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Title page

Endo Peripheral Arterial Tonometry (Endo-PAT 2000) use in Paediatric Patients – a systematic review.

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Key words: Endo-PAT 2000, peripheral artery tonometry, Endothelial dysfunction, paediatric diabetes mellitus, chronic diseases

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Abstract:

Objectives: Endo Peripheral Artery Tonometry (EndoPAT-2000) is a non-invasive technology for measuring endothelial dysfunction (ED). The reactive hyperaemia index (RHI) is resulted and is low when endothelial dysfunction is present. Microvascular ED, the early stage of atherosclerosis, precedes macrovascular ED and can be detected in children and adolescents.

Design:

A comprehensive systematic review was conducted to identify publications that investigated the use of Endo-PAT 2000 from 2015 to present.

Results:

156 articles were identified in our review. We have subdivided these papers into different systems for ease of reference and have reported our findings in 6 tables.

Conclusions:

A number of papers using Endo-PAT for children with various chronic diseases have evidence of ED. It should be concerning to paediatricians that children with various chronic diseases have evidence of ED. However, in many cases, there has only been a single cohort study using Endo-PAT. Further studies are required to validate these findings and to help characterise the cardiovascular risk profile of children with chronic disease. Further studies are also required that will characterise more completely the cardiovascular risk profile of these children with chronic disease.

Consensus on other vascular risk markers that could be included in future studies is ideal and if accomplished, this would facilitate meta-analyses of studies of conditions with relatively rare conditions.

Strengths and limitations:

- Comprehensive literature review on paediatric endothelial dysfunction from 2015 to present.
- All study types were reviewed and even the studies without results but had interesting points were included in our discussion.
- Awareness of ED in paediatric patients can aid an approach to cardiovascular risk assessment for young children and adolescents, in particular those with chronic diseases.
- Separate paediatric results were obtained where possible from studies with combined adult and paediatric data.
- Further studies would help characterise the cardiovascular risk profile of children with chronic diseases.

Introduction:

Endothelial dysfunction (ED) is an early predictor of cardiovascular disease(1). ED occurs when the endothelium loses its ability to promote vasodilation, fibrinolysis and anti-aggregation(2). It can be caused by oxidative stress with loss of vaso-active or inflammatory homeostasis within the body's vascular system. It may be secondary to mechanical stimuli, for example increased intraluminal pressure within the blood vessel or metabolic factors such as hormones (oestrogen's vasodilation action)(3). Damaged endothelium can release a cascade of substances which pose a risk of thrombosis, inflammation and ultimately atherosclerosis(4).

ED in paediatric populations has been associated with several conditions including type 1 diabetes (T1D), type 2 diabetes (T2D), renal impairment, obesity, metabolic syndrome, etc(5). ED can progress to atherosclerosis which is a chronic condition that poses severe risk of coronary artery disease, stroke, peripheral arterial disease, etc.

Improving glucose control can protect endothelial function. Persistent high sugars can impair endothelial function via oxidative stress, free-radicals, etc(2). Diabetic microangiopathy can result in

the outcomes of retinopathy, neuropathy and peripheral vascular neuropathy. Subclinical evidence of these complications can be seen in paediatric patients, especially in those with poor glycaemic control. In patients with T2D, obesity and metabolic syndrome, insulin resistance is one of the most importance factors contributing to ED(6). Metabolic syndrome is a pro-inflammatory state where dyslipidaemia, hyperuricemia, and hypertension occur and can predispose to ED(7). Unfortunately, there have been reports of T2D paediatric patients diagnosed with microangiopathic complications, particularly nephropathy(8). This early endothelial damage can be linked with increased morbidity and mortality(9). New onset diabetes after transplantation (NODAT) is characterised by insulin resistance and T2D(10).

In recent decades, the number of childhood cancer survivors is increasing(11). Treatments utilized such as haematopoietic stem cell transplantation have increased risk of cardiovascular disease(12, 13). Following chemotherapy, radiotherapy, immunosuppressive treatments the risk of insulin resistance has been noted(14). With advances in treating malignant paediatric conditions there are long term complications emerging in survivors. High dose chemotherapy including anthracyclines, alkylating agents and vinca alkaloids may disrupt the substances on the surface of the endothelium and impair its ability to dilate and constrict. Moreover, total body radiation poses a risk by damaging the elastic matrix. Heart disease in long-term cancer survivors is 5-10 times higher than their siblings(14).

Endo-PAT 2000:

Endo Peripheral Artery Tonometry (Endo-PAT 2000) is a non-invasive technology for measuring ED developed by Itamar Ltd. Non-invasive pneumatic probes which are placed on the both index fingers, which continuously records pulse wave amplitude. A blood pressure cuff is inflated to occlude blood flow and response after deflation is recorded. The reactive hyperaemic index (RHI) is resulted following this mini-ischemic stress to the vessel. The pulse wave amplitude (PWA) is measured and computes a RHI result automatically. RHI is calculated as the ratio of average PWA divided by the

average amplitude during the equilibration period. To compensate for any systemic changes, this ratio is normalized to a concurrent signal from the contralateral finger.

Numerous studies in both adult and paediatric literature reveal Endo-PAT's excellent reproducibility and reliability(15, 16). In ED, the RHI is low and pulse amplitude is high. PAT also provides results on the peripheral augmentation index (PAT-AIx). Bonetti et al report a RHI of <1.35 as indicative of coronary ED in adults(17). However, there is no reported RHI cut off value in paediatric patients. Endo-PAT can be used at the patient's bedside, without extensive training required of the operator.

Prior to Endo-PAT, ED had been assessed by flow-mediated vasodilation (FMD). 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 cuff. A reduction in FMD represents ED. FMD is technically challenging to perform, user-dependent and requires training. FMD results macro blood vessel reactivity whereas Endo-PAT results micro, which may account for the challenges in comparing the two techniques. Endo-PAT is easier to set up, is automated and less user-dependent.

Objective:

The aim of this study is to conduct a systematic review of the use of Endo-PAT 2000 with particular emphasis on paediatric populations.

Methods:

A comprehensive systematic review was conducted to identify publications that investigated Endo-PAT 2000. All papers published from 2015 to March 2021 in paediatric populations age birth to 16 years of age were analysed. PRIMSA study design was used.

The following scientific databases were searched: The Cochrane Database, MEDLINE EBSCO, EMBASE (Ovid), PUBMED and CINAHL EBSCO. The search was not limited by language. The search was limited by type of subjects (human), date (2015 to March 2021) and included all study types. Snowballing method was used. Authors of joint adult and paediatric papers were contacted by email to obtain separate paediatric data.

The database search was repeated several times using the combinations of keywords, MeSH terms and filters (child: birth-16 years). The following MeSH terms or key words were used for searching: Peripheral arterial tonometry, PAT test, endopat, adolescent, ado*, child, paediatric, pediatric, preschool, schoolboy, schoolgirl, boy, girl, teen, toddler, infant, baby.

Exclusion criteria were: 1. If a study used a different device for example 'Watch-PAT;' 2. If the study had no results. Inclusion criteria were: 1. Published in the English; 2. More than 50% of study subjects were in the paediatric age range; 3. Data relevant to paediatric age range children could be extrapolated from all data, where not all study subjects were children. A child was defined as up to 16 years, and this is consistent with PubMed's definition of a child. Where data relevant to children could not be extrapolated from the whole dataset, the study authors were contacted for additional information prior to study inclusion or exclusion.

Patient and public involvement:

No patient involved.

Data collection and analysis:

A total of 290 articles were obtained via the online database search (*Figure 1*: flow diagram). Following removal of duplicates, 158 articles remained. The second screening was conducted by 'Rayyan- systematic review software' Copyright © 2021 Rayyan. Two further duplicate articles were removed, with 156 remaining for review.

