PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Endo Peripheral Arterial Tonometry (Endo-PAT 2000) use in Paediatric Patients – a systematic review.
AUTHORS	Hayden, Jenny; O'Donnell, Gill; deLaunois, Isabelle; O'Gorman, Clodagh

VERSION 1 – REVIEW

REVIEWER	O'Neill, MB Mayo University Hospital, Department of Paediatrics
REVIEW RETURNED	03-Apr-2022

GENERAL COMMENTS	Review Comments 2 Abstract The actual objective of this SR was not stated explicitly The results section of the abstract in uninformative. The total number articles identified is stated however the number reviewed is not stated, nor are the contents of the 6 tables mentioned. In the conclusion line 36 should be removed 4 Methods There is some confusion as to whether searches were restricted to the English language or not. 10 Results 50 studies were included in the review however I counted 49 (23+5+5+4+4+8) Using tables to group the results is very useful, however important information that would inform the reader is absent. The authors must state the primary aim (aims) of each study and indicate the relevant study outcomes inclusive of ED dysfunction level from each study. Noting whether ED assessment was a primary or secondary outcome would be useful. The outcomes from the reviewed studies are vague with little numerical data provided. Numerical data should be provided on the ED outcomes from the studies to inform the reader. I am not sure why the Metabolic syndrome, with 23 articles, is the supplementary section. I was confused by the presentation of the supplemental material in pages 26 to 29. There was no data summary. Line 161 Results start here not the discussion. 11 Discussion. The discussion should commence with a commentary on the quality of the papers assessed and where they rank in terms of quality of evidence. The studies are mostly case controlled and readers should be informed that the results are more imprecise that RCTs. This SR aims to synthesise the literature on ED dysfunction as assessed by Endo PAT. A specific comment should be made to

	inform the reader as to why there are no studies that can be compared. The potential role EndoPAT should be explored in the discussion. 12 Limitations of the paper Lines 193-195 outlines the limitations of the study. The limitations of the studies should be highlighted. Specific comments should be made on the strength of the conclusions that can be drawn from the assessed studies.

REVIEWER	Palmieri, Vittorio Ospedali dei Colli Monaldi Cotugno CTO, Cardiac surgery and transplantation
REVIEW RETURNED	16-Jun-2022

GENERAL COMMENTS	When it comes to flow-mediated dilation (FMD) or plethysmography-based definition of endothelial dysfunction (ED), one of the controversial points is the definition of ED in itself (gold-standard), and consequently the definition of a metric of it. Endo-Path-2000 is relatively user friendly, and relatively automated. However, such a characteristic does not automatically define such a method as reliable for measuring ED I believe that readers show find more of the following, and less on a generic description of determinants and consequences of ED: Was plethysmography-based definition of endothelial dysfunction related to atherosclerosis more strongly than FMD? Was plethysmography-based definition of endothelial dysfunction a correlate of hsCRP, LDL or Lpa, or homocysteine, stronger than FMD? For instance, in the table readers find endothelial dysfunction prevalence up to 76% among patients with Type 1 DM, unresponsive to treatments, and 22% among patients with systemic lupus erythematosus, absolutely well known to be related to coronary atherosclerosis and myocardial infarction potentially more than with type 1 DM. Page 6, lines 105-110: please, report correlations (i.e. R2), if any, between FMD and plethysmography, so that readers may appreciate the extent to which the two methods actually correlate. More in general, it is clear that the study reports on Endo-path-2000 as a relatively easy method to study endothelial function in a number of pathophysiologic conditions characterized mainly by inflammation or metabolic disorders or both. What really matter is the extent to which cardiovascular events occur in those pathophysiologic models, whether endo-path-2000 is a correlate of those stronger that FMD is, and whether treatments impact such a metric and findings.

