with Ikram's 2012 review, from which we had expected that CRAE would be substantially larger by midlife, but that CRVE would remain static (prior to both reducing into old age).[10] Because these results were surprising, we therefore conducted some unplanned post-hoc analyses to determine how our findings fit within the exisitng literature. These should be considered as exploratory and hypothesis-generating only."	Page 15

	We haven't analysed further and do not want to imply that this is the last word. We do think that our inclusion/exclusion criteria are clearly stated. We hope the reviewer will concur (Discussion) that more and better research on retinal parameters in mid-life is needed that would support high-quality individual participant meta-analysis. We do think this adds interest and will stimulate discussion, and note that Reviewer 1 did not ask for this section to be removed or strengthened. Please let us know if you would nonetheless prefer that we remove it, as we acknowledge it's not a standard approach.	
R.2.13. Conclusion, first paragraph: The first sentence talks about "demonstrating an age-related decrease in both mean CRAE and CRVE between mid-childhood and mid-life." Again, this doesn't seem like something that was evaluated in this study (see comment #7 above).	See above comments. We have amended the Conclusion, Unanswered questions and future research paragraph slightly to now read: " We provide normative values for retinal vessel calibre for Australian 11-12 year olds and mid-life adults using standardised protocols. Our findings make explicit a need for reliable age-specific normative reference values across the lifecourse. Ideally, this would extended to large long-running cohort studies with access to clinical outcomes; to exploration of other retinal vascular features such as branching angles, tortuosity and fractal dimension; and to consider other factors such as polygenic risk scores and macrovascular risk. Such studies could help retinal calibre realise its potential as a clinical, population screening and/or risk stratification tool for cardiovascular disease."	Page 17
R.2.14. Conclusion, Limitations: In what would the correlations have changed for smoking status, sedentary lifestyle and diet? Why didn't you control for refractive errors? Why would you expect the effects from refractive error to be small? How about axial length? Does this influence your measurements?	Previous studies have shown associations between smoking, decreased physical activity and dietary changes and retinal vessel caliber, though the evidence is mixed and this remains is a topic of current research. The text now reads: "We adjusted only for a limited range of potential confounders. While the distributions of parent retinal vessel caliber might have changed slightly had we further adjusted for smoking status, sedentary	Page 16

	lifestyle and diet (all previously associated with altered retinal vessel calibre[36]), their impact on concordance would likely be small because these factors are all strongly socially patterned. By 2019 this cohort will also be able to consider genome-wide association data, potentially shedding further light on the roles of genetic and shared environmental factors. We were not able to measure refractive errors in this study. Retinal vascular calibre measurements may be influenced by refractive errors and refraction is different between children and adults.[37, 38] However, we would expect these effects to be small, particularly as other sources of systematic bias were minimised (measured on the same day with the same equipment by the same person who was blind to dyadic membership).	