Two independent authors separately performed a blind screen on the 156 abstracts. 65 articles were initially excluded based on title or abstract: 37 adult studies, 18 'PAT' did not represent peripheral arterial tonometry (e.g. prism adaptation test, psychosocial assessment tool), 6 Watch-PAT, 2 sleep studies and 2 had no results available.

The remaining 91 articles were analysed viewing full text articles for further information. A further 20 were screened out as did not fit inclusion criteria or have results to report. Some of these articles that included Endo-PAT 2000 in paediatrics were referenced in the discussion.

Twenty-eight authors of studies including both adults and paediatric patients were contacted twice by email to gather separate information on the paediatric participants. Twenty authors did not reply and were thus excluded. Eight authors replied: three providing results, four unable to give separate paediatric data and one author's research was on adult patients so was excluded. Three of the articles whose authors replied with data were included in our review.

Four studies were obtained via snow balling searching. Some articles did not have results for the systematic review but had reviews and conclusions that were relevant to the paper and so were excluded from analysis.

A total of 50 articles were included in our results and are represented in tables 1-6. For each eligible study the following data was reported: author, year of publication, design of the study, population studied, control group (if available), RHI results.

Discussion

This literatures search identified 156 articles in the published paediatric literature on ED in children. We have subdivided these papers into different systems for ease of reference (*Tables 1-5, supplementary material, supplementary table 1*).

Title, lead author	Year	Study design	Population: n=sample size, age; mean ± SD or median (range), [F/M]	Control group: n=sample size, age; mean ± SD or median (range), [F/M]	Results: RHI reported. If RHI not specified, we reported p/r values	Outcomes
Adolescents and young adults with type 1 diabetes display a high prevalence of endothelial dysfunction. Scaramuzza et al (18)	2015	Cohort prospective observational study	n=73 T1D adolescents, diagnosed > 1 year, 16.2 +/- 3.5 years, [F/M 25/48]	No controls. Results at baseline and after a 1-year follow-up	56 (76.7%) had ED, with lower mean RHI scores (1.26 ± 0.22 versus 2.24 ± 0.48, $p < 0.0001$). More with ED had abnormal cardiac autonomic tests ($p = 0.02$) and were more sedentary. After 1 year follow-up in 64/73 patients, 81.8% had ED, despite some improvement in	T1D adolescents had evidence of ED. Good metabolic control (HbA1c ≤7.5%) and regular physical activity might be protective. ED progression despite some improvement to HbA1c.

					HbA1c. ED rate recorded at baseline was 76.7%.	
Alpha-Lipoic Acid and Antioxidant Diet Help to Improve Endothelial Dysfunction in Adolescents with Type 1 Diabetes: A Pilot Trial. Scaramuzza et al (19)	2015	Double-blind, randomized controlled trial – snow balling	n=71 T1D patients, followed for at least 1 year, age 16.3 ± 3.4 years, [F/M 29/42]	Results at baseline and after follow-up.	3 double-blind study arms: 10,000 ORAC antioxidant diet +/- lipoic acid: RHI 1.40 ± 0.68 vs 1.72 ± 0.66 (P<0.05) (baseline vs af ter 6 months). 10,000 ORAC antioxidant diet + placebo: RHI 1.39 ± 0.41 vs 1.58 ± 0.40 (P>0.05) (baseline vs af ter 6 months). Controls: RHI 1.58 ± 0.64 vs 1.54 ± 0.42 (P>0.05).	Positive association between ED parameters and alpha-lipoic acid administration.
Effect of metformin on endothelial function in overweight adolescents with type 1 diabetes (T1D). Nadeau et al(20)	2016	Conference abstract	Total n=70 overweight T1D patients. n= 41 on metformin (up to 2000 mg/day), 12-19 years (mean 15.8)	n=29 placebo group. Endo-PAT scores at baseline and 13 weeks	Mean baseline RHI score was 1.8 +/- 0.6 in the metformin group and 1.7 +/- 0.6 placebo group. At 13 weeks, no significant change from baseline RHI (+0.1 in metformin vs. -0.0 in placebo, P = 0.08). There was some improvement in endothelial function among males.	Metformin may improve endothelial function in overweight T1D males.
Assessment of biomarkers of inflammation and premature atherosclerosis in adolescents with type-1 diabetes mellitus. Babar et al (21)	2019	Cross-sectional study	T1D adolescents ≥12 years. Two groups based on different HbA1c ranges. (HbA1c) ≤8.5% (n=27)	HbA1c ≥9.5% (n=25)	PAT results were not significantly different between the groups. Pearson correlation showed a significant direct relationship between rising HbA1c and PAT (p=0.03, r=0.31).	Suboptimal glycemic control as evidenced by a rising HbA1c causes early atherosclerosis.
Improvements in peripheral vascular function with vitamin D treatment in deficient adolescents with type 1 diabetes. Deda et al (22)	2018	Research article – snow balling	n=21 T1D patients followed for ~2 years. 25-OH-Vit. D levels < 37.5 nmol/L. Age 15.7 ± 1.4 years, [F/M 19/12]	Controls: matched age, sex and T1D tested spring and in fall (no significant difference noted).	After 4.8 ± 1.3 months of Vit. D supplementation RHI improved: 1.83 ± 0.42 vs 2.02 ± 0.68 (P<0.05).	Vit. D supplementation associated with improvement to endothelial function and reduced expression of urinary inflammatory markers.

Table 1: Endo-PAT 2000 in paediatric type 1 diabetes mellitus (T1D) patients (5 studies). Reactive hyperemia index (RHI), type 1 diabetes mellitus (T1D), augmentation index (AIx) (vascular stiffness), endothelial dysfunction (ED), Oxygen radical absorbance capacity units (ORAC).

Title, lead author	Year	Study design	Population: n=sample size, age; mean ± SD or median (range), [F/M]	Control group: n=sample size, age; mean ± SD or median (range), [F/M]	Results: RHI reported. If RHI not specified, we reported p/r values	Outcomes
Nocturnal blood pressure dipping as a marker of endothelial and cardiac function in pediatric-onset systemic lupus erythematosus (SLE). Chang et al (23)	2020	Cross-sectional study – author contacted for separate paeds data	n=20, 9-19 years (mean 16.5), (7 were age 16 or under). Average disease duration 3.2 years (± 2.1). [F/M 17/3]	Separated into 2 groups based on nocturnal BP dipping status.	Mean ln(RHI) for n=7 (aged 16 and under): 0.529. 22% had ED. Reduced diastolic BP dipping was associated with poorer endothelial function (r 0.5, p = 0.04).	SLE cohort, isolated nocturnal BP non-dipping is prevalent and associated with ED and atherosclerotic changes. Potential role for routine ABPM for youth with SLE.
Endothelial Function and Arterial Stiffness Relate to Functional Outcomes in Adolescent and Young Adult Fontan Survivors. Goldstein et al (24)	2016	Cross-sectional prospective observational study	n=60, 8-25 years (mean 13.9±4.1), [F/M 29/31]	No controls	AI (P<0.05) was negatively associated with peak VO2 (02 consumption). PAT derived baseline pulse amplitude (P<0.05) was negatively associated with the ratio of minute ventilation to C02 at anaerobic threshold. PAT-AI (P<0.05) was negatively associated with parent-reported Paeds QOL total and physical heath summary scores.	Worse vascular measures were associated with worse functional measures. Increased arterial stiffness and decreased endothelial function are associated with lower aerobic capacity, physical activity, and quality of life in Fontan survivors.
Natural history of vascular function in adolescent and young adult Fontan survivors: A longitudinal assessment of endothelial function and arterial stiffness. Goldstein et al (25)	2017	Prospective single-centre longitudinal study, conference abstract	n=50, mean 13.7 +/- 4.2 years, [F/M 23/27]	Paired testing at a mean interval of 2.0 +/- 0.2 years of Fontan survivors.	Decreases in RHI (0.002 +/- 0.01/yr) were not significant. AIx improved by 0.74 +/- 0.3/yr (p=0.02). Changes RHI and AI did not correlate with change in peak VO (peak 02 consumption). Change in BMI was a predictor for RHI (R 0.17, p=0.007). Change in resting O2 saturation was the only predictor of the rate of change in AI (R 0.09, p=0.04).	Vascular function does not change uniformly in Fontan survivors. Changes in vascular function do not relate to changes in aerobic capacity but are associated with changes in anthropometric measures and O2 saturation.
Vascular function long term after Kawasaki disease: another piece of the puzzle? Pinto et al (26)	2017	Single-centre prospective study	n=43 Kawasaki patients, age >11 years, diagnosed >5 years ago, with no coronary lesions or any other risk factors for cardiovascular disease.	n= 43 control group of individuals without cardiovascular risk factors.	Kawasaki patients had decreased RHI compared with controls (1.59±0.45 versus 1.98±0.41; p<0.001). AI was similar in both groups (-4.5±7 versus -5±9%; p 0.6).	Children with Kawasaki disease may have long-term sequelae, even when there is no discernible coronary artery involvement in the acute stage of the disease.
Endothelial function in	2017	Observational	n=19 with HSP, 13.5 ± 3.9 years,	n=23 healthy children, 12.8 ±	Mean RHI 1.81 in the study group and 1.87 in	This study suggests that HSP causes short