REVIEWER	Ellins, Elizabeth Swansea University, Institute of Life Sciences
REVIEW RETURNED	24-Jun-2022

GENERAL COMMENTS	Abstract: The abstract needs further work. The objective of the study is not clear, and more details could be included about the methods and results, e.g., what themes were the papers were divided into. Strengths and limitations: no limitations of the study are mentioned. For the first point in the section, it is a comprehensive of the literature for endothelial function as assessed by the Endo-Pat not just endothelial function. Papers using other methods of assessment such as flow-mediated dilatation are not included. Introduction:

	It is not clear from the introduction what the actual purpose of the study is, why do the authors want to review the literature on Endo-Pat in paediatric populations? With regard to the differences between FMD and Endo-PAT endothelial function assessment. Brachial artery FMD is driven by NO whereas other pathways also influence microvascular endothelial function as determined by Endo-PAT. This should also be highlighted when discussing the two techniques. Please find alternative endings to sentences rather than etc. Reference 5 is a study investigating augmentation index as an indicator of arterial stiffness in children with type 1 diabetes, therefore further references are required for the other disease types listed in the sentence. Methods: Were the papers included assessed for quality? Results: Not included. The summary of the literature search could have been included here. Also, a summary of the findings from the studies included in the review and the tables of the papers. Discussion: This section came across as more of a results section than a comparison with the literature. Tables some of the information in the study design column needs checking as conference abstract and research article aren't types of study. Also, the control group column contains study design information. Not all tables were referred to in the text. I would question the use of the term metabolic syndrome as a theme. Patients with polycystic ovarian syndrome or familial hypercholesterolaemia may have some of these risk factors but this does not mean they meet the definition of metabolic syndrome. A lot of the articles included in the review are conference abstracts so there is limited information available on methods and results. This is not discussed in the discussion. Additionally, if a weakness of the study is that it only included papers after 2015 why was this cut off used?

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

The actual objective of this SR was not stated explicitly.

The objective of our study has been revised:

We aim to synthesise the literature on paediatric ED that utilised Endo-PAT analysis.

The results section of the abstract in uninformative. The total number articles identified is stated however the number reviewed is not stated, nor are the contents of the 6 tables mentioned.

The results section of the abstract has been adjusted:

Following the removal of duplicates, 156 articles were initially identified. Based on our exclusion criteria, 50 articles were included for review. We have subdivided these 50 papers into different systems for ease of reference and have reported our findings in 6 tables. The tables include patients with type 1/2 diabetes, obesity, cardiovascular, respiratory, psychiatric conditions and miscellaneous

diseases. For each, the study design, population, control group (if available), RHI results and conclusions were reported.

In the conclusion line 36 should be removed

This line has been removed.

Methods: There is some confusion as to whether searches were restricted to the English language or not.

Only English papers were searched, this has been adjusted.

50 studies were included in the review however I counted 49 (23+5+5+4+4+8).

There are a total of 50 studies as there are 24 studies in the supplementary table. The tables have been readjusted so there is no longer a supplementary table as it was taking from the main results.

Using tables to group the results is very useful, however important information that would inform the reader is absent. The authors must state the primary aim (aims) of each study and indicate the relevant study outcomes inclusive of ED dysfunction level from each study. Noting whether ED assessment was a primary or secondary outcome would be useful.

We haven't done a column in the tables to state the primary aim as we are limited to space and felt the title of most articles does explain the aims. However, if this was felt to improve the table I can add in an extra column? Each result column has the EndoPAT result entered and a conclusion comment with the studies main findings. We have not added in whether ED was a primary or secondary aim as again we are limited for space and most papers were assessing ED as a primary aim.

The outcomes from the reviewed studies are vague with little numerical data provided. Numerical data should be provided on the ED outcomes from the studies to inform the reader.

All papers have numbers in case and control groups (if available), male/female numbers, age, RHI and augmentation index results are reported when available. If any other results would be helpful to add I can certainly do so?

I am not sure why the Metabolic syndrome, with 23 articles, is the supplementary section.