children with a history of Henoch Schonlein purpura (HSP). Butbul Aviel et al (27)		prospective study	[F/M 8/11]	4.5 years, [F/M 7/16]	the control group (p = 0.18). RHI was higher in patients who had endothelial function measured >6 years since HSP diagnosis compared with those patients with less than 6 years follow up (1.98 + 0.74 vs. 1.38 ± 0.43 P = 0.037).	term endothelial dysfunction that improves with time.

170 Table 2: Endo-PAT 2000 in paediatric patients with cardiac and vascular conditions (5 studies). Reactive

171 hyperemia index (RHI), augmentation index (AI), systemic lupus erythematosus (SLE), ambulatory blood

Title, lead author	Year	Study design	Population: n=sample size, age; mean ± SD or median (range), [F/M]	Control group: n=sample size, age; mean ± SD or median (range), [F/M]	Results: RHI reported. If RHI not specified, we reported p/r values	Outcomes
Vascular function in asthmatic children and adolescents. Augusto et al (28)	2017	Cross-sectional controlled study	n=19 asthmatic patients, age 13.6 ± 0.6 years. [F/M 0/19]	n=18 controls. 14.9 ± 0.7 years. [F/M 0/18]	LnRHI were similar between groups (p = 0.23). The augmentation index (AIx@75%) was significantly higher in the asthmatic group (-7.75 ± 1.7) compared to the control group (-15.25 ± 1.8), p < 0.04.	The increased AIx@75 without changes in LnRHI in asthmatic patients could mean that an early detection of vascular impairment may precede ED.
The effect of weight loss on endothelial function and sleep disordered breathing (SDB) in obese children. Ysebaert et al(29)	2018	Conference abstract	n=62 obese, age 11-19 (mean 15.8) years, [F/M 20/42]	No controls. Children reassessed after 6-month weight loss programme.	Endo-Pat used. At baseline 39% had SDB. After 6 months, 86% had resolution of earlier diagnosed SDB. All showed significant improvement of endothelial function after programme (p < 0.001). No correlations between presence of SDB and improvement in endothelial function found.	Endothelial function significantly improves after weight loss.
Polysomnographic correlates of endothelial function in children with obstructive sleep apnoea (OSA). Zhang et al (30)	2018	Cross sectional study	n=121 mild OSA, 6.2 ± 1.6 years, [F/M 37/84]. n=127 moderate-severe OSA, 6.0 ± 1.6 years, [F/M 31/96]	n=107 primary snorers (PS), age 6.4 ± 1.8 years, [F/M 37/70]	OSA groups lower RHI than PS (P < 0.001, P = 0.001). RHI positively correlated with age (r = 0.17, P = 0.002), BMI z score (r = 0.14, P = 0.008) and oxygen saturation (r = 0.15, P = 0.006).	Children with OSA are at increased risk for abnormal endothelial function than habitually snoring children.
Endothelial	2020	Cross	n=248 OSAS, age	n=107 primary	OSAS had lower RHI	OSAS have

172 pressure monitoring (ABPM), Henoch Schonlein purpura (HSP).

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dysfunction in children with obstructive sleep apnoea syndrome (OSAS). Xu et al(31)		sectional study	3-11 years	snorers (PS). No significant differences in age/gender.	1.1±0.1 vrs 1.2±0.2 (P<0.01). RHI independently correlated with age, gender, obstructive apnoea hypopnea index, oxygen desaturation index (*P*<0.01).	significant ED compared with PS. Frequent arousals due to obstructive respiratory events during sleep may be a candidate risk factor for ED.

Table 3: Endo-PAT 2000 in paediatric patients with respiratory conditions (4 studies). Natural logarithm of RHI (LnRHI), endothelial dysfunction (ED), reactive hyperaemia index (RHI), augmentation index (AI), heart rate-corrected augmentation index (AIx@75), primary snorers (PS), obstructive sleep apnoea (OSA), obstructive sleep apnoea syndrome (OSAS).

Title, lead author	**Year**	**Study design**	**Population: n=sample size, age; mean ± SD or median (range), [F/M]**	**Control group: n=sample size, age; mean ± SD or median (range), [F/M]**	**Results: RHI reported. If RHI not specified, we reported p/r values**	**Outcomes**
Do self-reported stress and depressive symptoms effect endothelial function in healthy youth? The LOOK longitudinal study. Olive et al(32)	2018	Longitudinal cohort study	n=203, 7.6 ± 0.3 years, [F/M 111/92].	LOOK longitudinal study, who were followed through to adolescence (16 years).	All relationships occurred in the hypothesised direction, but no cross-sectional or prospective evidence of early psychological stress or depression was associated with ED (all p > 0.05).	Contrast to previous findings in adolescents, little evidence between current or previous psychosocial stress or depression and endothelial function in 16-year-old adolescents.

Cerebrovascular reactivity is associated with peripheral endothelial function (EF) among adolescents. Urback et al(33)	2016	Conference abstract	n=11 with bipolar disorder. EF measured by PAT and cerebrovascular reactivity (CVR) by blood-oxygen-level dependent fMRI.	n=35 healthy controls	EF was positively correlated with CVR in grey matter (r=0.41, p=0.012), and a peak voxel in the left-medial-frontal gyrus (r=0.35, p=0.036).	Breath-hold CVR and peripheral EF are linked, suggesting that vascular function may be a multi-systemic phenotype. EF may be a potential proxy for cerebral blood vessel function with greater accessibility and lower cost than fMRI.
Retinal-vascular photography as a window into the cardiovascular and neurocognitive burden of adolescent bipolar disorder (BD). Naiberg et al (34)	2017	Cross-sectional study, author emailed for separate paeds data-most were teenagers	n=30 with bipolar disorder, 17.97±1.86 years	n=32 healthy controls, 16.00±1.62 years	In BD group, higher endothelial function associated with higher arterio-venular ratio (r=0.375, p=0.041).	Retinal photography may help assessing cardiovascular and neurocognitive burden of BD.
Impact of psychological health on peripheral endothelial function and the HPA-axis activity in healthy adolescents. Chen et al(35)	2017	Longditudinal 3-year follow-up study	n=162, 14.5 ± 1 years. [F/M 94/68].	Baseline and three-year follow-up.	Lower peripheral endothelial function was associated with high level of anger ($\beta = -0.332$, $p = 0.018$) and disruptive behaviour ($\beta = -0.390$, $p = 0.006$) over three years in males, but not in females, adjusted for covariates.	High amounts of negative emotions may have adverse effects on peripheral endothelial function and regulation of the HPA-axis activity. High level of self-concept might be protective.

183 Table 4: Endo-PAT 2000 in paediatric patients with psychiatric conditions (4 studies). Endothelial dysfunction

184 (ED), endothelial function (EF), cerebrovascular reactivity (CVR), bipolar disorder (BD), functional magnetic

185 resonance imaging (fMRI), hypothalamic–pituitary–adrenal HPA.

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Title, lead author	**Year**	**Study design**	**Population: n=sample size, age; mean ± SD or median (range), [F/M]**	**Control group: n=sample size, age; mean ± SD or median (range), [F/M]**	**Results: RHI reported. If RHI not specified, we reported p/r values**	**Outcomes**

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Vascular endothelial function in inflammatory bowel disease (IBD). Winderman et al(36)	2018	Case-control study	n=16 with IBD (all in clinical remission), age 16.7 +/- 2.6 years, [F/M 8/7]	n=16, age 15.1 +/- 2.8 years, [F/M 7/8]	RHI IBD vs controls 1.66 vs 2.02 (P =0.036). IBD group had a mean RHI within the range associated with VD risk in adults (1.67).	IBD group lower RHI compared with controls. IBD patients may need to be monitored for thromboembolic phenomena.
Endothelial health in childhood acute lymphoid leukaemia (ALL) survivors: pilot evaluation with peripheral artery tonometry. Ruble et al (37)	2015	Case control study	n=16 ALL survivors, age 8-20 years (12.9+/-0.9), [F/M 8/8].	n=16 healthy sibling pairs 13.8 (0.9), [F/M 10/6].	Both groups similar in cardiovascular risk measures but survivors had lower RHI (1.54 vs. sibling 1.77; P=0.0474).	Evidence of poorer vascular health in cancer survivors.
Microvascular endothelial function in Japanese early adolescents. Odanaka et al (38)	2017	Control study	n=157 healthy adolescents divided by gender. Females n=82, median age 14 (1), 13.7 ± 0.9 years	Males n= 75, median age 14 (2) years	No difference in RHI according to sex: boys and girls 1.85 ±0.6, 1.82 ±0.66 and 1.87± 0.54. RHI was significantly associated with systolic and diastolic BP, and had no correlation with anthropometric parameters and arterial stiffness markers.	RHI among adolescents were similar to those reported in previous studies on children and early adolescents.
Endothelial Dysfunction and the Effect of Arginine and Citrulline Supplementation in Children and Adolescents With Mitochondrial Diseases. Al Jasmi, et al (39)	2020	Case control study	9 participants, age 6-17 years (mean 9.6).	3-15 years (mean 9.4). Baseline endothelial dysfunction was assessed in control individuals.	Lower RHI with mitochondrial diseases. RHI increased with arginine or citrulline supplementation	Supplementation with NO precursors may improve ED by enhancing NO production. First study to use Endo-PAT methodology in mitochondrial diseases.
Assessment of traditional and non-traditional risk factors for premature atherosclerosis in children with juvenile dermatomysoitis (JDM) and pediatric controls. Wahezi et al (40)	2020	Retrospective controlled study	n=40 JDM, age 6-22 (mean 12.4± 4.1) years, [F/M 28/12]	n=20 controls, age 12.7± 3.9 years, [F/M 14/8]	75% controls had ED compared to 50% JDM group. RHI controls and JDM groups: 1.43 [1.2, 1.7] and 1.57 [1.2,1.9]. When controlled for lipoprotein A (atherogenic confounder), JDM patients had 41% RHI increase, thus indicating less ED compared to controls.	Rheumatology childhood disorders may be at increased risk of developing premature atherosclerosis, but traditional and sociodemographic features may play a greater role in the ultimate development of cardiovascular disease.
Vascular Health of Children Conceived via In Vitro Fertilization (IVF). Zhang et al	2019	Cross-sectional pilot study	n=17 IVF children, 10-14 years. Also used carotid ultrasound and pulse wave velocity	Compared to published norms or to historical Stanford controls	Mean Endo-PAT index in the IVF cohort was 1.66+/-0.52, 71% had abnormal values (<1.9). Mean RHI was not significantly	Children conceived by IVF seem to have evidence of abnormal vascular health.

(41)			measurements.		different between IVF and controls.	
Endothelial dysfunction in South African youth living with perinatally acquired human immunodeficiency virus (PHIV) on antiretroviral therapy. Mahtab et al (42)	2020	Case control study	n= 431 PHIV, median 14.1 (12.8, 15.5) years, [F/M 213/218]	n=93 without HIV, median 13.9 (12.1, 15.3) years, [F/M 53/40]	PHIV had higher rates of ED (50% vs 34%; P = .01); relationship persisted after adjusting for age, sex, BMIZ, elevated BP, and hypercholesterolemia (RR, 1.43; P =0.02). PHIV, CD4 count, viral load and current ART class were not associated with ED after adjustment.	PHIV appear to have increased risk of ED. These findings have important implications as HIV has increased risk of premature CVD and complications.
Soluble CD14 (sCD14) is associated with endothelial dysfunction in South African youth on ART. Dirajlal-Fargo et al (43)	2020	Case control study	n=283 perinatally acquired HIV (PHIV), 9-14 years.	n=69 age-matched without HIV	PHIVs had lower RHI despite viral suppression (RHI=1.36 vs 1.52, p<0.01). sCD14 at 24 months correlated with ED (p≤0.04). PHIV with ED, sCD14 was associated with lower RHI (β-0.05, p=0.01).	Higher sCD14 is independently associated with ED in PHIVs.

Table 5: Endo-PAT 2000 in paediatric patients with other miscellaneous paediatric conditions (8 studies). Reactive hyperemia index (RHI), augmentation index (AIx) (vascular stiffness), endothelial dysfunction (ED), inflammatory bowel disease (IBD), acute lymphoid leukaemia (ALL), nitric oxide (NO), perinatally acquired human immunodeficiency virus (PHIV), In Vitro Fertilization (IVF), soluable CD 14 (sCD14).

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.

Several factors may impact microvascular function. For example, puberty, which is of particular interest in our paediatric review. Bhangoo et al report improved RHI in correlation with an increase in Tanner stages and postulated that this may be due to sex steroids(44).

Strengths of the paper include a comprehensive literature search including contacting authors by email for separate paediatric results in studies with combined adult and paediatric data. All study types were reviewed and even the studies without results but had interesting points were included in our discussion. Also, we do not think that this paediatric Endo-PAT review has been done before.

Weaknesses of the paper include 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 date were included.

Endothelial dysfunction in paediatric type 1 diabetes mellitus patients:

Table 1 presents 5 studies in T1D patients. 3/5 studies reported RHI changes in the T1D group. 1/5 studies included only adolescent and reported that RHI negatively correlates with impaired metabolic control and subclinical signs of autonomic neuropathy. However, regular physical activity is protective(18). Suggesting that good metabolic control (HbA1c ≤7.5%) and regular physical activity might be protective against ED.

1/5 studies report the positive association between ED parameters and alpha-lipoic acid administration and antioxidant diet(19). 1/5 reported RHI values after metformin use and this may improve endothelial function in overweight T1D males(20).

Barber et al report suboptimal glycaemic control as evidenced by a rising HbA1c causes early atherosclerosis(21). 1/5 studies noted an improvement in RHI post vitamin D supplementation in T1D patients with vitamin D deficiency(22).