The metabolic syndrome table was too big to put into the main document as per the BMJ guidelines as it would have taken up more than two pages, but now it has been reduced in size and is entitled 'overweight/obesity' so it is smaller. The other studies have been moved to other tables. Therefore, the obesity/overweight patients are in one table (Table 2) and have been added to the main results. This overweight/obesity table now includes 14 studies. The PCOS and hyperlipidaemia studies were added to the miscellaneous and cardiovascular tables.

I was confused by the presentation of the supplemental material in pages 26 to 29.

This is no longer in the supplementary material section. It is in the main results section as there are some interesting and relevant studies that are easier to see now.

There was no data summary.

The data summary in the results and discussion sections have been adjusted and extended. Most studies have only a single paper on a disease so we haven't gone into discussion on all papers, therefore some are just referenced in the tables. If there is more than one paper on a topic, I have discussed those results in separate paragraphs to summarise the findings. The results section is

separated into the categories for example diabetes, obesity, cardiovascular. This is to ease the reader to reference the text and tables together.

Line 161 Results start here not the discussion.

The results section has been adjusted and now includes multiple sections separated into 6 categories that reflect the 6 tables for ease of reference to the reader.

Discussion. The discussion should commence with a commentary on the quality of the papers assessed and where they rank in terms of quality of evidence.

This has been adjusted: see comment below on weaknesses/limitations of the paper.

The studies are mostly case controlled and readers should be informed that the results are more imprecise that RCTs. This SR aims to synthesise the literature on ED dysfunction as assessed by Endo PAT. A specific comment should be made to inform the reader as to why there are no studies that can be compared. Limitations of the paper. Lines 193-195 outlines the limitations of the study. The limitations of the studies should be highlighted. Specific comments should be made on the strength of the conclusions that can be drawn from the assessed studies.

Weaknesses of the paper include the quality of the papers are limited and varied; 11 are conference abstracts that had little information available on methods or results and have limited analysis. Observational studies are also limited in research value. Many are case-control studies which are not as valuable as randomised controlled trials (RCT). The studies cannot be compared for a meta-analysis as they are not RCT level research of high enough quality. Therefore, the conclusions drawn from many of these studies are limited.

There may be significant findings in studies in the grey literature or in conference presentations that was not included, for example in the studies where 25 authors did not respond to emails. Only papers from 2015 to March 2021 were included. Papers using other methods of ED assessment such as flow-mediated dilatation are not included. Many of the papers did not include other factors that would be important in a cardiovascular assessment of children, for example family history, cholesterol and blood pressure parameters and Body Mass Index (BMI) and standardised BMI (SDS) measurements. So, in many studies it cannot be excluded that there were confounding variables affecting the ED score. Regardless, this study indicates that there are a significant number of published paediatric papers that indicate the presence of ED in children as young as 8 years old.

The potential role EndoPAT should be explored in the discussion.

The future potential role of Endo-PAT has been discussed in the discussion section and I reference papers highlighting the usefulness of it in many paediatric conditions.

Reviewer: 2

When it comes to flow-mediated dilation (FMD) or plethysmography-based definition of endothelial dysfunction (ED), one of the controversial points is the definition of ED in itself (gold-standard), and consequently the definition of a metric of it. Endo-Path-2000 is relatively user friendly, and relatively automated. However, such a characteristic does not automatically define such a method as reliable for measuring ED. I believe that readers show find more of the following, and less on a generic description of determinants and consequences of ED:
Was plethysmography-based definition of endothelial dysfunction related to atherosclerosis more strongly than FMD?

There are some studies related to this. It is not definite which method is superior in paediatric patients. One of the studies in our review by Fusco et al reported that RHI did not correlate with LDF(1). Wilk et al reported that RHI correlated with FMD (r = 0.35, P <0.01)(2). They concluded that FMD is a more sensitive method than EndoPAT in evaluating the effect of classical atherosclerotic risk factors on vascular endothelial function. Another study by Allan et al favours FMD over EndoPAT but it was conducted in elderly patients and cannot be extrapolated to paediatric populations(3).

Was plethysmography-based definition of endothelial dysfunction a correlate of hsCRP, LDL or Lpa, or homocysteine, stronger than FMD? For instance, in the table readers find endothelial dysfunction prevalence up to 76% among patients with Type 1 DM, unresponsive to treatments, and 22% among patients with systemic lupus erythematosus, absolutely well known to be related to coronary atherosclerosis and myocardial infarction potentially more than with type 1 DM.

I am not sure which assessment, FMD or EndoPAT, is superior in assessing correlations between hsCRP, LDL, etc. Most studies in our review have not correlated the two techniques against blood results such as cholesterol levels or inflammatory markers. Wilk et al reported no significant correlation between RHI and IMT, SCORE, or the number of atherosclerotic risk factors including hypercholesterolemia. This study age group was 43.6 ±14.8 years. FMD significantly correlated risk factors (r = -0.55, P <0.05). in another study, obese adolescents were noted to metabolic syndrome more than normal weight kids but this is a study on EndoPAT only(4).

Page 6, lines 105-110: please, report correlations (i.e. R2), if any, between FMD and plethysmography, so that readers may appreciate the extent to which the two methods actually correlate. More in general, it is clear that the study reports on Endo-path-2000 as a relatively easy method to study endothelial function in a number of pathophysiologic conditions characterized mainly by inflammation or metabolic disorders or both. What really matter is the extent to which cardiovascular events occur in those pathophysiologic models, whether endo-path-2000 is a correlate of those stronger that FMD is, and whether treatments impact such a metric and findings.

Wilk et al reported that RHI correlated with FMD (r = 0.35, P <0.01)(2). This has been added to the paper in the EndoPAT section. They concluded that FMD is a more sensitive method than EndoPAT in evaluating the effect of atherosclerotic risk factors on vascular endothelial function. However, another study assessing ED and ageing did not report a correlation between FMD and RHI (*r* = -0.15; *P* = 0.35)(5).

Reviewer: 3

Abstract:
The abstract needs further work. The objective of the study is not clear, and more details could be included about the methods and results, e.g., what themes were the papers were divided into.

The abstract has been adjusted based on these and other reviewer comments.

Strengths and limitations: no limitations of the study are mentioned.

The limitations section is at the start of the discussion now and has been expanded.

For the first point in the section, it is a comprehensive of the literature for endothelial function as assessed by the Endo-Pat not just endothelial function. Papers using other methods of assessment such as flow-mediated dilatation are not included.

This point has been adjusted.

Introduction: It is not clear from the introduction what the actual purpose of the study is, why do the authors want to review the literature on Endo-Pat in paediatric populations?
With regard to the differences between FMD and Endo-PAT endothelial function assessment. Brachial artery FMD is driven by NO whereas other pathways also influence microvascular endothelial function as determined by Endo-PAT. This should also be highlighted when discussing the two techniques.

This introduction has been adjusted: We aim to synthesise the literature on paediatric ED that utilised Endo-PAT analysis.

We have added to the FMD section:

FMD uses an ultrasound to assess the change in brachial artery diameter in response to increased flow after a period of vascular occlusion by a blood pressure cuffand is highly dependent on nitric oxcide (NO) bioavailability. ED is identified by less vasodilatation (reduced FMD) of the brachial artery.

Please find alternative endings to sentences rather than etc.

This has been adjusted.

Reference 5 is a study investigating augmentation index as an indicator of arterial stiffness in children with type 1 diabetes, therefore further references are required for the other disease types listed in the sentence.

This has been adjusted and 3 further references have been added.

Methods: Were the papers included assessed for quality?

The papers were not formally assessed for quality but a general assessment based on study design and number of participants can help identify the quality of the paper. I have mentioned in the limitations the quality of the papers is limited in the discussion.

Results: Not included. The summary of the literature search could have been included here. Also, a summary of the findings from the studies included in the review and the tables of the papers.

The results section has the literature search results and this has been adjusted.

Discussion: This section came across as more of a results section than a comparison with the literature.