Endothelial dysfunction and Metabolic Syndrome:

24 studies (*Supplementary Table 1*) describe the use of Endo-PAT 2000 for metabolic syndrome. These studies included measurement of the following parameters: BMI, T1D, T2D, gender, pubertal stage, age, polycystic ovary syndrome, blood pressure values, non-alcoholic fatty liver disease,

obstructive sleep apnoea, lipid profile, insulin, plasma glucose levels, inflammatory markers (urinary markers, CNP, micro-RNA-126, E-Selectin).

19 studies focused on obesity/overweight paediatric patients. In numerous studies, RHI was significantly lower in the obese group(45-50). ED may mediate the link between obesity-related insulin resistance and early microalbuminuria(51). Exercise and diet control improves glycolipid metabolism. Noma et al (2017) report the beneficial effects of exercise in paediatric patients and is an important message in reducing future endothelial complications(52).

6 studies focused on patients with impaired glucose tolerance or T2D. Studies also compared RHI in T1D and T2D patients. Kochummen *et al* (2019) mean RHI in obese adolescents without diabetes was similar to T1D and T2D patients(45). Tomsa et al (2016) RHI was higher if HbA1c was less than 5.5%, and was lower in T2D obese(53).

Lower insulin sensitivity in these patients poses a risk of diabetic nephropathy(6). Microangiopathic renal damage increases oxygen consumption and increases resistance in the afferent arterioles. Shah et al (2017) report T2D patients have greater vascular thickness and stiffness and worse endothelial function compared to obese and lean children(54). This is raising concern that adolescents with T2D are already at risk of developing early onset cardiovascular disease.

Berardinelli-Seip syndrome is a rare condition characterized by severe insulin resistance and absence of subcutaneous fat since birth or early childhood. Lipids can deposit in muscle, liver and arterial walls; explaining its clinical complications of diabetes, hepatic injury, hyperlipidaemia and premature atherosclerosis. Fernandes et al (2015) used Endo-PAT 2000 in a cohort with this syndrome(55). 50% had ED (natural logarithm RHI index of 0.49±0.15). Their results support the risk of ED in this cohort and highlights the necessity of early intervention to avoid cardiovascular complications.

Endothelial dysfunction in cardiac and vascular conditions:

Dietz et al (2015) systematic review and metanalysis on peripheral ED in Kawasaki disease, report that patients with coronary arterial aneurysms had higher surrogate markers for cardiovascular disease risk(56). This may indicate these patients should be monitored for CVD in adulthood, however

significant heterogeneity was reported. Two studies include ED in patients with systemic lupus erythematosus (SLE) and Henoch Schonlein purpura (HSP).

Goldstein et al (2016) by using Endo-PAT identified multiple patient and procedural factors for Fontan survivors(57). Some determinants of RHI included prior Norwood procedure, systolic blood pressure, resting heart rate and oxygen saturation. Targeted intervention of modifiable risk factors may improve long-term vascular health and functional status in Fontan survivors. Further research by Goldstein et al (2015) noted increased arterial stiffness and decreased endothelial function are associated with lower aerobic capacity, quality of life (QOL) and physical activity in adolescent and young adult Fontan survivors(58).

'The LOVE-COARCT study' (Long-term Outcomes and Vascular Evaluation After Successful Coarctation of the Aorta Treatment) compares vascular function in patients with coarctation of the aorta treated with surgery, balloon dilation or stenting. Endothelial function was similar among groups(59).

Negishi et al (2016) used Endo-PAT to compare Fontan survivors and healthy controls. The Fontan patients were aged 15 to 32 years. Mean RHI 0.56+ /- 0.26 in Fontan patients and 0.78+ /- 0.31 in controls (p= 0.09). RHI in Fontan patients was associated with diastolic blood pressure, heart rate and haemoglobin A1c level(60). Endothelial function in Fontan patients was associated with abnormal glucose tolerance and arterial stiffness and therefore concluded that glucose regulation might be a potential target to improve ED in this cohort.

Adult patients with repaired coarctation of aorta have a high risk of late HTN. Nozaki et al (2018) assessed ED in conduit and resistance arteries and used FMD and Endo-PAT in paediatric patients with repaired coarctation of aorta(61).

Endothelial dysfunction in respiratory conditions:

Augusto et al noted an increased augmentation index without changes in LnRHI in asthmatic patients; indicating early detection of vascular impairment may precede ED, and different mechanisms may contribute to the pathogenesis and progression of cardiovascular events in this population(28).

1/4 reported an improvement in sleep disordered breathing post weight loss(29). Also, endothelial function significantly improves after weight loss. 2/4 studies report children with OSA compared to habitual snorers are at increased risk for ED(30, 31). Frequent wakening due to obstructive respiratory events may be a risk factor for ED in OSA.

Endothelial dysfunction and psychological conditions:

Potential limitations in this area are self-reported methods for detecting psychological distress of children, for example in the LOOK longitudinal study(32). Naiberg et al (2017) utilised retinal vascular photography, a proxy for cerebral microvasculature, and Endo-PAT to assess cardiovascular and neurocognitive burden in bipolar disorder (BD) adolescents (mean age 16-17 years)(34). In the BD group, better endothelial function was associated with higher arterio-venular ratio (r=0.375, p=0.041). Retinal vascular calibre was significantly associated with endothelial function in BD and it has been suggested that it may be used as an assessment tool in this cohort.

Olive L.S. (2017) published 'The emerging field of paediatric psycho-cardiology' highlighting the importance of the childhood origins of adult CVD(62). This article highlights that psychological distress can influence CVD risk, directly by physiological change that can negatively impact the integrity of the cardiovascular system.

Conclusion:

There are a number of papers in the paediatric literature describing ED at young ages using Endo-PAT. It should be concerning to paediatricians that children with various chronic diseases have evidence of ED. However, in many cases, there has only been a single cohort study using Endo-PAT. Further studies are required to validate these findings. Additionally, longitudinal studies are required to evaluate how this ED may change as the child ages and their chronic conditions changes. Further studies are also required that will characterise more completely the cardiovascular risk profile of these children with chronic disease. Consensus on other vascular risk markers that could be included in future studies is ideal and if accomplished, this would facilitate meta-analyses of studies of conditions with relatively rare conditions. Paediatricians should start to include an approach to cardiovascular

risk assessments in their assessments of young children and adolescents, including but not limited to those with chronic diseases.

Statements and declarations:

a. Authorship contributions:

All authors contributed to the initial search strategy protocol. I deLaunois performed the online database search. J Hayden and G McDonnell separately performed a blind screen of the abstracts and analysed the papers. G McDonnell contacted the authors of joint adult and paediatric papers to obtain separate paediatric data. J Hayden wrote the initial manuscript which was revised by C O'Gorman. All authors reviewed the manuscript prior to submission.

b. There are no competing interests to declare.

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d. Data sharing: search technique and data analysis are available from Rayyan software and the corresponding author.

e. Competing Interest: None declared

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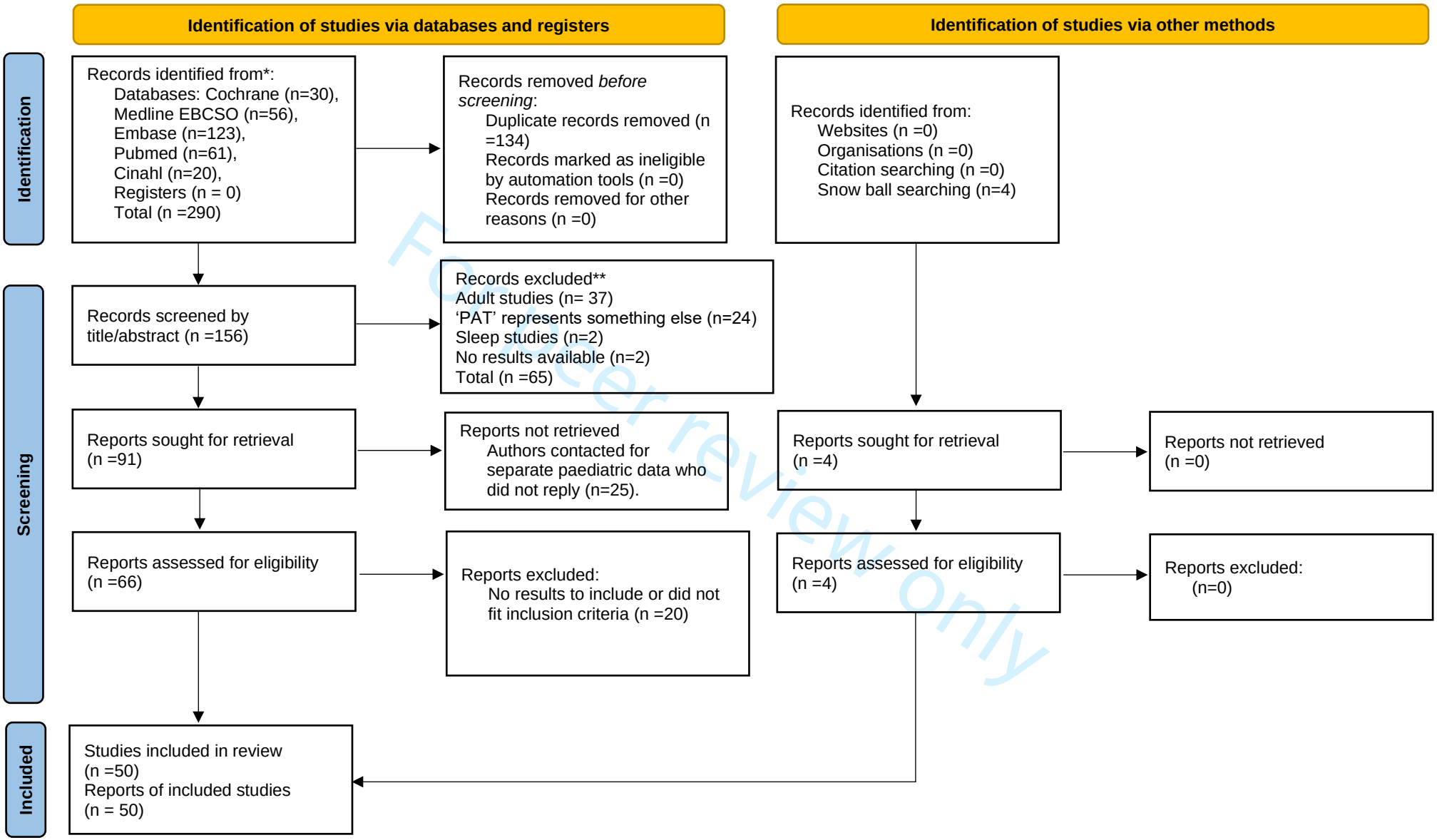
Figure 1: PRISMA 2020 Flow diagram of systematic search for Endo-PAT 2000 in paediatric populations.

PRISMA 2020 flow diagram for new systematic reviews which included searches of databases, registers and other sources



*Consider, if feasible to do so, reporting the number of records identified from each database or register searched (rather than the total number across all databases/registers).
**If automation tools were used, indicate how many records were excluded by a human and how many were excluded by automation tools.

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71. For more information, visit: http://www.prisma-statement.org/

Supplemental material:

Endothelial dysfunction and other paediatric conditions

Childhood cancer survivors:

Chemotherapy causes cardiomyocyte damage and also negatively affects endothelial function. Broberg et al (2018) utilised Endo-PAT 2000 in childhood cancer survivors and noted a lower RHI in this cohort compared to controls(1). Brouwer et al (2013) studied cancer survivor patients after potential cardiovascular toxic treatment (e.g. anthracyclines, platinum) and/or radiotherapy and noted a higher risk of ED compared with sibling controls(2). Broberg et al (2016) identified one-third of cancer survivors (31.2%) compared to 8% of controls (p= 0.02) had ED in their study(3). They concluded this may be a useful screening tool of cardiovascular disease in asymptomatic cancer survivor patients.

Pao et al (2018) assessed the relationship between blood pressure and ED using Endo-PAT 2000 in haematopoietic stem cell transplant recipients. Hypertension on ambulatory blood pressure monitoring (p= 0.045) and blunted nocturnal dipping (p= 0.04) were associated with a lower Endo-PAT scores(4). Jehlicka et al (2011) used Endo-PAT and noted ALL patients had lower RHI compared to controls (1.57±0.50, 1.96±0.63; p≤0.05)(5).

Autoimmune conditions:

Children with autoimmune diseases may have a high tendency to develop ED which was highlighted in a study using a novel technique(6). Atherosclerosis is an emerging cause of morbidity and mortality in patients with rheumatological conditions such as juvenile idiopathic arthritis, systemic lupus erythematous (SLE), dermatomyositis, etc. Borenstein-Levin et al assessed a cohort with autoimmune conditions compared to controls and 29% in the study group had ED compared to 6% in the control group (p <0.05)(6).

In SLE patients, ED may occur from impaired clearance of apoptotic cells, oxidative stress, B cell activation with different circulating autoantibodies, etc(7). Regular ED assessment in SLE patients has been recommended due to risk of subclinical atherosclerosis(7).

Metabolic diseases:

Yano et al research in Fabry disease patients demonstrated that early diagnosis of ED can help determine the timing of initiating enzyme replacement therapy(8). Utilizing RH-PAT as a screening tool for early renal involvement may be helpful as it may detect abnormalities even prior to microalbuminuria(9). This can provide guidance on enzyme replacement therapy which is required to prevent irreversible progressive renal failure.

Al Jasmi et al research in mitochondrial diseases reported that arginine or citrulline supplementation may improve ED, which provides evidence that these amino acids may be therapeutic(10).

Turner syndrome:

Turner syndrome (TS) patients have increased cardiovascular risk factors which predispose to cardiac and cerebrovascular complications. A literature review concluded that TS have unfavourable cardiometabolic risk factors which predispose them to adverse cardiac and cerebrovascular outcomes in young adulthood(11). It is unclear whether this is secondary to the syndrome itself or from modifiable risk factors such as obesity, hypertension, etc. Moreover, congenital heart disease is a clinical feature in 30% of cases of TS patients. There is a huge emphasis on the importance of regular screening in this cohort and also further research into whether any variables could potentially be altered to reduce the atherosclerosis risk in adulthood.

O'Gorman et al (2012) published a case-control study on TS patients(12). This paper excluded any with structural congenital heart disease. Lower RHI scores in TS compared with controls 1.64 (0.34) vs 2.08 (0.32) ($P<0.005$). Growth hormone may protect endothelial function in TS patients as GH-untreated RHI 1.44 (0.26) versus GH-treated 1.86 (0.28) (p $P<0.05$).

Inflammatory bowel disease:

One study in our review (*Table 6*) highlights that IBD patients had lower RHI compared with controls(13). Petr et al (2014) provided evidence of increased ED in children with Crohn's disease compared to healthy controls(14). RHI values were significantly lower in the patients with Crohn's than controls ($p< 0.05$).

Infectious diseases:

Dirajlal-Fargo et al (2017) used Endo-PAT 2000 to assess ED in perinatally acquired HIV patients. Perinatally acquired HIV patients appear to have higher levels of ED (lower RHI 1.34 (1.20, 1.42) compared with control group 1.52 (1.27, 1.80) ($p<0.01$))(15).