This has been adjusted. The results section now has the separate sections summarising the tables.

Tables some of the information in the study design column needs checking as conference abstract and research article aren't types of study.

I have gone through the abstracts available from these studies and unfortunately, they do not state exactly the type of study design used. I can adjust this again if needed I just wasn't sure and I had seen other reviews with tables that stated conference abstracts/RAs so I used that in that column but I can adjust accordingly?

Also, the control group column contains study design information.

Any study design information has been moved from the control group column to the study design column.

Not all tables were referred to in the text.

All tables are now referred to in the text in the results section.

I would question the use of the term metabolic syndrome as a theme. Patients with polycystic ovarian syndrome or familial hypercholesterolaemia may have some of these risk factors but this does not mean they meet the definition of metabolic syndrome.

The studies for PCOS and familial hypercholesterolaemia (FH) have been moved to a different table – PCOS to miscellaneous table 6 and FH to the cardiovascular table 3.

A lot of the articles included in the review are conference abstracts so there is limited information available on methods and results. This is not discussed in the discussion.

This has been added as a limitation in the discussion section.

Additionally, if a weakness of the study is that it only included papers after 2015 why was this cut off used?

We used this cut off because most of the papers published using EndoPAT in paediatric populations are relatively recent and we thought we would get a good yield from that date of 2015.

References:

1. Fusco E, Pesce M, Bianchi V, Randazzo E, Del Ry S, Peroni D, et al. Preclinical vascular alterations in obese adolescents detected by Laser-Doppler Flowmetry technique. Nutrition, Metabolism & Cardiovascular Diseases. 2020;30(2):306-12.
2. Wilk G, Osmenda G, Matusik P, Nowakowski D, Jasiewicz-Honkisz B, Ignacak A, et al. Endothelial function assessment in atherosclerosis: comparison of brachial artery flow-mediated vasodilation and peripheral arterial tonometry. Pol Arch Med Wewn. 2013;123(9):443-52.
3. Allan RB, Vun SV, Spark JI. A Comparison of Measures of Endothelial Function in Patients with Peripheral Arterial Disease and Age and Gender Matched Controls. Int J Vasc Med. 2016;2016:2969740.
4. Donghui T, Shuang B, Xulong L, Meng Y, Yujing G, Yujie H, et al. Improvement of microvascular endothelial dysfunction induced by exercise and diet is associated with microRNA-126 in obese adolescents. Microvasc Res. 2019;123:86-91.
5. Babcock MC, DuBose LE, Witten TL, Brubaker A, Stauffer BL, Hildreth KL, et al. Assessment of macrovascular and microvascular function in aging males. J Appl Physiol (1985). 2021;130(1):96-103.

VERSION 2 – REVIEW

REVIEWER	O'Neill, MB Mayo University Hospital, Department of Paediatrics
REVIEW RETURNED	12-Oct-2022

GENERAL COMMENTS	The improvements in the paper since the last revision are welcome and provide clarity for the reader. The limitations (weaknesses) cited in the discussion help contextualise the limited literature for reader who maybe unfamiliar with Endo PAT in ED.

REVIEWER	Palmieri, Vittorio Ospedali dei Colli Monaldi Cotugno CTO, Cardiac surgery and transplantation
REVIEW RETURNED	31-Aug-2022

GENERAL COMMENTS	I appreciated very much the effort to revise the manuscript in response to raised criticisms and comments. My only persisting concern is the fact that the Authors put upfront a clinical issue, such as endothelial dysfunction, very relevant, and went straight to plethysmography-based method to define it. Subsequently, they granted RHI as a metric of endothelial dysfunction and reported on it in the pediatric population. However, as the authors recognized in their replies, RHI cannot be tout court considered a reliable measure of endothelial dysfunction. More so if such a diagnosis is based on specific cut-points of the hyperemic reaction that may change among studies, and influence the prevalence of it in subgroups. Nevertheless, as I recognize that there is a lot of work done, I strongly suggest to declare in Introduction and Discussion the limitations of the RHI as reliable method for defining endothelial dysfunction, and the peculiarity of the pediatric population.

REVIEWER	Ellins, Elizabeth Swansea University, Institute of Life Sciences
REVIEW RETURNED	13-Sep-2022

GENERAL COMMENTS	Introduction. I am not sure what paragraphs 3 and 4 add to the introduction. Line 119. Wilk et al needs its reference including as do the studies mentioned that do not report the same correlation. The introduction would be improved by bringing together the points to identify why the authors are carrying out this literature review as it isn't really very clear at the moment. What will it add above other systematic reviews and meta- analyses of endothelial function assessments in paediatric populations? Would it be possible to potentially identify a cut off for RHI in the paediatric population, a gap identified by the authors? Results The RHI should be reported for both controls and patients. This is not done so in all cases. I am not sure why augmentation index is reported in one study. Line 253: Not sure this point needs to be in the results as it relates to adult patients and isn't about RHI. Make sure acronyms are defined in the text. Line 260: "Also, endothelial function significantly improves after weight loss". Is this a finding from studies in this section or a discussion point (no references)? Line 270: More of a discussion point than results. Line 278: Childhood cancer survivors- this section seems to provide more results regarding papers not included in the table and that do not meet the inclusion criteria of the study i.e.

	published in 2013 & 2011 for example and therefore should not be in this section. Discussion A full revision of the discussion is needed. It would be helpful rather than jumping straight into weaknesses and limitations to provide a summary of the review and its main findings and to then discuss the context of these. Why is not including papers of other methods of endothelial function assessment a weakness if the purpose of the study is to review this one technique? Line 344. This sentence isn't very clear. Cholesterol and blood pressure are cardiovascular risk factors. Line 352, what are the multiple benefits in obesity? The paragraph then goes on to talk about about a rare condition where patients lack subcutaneous fat and does not seem to be linked to the obesity. Then there is a section on Turners presenting results which are not included in the tables in the results section. What is the relevance with the topics covered in the review? There are also a number of other studies introduced in the discussion which the relevance of which to the review isn't clear. The authors have plenty they wish to discuss about this interesting area but maybe a narrative review would be better rather than constraining themselves to a systematic review.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 1
Dr. MB O'Neill, Mayo University Hospital

We thank reviewer 1, Dr O'Neill, for these comments.

The improvements in the paper since the last revision are welcome and provide clarity for the reader. The limitations (weaknesses) cited in the discussion help contextualise the limited literature for reader who maybe unfamiliar with Endo PAT in ED.

Reviewer: 2
Dr. Vittorio Palmieri, Ospedali dei Colli Monaldi Cotugno CTO

We thank Dr Palmieri for these very useful and insightful comments and trust that we have addressed them to your satisfaction.

I appreciated very much the effort to revise the manuscript in response to raised criticisms and comments. My only persisting concern is the fact that the Authors put upfront a clinical issue, such as endothelial dysfunction, very relevant, and went straight to plethysmography-based method to define it.

We have expanded further on the definition of endothelial dysfunction and the use of Endo-PAT.

Subsequently, they granted RHI as a metric of endothelial dysfunction and reported on it in the pediatric population. However, as the authors recognized in their replies, RHI cannot be tout court considered a reliable measure of endothelial dysfunction. More so if such a diagnosis is based on specific cut-points of the hyperemic reaction that may change among studies, and influence the prevalence of it in subgroups.
Nevertheless, as I recognize that there is a lot of work done, I strongly suggest to declare in Introduction and Discussion the limitations of the RHI as reliable method for defining endothelial dysfunction, and the peculiarity of the pediatric population.

We have made adjustments to the introduction and discussion regarding RHI.

Reviewer: 3
Dr. Elizabeth Ellins, Swansea University

We thank Dr Ellins for the useful and thorough comments and suggestions and trust that we have addressed them to your satisfaction.
Introduction.
I am not sure what paragraphs 3 and 4 add to the introduction.