The pathogenesis of severe Plasmodium vivax malaria is poorly understood. ED and reduced nitric oxide (NO) bioavailability characterize severe falciparum malaria. Barber et al (2016) identified that endothelial function was impaired in proportion to disease severity. Those with severe vivax malaria, non-severe and healthy controls median RH-PAT index 1.49, 1.73, and 1.97 respectively ($p=0.018$)(16). ED in this cohort was associated with reduced L-arginine bioavailability, which may contribute to microvascular pathogenesis.

Haematological conditions:

Sivamurthy et al (2009) reported lower RHI in the majority sickle cell disease in a paediatric population (1.53 and 1.71; p value .032). RHI was not normal in children with chronic transfusions or hydroxyurea(17).

Very low birthweight babies:

Harris et al (2020) assessed cardiovascular outcomes for those born with very low birth weights (VLBW) <1500g. The VLBW cohort (n = 229; 71% of survivors) and term-born controls (n = 100), were assessed at age 26-30 years. The VLBW cohort had lower RHI compared to controls(18).

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Title, lead author	Year	Study design	Population: n=sample size, age; mean ± SD or median (range), [F/M]	Control group: n=sample size, age; mean ± SD or median (range), [F/M]	Results: RHI reported. If RHI not specified, we reported p/r values	Outcomes
Assessment of Microvascular Function in Children and Adolescents with Diabetes and Obesity. Kochummen et al(1)	2019	Cross-sectional study	DM group. n=33 T1D with normal weight. n=8 obese T2D, age 12.7 (3.8) years, [F/M 25/16]	n=17 obese, non-DM children (normal BGL, BP and lipid profile), 12.8 (2.7) years, [F/M 9/8]	For every 1% increase in HbA1C, RHI decreased by 0.097 (P = 0.01). RHI of DM group with HbA1C <10% (1.70 ± 0.58) versus those with ≥10% (1.21 ± 0.19) (p= 0.02).	Poorly-controlled DM (HbA1C ≥ 10%) had lower RHI. RHI negatively related with HbA1C. RHI similar between obese and normal weight with T1D. Similar between T1D and T2D.
Effects of a dietary strawberry powder on parameters of vascular health in adolescent males. Djurica et al (2)	2016	Randomised, double-blind, cross-over study	n=15 overweight /obese males, 14-18 years (mean 16). 1 week of 50 g of a freeze-dried strawberry powder (FDSP) or a control powder, daily. Before and after plasma nitrate/nitrite levels measured.	10 control powder, 14-18 years (mean 16).	Acute plasma nitrate/nitrite levels increased 1 h after consuming the FDSP (P<0.001). When nitrate levels increased after FDSP intake compared to controls, had an increase in RHI (P=0·014).	Strawberries can provide vascular health benefits to heavier adolescent males.
Non-alcoholic Fatty Liver Disease in Hispanic Youth with Dysglycemia: Risk for Subclinical Atherosclerosis? Bacha et al (3)	2017	Cross-sectional study	n=23 overweight/ obese with NAFLD, age 15.3 ± 0.4 years. n=20 prediabetes, n=16 T2D, [F/M 12/11]	n=13 overweight/ obese without NAFLD, age 15.3 ± 0.4 years, with pre-diabetes or T2D, [F/M 3/10]	NAFLD group had lower RHI (1.4 ± 0.05 vs 1.7 ± 0.09, p= 0.002), greater AIx (-6.0 ± 1.6 vs -12.0 ± 2.1, P = 0.03). Hepatic fat inversely related to RHI (r = -0.49, P = 0.002) and positively related to AIx (r = 0.45, P = 0.006).	Hepatic fat and AST/ALT levels were inversely related with RHI. If dysglycemia, NAFLD is associated with worse endothelial function.
Circulating fibroblast growth factor-21 (FGF-21): A biomarker of subclinical atherosclerosis in obese youth with non-alcoholic fatty liver disease (NAFLD)? Bacha et al (4)	2017	Conference abstract	Obese adolescents with NAFLD, 15.4+/-0.3 years. n=13 normal glucose tolerance, n=19 prediabetes, n=16 T2D patients	Control group: no NAFLD. No difference in age/gender between groups.	Lower RHI in NAFLD group and higher AIx-75. FGF-21 concentrations were related to RHI (r=-0.33, p=0.03) and AIx (r=0.45, p=0.02).	Circulating FGF-21 levels are elevated in obese youth with NAFLD and are associated with measures of insulin sensitivity and ED. FGF-21 may constitute a biomarker of higher risk for vascular dysfunction in these youth.
Flow-mediated dilation in obese adolescents: Correlation with waist circumference (WC) and systolic blood pressure	2018	Case control study	n=20 obese patients, median age 14 years	n=10 normal weight, median age 15 years, paired for gender	No difference in RHI between groups. 35% obese group had metabolic syndrome, none in control group. OSA in 86.6% obese and 50% of normal weight group.	Obese group had evidence of ED and metabolic syndrome. Increased WC and SBP seem to be involved in this finding.

(SBP). Hussid et al (5)						
Improvement of microvascular endothelial dysfunction induced by exercise and diet is associated with microRNA-126 in obese adolescents. Donghui et al (6)	2019	Quasi-randomized study	n=57 obese male adolescents, 12-18 (15.38 ± 2.82) years, [F/M = 0/57], 6-week exercise program with dietary intervention.	n=10 normal weight adolescents, 15.38 ± 2.82 years, maintained sedentary. Age 12-20, *n* = 10 [F/M 0/10]	Obese group 1.43 (0.35) vs controls 1.67 (0.36) ($p< 0.05$). After 6 weeks intervention RHI increased ($p <0.01$), while microRNA-126 significantly decreased ($p<0.01$). miRNA-126 positively correlated with ΔRHI ($r = 0.69$, $p<0.05$).	RHI higher in controls. RHI improved in obese group after exercise and diet interventions. Findings might be related to changes in serum miRNA-126.
Distribution of peripheral arterial stiffness and endothelial function as well as their correlations with cardiovascular risk factors in children and adolescents. Mu et al (7)	2016	Cross-sectional population-based study, conference abstract	n=94 obese, 7-17 years, used automatic waveform analyser (BP-203RPE-I) and Endo-PAT 2000.	n=452 normal-weight	In normal weight group, RHI increased with age ($r=0.33$, $P<0.01$; $r=0.36$, $P<0.01$). RHI positively correlated with BMI ($r=0.10$, $P=0.018$) but negatively correlated with DBP ($r=-0.10$, $P= 0.016$). RHI did not differ between genders.	Brachial-ankle pulse wave velocity (baPWV) and RHI increased along with age; arterial stiffness and endothelial function continued to develop in the normal weight group.
Urine Albumin-to-Creatinine Ratio (UACR): A Marker of Early Endothelial Dysfunction in Youth. Bartz et al(8)	2015	Control study	15.6 ± 0.2 years, n=25 overweight with normal glucose tolerance, [F/M 17/8]. n=20 overweight with prediabetes, [F/M 11/9].	n=13 normal weight, 16.3 ± 0.4, [F/M 7/6].	Fasting UACR was analysed. Normal weight group had higher RHI (1.84 ± 0.1, 1.56 ± 0.1, and 1.56 ± 0.1, $P = .04$). UACR was related to RHI ($r = -0.33$, $p = .01$).	UACR is an early marker of endothelial dysfunction in youth, independent of glycemia.
Urinary biomarkers as indicator of chronic inflammation and endothelial dysfunction in obese adolescents. Singh et al(9)	2017	Research article, control study	n=63 total. n=14 overweight (OW), n=29 obese, age 13.8 (2.4), [F/M 23/20]	n=20 normal weight (NW), age 13.9 (2), [F/M 8/12]	There were no differences in RHI levels among the study groups. NW 1.6 (0.1), OW 1.66 (0.1) and obese 1.67(0.1). NW girls RHI 1.9 vs NW boys 1.25.	No significant correlation between RHI and urinary markers. RHI higher in NW female adolescents.
Prevalence of Type D personality in obese adolescents and associated cardiovascular risk. Bruyndonckx et al(10)	2018	Control study, conference abstract	Obese adolescents-no definite numbers (conference abstract only)	Healthy normal weight children	Positive correlation in obese adolescents between negative affectivity and vascular stiffness ($r= 0.28$; $p= .04$)	Obese adolescents have worse cardiovascular risk profile with ED.
Endothelial function and arterial stiffness in obese adolescents - A relation to barorefex function.	2017	Conference abstract	n=22 obese, 15.28 +/- 2.8 years, [F/M 10/12]	n=22 non-obese, 15.98 +/- 2.46 years, [F/M 10/12]	No significant difference in RHI ($p = 0.473$). Baro-reflex sensitivity was also calculated.	No difference in RHI between groups. Findings require further study.