These paragraphs have been moved to the discussion.

Line 119. Wilk et al needs its reference including as do the studies mentioned that do not report the same correlation.

This reference has been added to the end of the sentence.

The introduction would be improved by bringing together the points to identify why the authors are carrying out this literature review as it isn't really very clear at the moment. What will it add above other systematic reviews and meta- analyses of endothelial function assessments in paediatric populations?

The introduction has been adjusted.

Would it be possible to potentially identify a cut off for RHI in the paediatric population, a gap identified by the authors?

This is a most interesting question. Unfortunately, we think that an answer or a suggestion about a possible cut off for RHI is beyond the scope of this systematic review. It would be helpful if such a cut off could exist, but it is likely to only be clinically meaningful if estimated and reviewed in an iterative process, as part of a prospective longitudinal study, whereby "normal" is reviewed repeatedly in the context of sickness/wellness as they exist in the study cohort. Or alternatively, if estimated by a meta-analysis. Meta-analysis of the results of the included studies in our paper was not possible due to the heterogeneity of reported studies and reported results. We have included a comment in the conclusions section suggesting that if authors could standardise reporting in future studies, that a future meta-analysis might be possible. The importance of a cut-off RHI would be its clinical significance and, much as normal values for other continuous variables, e.g. fasting blood sugar, have changed over time, so too would an estimate for RHI need to be reviewed iteratively as it relates to clinically meaningful results (or results, e.g. vascular endpoints achieved or not achieved) over time.

Results
The RHI should be reported for both controls and patients. This is not done so in all cases. I am not sure why augmentation index is reported in one study.

The augmentation index results have been removed. Some of the papers including the conference abstracts had no RHI results to add in. Some had no controls and therefore just the RHI for the study group is reported.

Line 253: Not sure this point needs to be in the results as it relates to adult patients and isn't about RHI.

This line has been removed.

Make sure acronyms are defined in the text.

All acronyms are defined within the text.

Line 260: "Also, endothelial function significantly improves after weight loss". Is this a finding from studies in this section or a discussion point (no references)?

This was a finding from a study - Ysebaert et al - the reference has been added.

Line 270: More of a discussion point than results.

This point has been moved to the discussion.

Line 278: Childhood cancer survivors- this section seems to provide more results regarding papers not included in the table and that do not meet the inclusion criteria of the study i.e. published in 2013 & 2011 for example and therefore should not be in this section.

These have been removed from the results section to the discussion.

Discussion
A full revision of the discussion is needed. It would be helpful rather than jumping straight into weaknesses and limitations to provide a summary of the review and its main findings and to then discuss the context of these.

The discussion has been revised.

Why is not including papers of other methods of endothelial function assessment a weakness if the purpose of the study is to review this one technique?

We agree with the reviewer and have removed this line from the discussion as a limitation of this systematic review. Notwithstanding, it is an interesting question, but it is beyond the scope of this study.

Line 344. This sentence isn't very clear. Cholesterol and blood pressure are cardiovascular risk factors.

This sentence has been edited.

Line 352, what are the multiple benefits in obesity? The paragraph then goes on to talk about a rare condition where patients lack subcutaneous fat and does not seem to be linked to the obesity.

This section has been edited.

Then there is a section on Turners presenting results which are not included in the tables in the results section. What is the relevance with the topics covered in the review? There are also a number of other studies introduced in the discussion which the relevance of which to the review isn't clear.

We have edited the section on Turner's syndrome in the discussion. The other cardiac studies mentioned, some were narrative reviews or meta-analysis and did not have results to put into the tables, therefore we added in a few of these studies into the discussion as they were of interest to Endo-PAT use in paediatrics.

The authors have plenty they wish to discuss about this interesting area but maybe a narrative review would be better rather than constraining themselves to a systematic review.

We thank the reviewer for this helpful suggestion. However, we have conducted a rigorous systematic review, and have thus limited the perhaps more narrative parts of our discussion to a report of the systematic review.