Czippelova et al(11)						
Obesity in children and adolescents: A relation to endothelial function and arterial stiffness. Czippelova et al(12)	2016	Conference abstract	n=16 obese adolescents,15.22 +/- 2.2 years, [F/M 7/9]	n=16 non-obese, 16.22 +/- 1.5 years, [F/M 7/9]	Significant difference in RHI (p = 0.018) with RHI higher in obese group (1.66 +/- 0.28 vrs 1.4 +/- 0.25).	Less early atherosclerotic changes in obese group which was in contrast to expectations: requires further study.
Endothelial function in youth: A Biomarker modulated by adiposity-related insulin resistance. Tomsa et al (13)	2016	Cross sectional study	Total n = 60. n=25 obese without DM, n=19 obese with impaired glucose tolerance, n=16 obese T2D but HB1Ac < 8%. Age 15.5 (0.2), [F/M 37/23]	n=21 normal weight, age 15.5 (0.2), [F/M 9/12]	RHI inversely related to % body fat (r = -0.29, P = .008), total (r = -0.37, P = .004), subcutaneous (r = -0.39, P = .003), and visceral abdominal fat (r = -0.26, P = .04). AIx at heart rate 75 bpm was higher (worse) in the lower RHI groups (P = .04).	Childhood obesity is associated with ED: lower RHI and higher AIx. RHI lower in obese and T2D. RHI negatively related with percentage body fat, WC, Leptin, TNF-alpha, blood glucose.
Free Vitamin D: Relationship to Insulin Sensitivity and Vascular Health in Youth. Bacha et al (14)	2019	Cross-sectional study	n=79, age 15.4 ± 0.2 years, [F/M 45/34]. n=30 overweight. n=31 overweight with prediabetes	n=18 normal weight and normal glucose tolerance. Across tertiles of free 25(OH)D concentrations	The lowest tertile group had lower RHI (1.42 ± 0.06, 1.54 ± 0.06, and 1.77 ± 0.09, P = 0.002), compared with the second and third tertiles.	Youth with low free 25(OH)D or BioD concentrations have lower insulin sensitivity and worse endothelial function.
Preclinical vascular alterations in obese adolescents detected by Laser-Doppler Flowmetry technique. Fusco et al (15)	2020	Research article	n=22 obese adolescents, 14.11 +/-2.53, [F/M 13/9]	n=24 normal-weight, 15.2 +/- 1.56, [F/M 11/13]	Similar RHI between obese and non-obese groups (1.80 +/- 0.62 and 1.86 +/- 0.51).	RHI not different between groups. RHI did not correlate with LDF (that is impaired in obese).
Impaired endothelial function in adolescents with overweight or obesity measured by peripheral artery tonometry. Pareyn et al (16)	2015	Cross sectional study	n=27 overweight or obesity, 14.7 (13.0–16.4) years, [F/M 11/16]	n=25 normal weight controls, 15.5 (13.9–16.2) years, [F/M 13/12]	RHI normal weight 1.88 (1.7-2.4) vs OW/obese 1.5 (1.3-1.9) (P< 0.05). Lower RHI if OW/obese, and higher baseline pulse amplitude (p = 0.027 and p < 0.0001). Significant positive correlation between baseline pulse amplitude and BMI in OW/obese group. RHI positively correlated with age and tanner stage (P< 0.05). RHI negatively correlated with DBP (P< 0.05).	ED measured by lower RH-PAT score and higher baseline pulse amplitude, was present in OW group. First time in the literature to report significant difference in baseline pulse amplitude between OW adolescents compared to their peers.

C-type natriuretic peptide (CNP) plasma levels and whole blood mRNA expression show different trends in adolescents with different degree of endothelial dysfunction. Del Ry et al(17)	2020	Research article -snow balling	n=16 primary obesity, not DM, age 13.3 (0.5) years, [F/M 8/8].	n=24 normal weight, age 14.3 (0.4) years, [F/M 14/10].	RHI normal weight 2.1 (0) vs obese 1.4 (0) (P< 0.005). RHI negatively associated with CNP and diastolic BP (P< 0.005).	RHI significantly lower in obese group. RHI negatively related with CNP, DBP, fat mass and HbA1C.
C-type natriuretic peptide (CNP) is closely associated to obesity in Caucasian adolescents. Del Ry et al (17)	2016	Research article -snow balling	n=10 overweight, age 12.8 (1.6) years, [F/M 5/5]. n=45 obese, 12.8 (1.6) years, [F/M 19/26]	n=27 normal weight, age 12.8 (1.4) years, [F/M 14/13]	Normal weight group RHI 2.1 (0.2) vs OW 1.6 (0.4) (P< 0.05). Normal weight vs obese group RHI 1.4 (0.3) (P< 0.005). RHI negatively associated with CNP (P< 0.005).	RHI significantly lower in overweight/obese groups. CNP negatively related with RHI.
Arterial Stiffness and Endothelial Function in Young Obese Patients - Vascular Resistance Matters. Czippelova et al (18)	2019	Research article	Author contacted for separate paeds data. n=16 obese group, age <16 years, [F/M 7/9]	n=15 controls, age <16 years, [F/M 7/8]	RHI control vrs obese groups: 1.320 ± 0.427 and 1.457 ± 0.280. RHI obese girls and boys: 1.410 ± 0.253 and 1.494 ± 0.308. RHI control girls and boys: 1.171 ± 0.210 and 1.436 ± 0.524	RHI was influenced by vascular tone and resistance. RHI in obese positively related with SVR. RHI is influenced by vascular tone.
Physiological changes in blood pressure (BP) impact peripheral endothelial function during adolescence. Deda et al (19)	2015	Control study	n =90 healthy adolescents to assess normal RHI response, 14.2±1.91 years, [F/M 46/44].	No controls. Assessing association between RHI and known cardiovascular risk factors.	Mean arterial pressure significantly associated with RHI (p=0.01). Positive correlation between RHI and age in the females (r=0.33, p<0.02). RHI correlated with pubertal status in males (r=0.411, p=0.03) and females (r=0.36, p =0.03).	Physiological changes in BP significantly impact PAT results.
Role of insulin resistance and hyperandrogenemia in early vascular dysfunction in adolescents with PCOS. Bartz et al (20)	2015	Conference abstract	14 adolescents with PCOS (on no treatment).	7 non-PCOS. Both groups had similar age, tanner stage, race, glucose tolerance status, BMI (34.1 +/- 1.1 vs. 30.4 +/- 1.6 kg/m^2).	Despite higher peripheral and hepatic insulin resistance in adolescents with PCOS, RHI is not significantly lower when compared with controls of similar total body and abdominal adiposity.	PCOS has evidence of increased vascular inflammation. Hyperandrogenemia, as well as insulin resistance, may play an important role in modulating vascular inflammation.
Cardiovascular adaptations after 10 months of intense school-based physical training for 8- to	2018	Randomised control study	n=93 small-sided games group, 9.3+/-0.4 years. n=83 circuit strength training group, 9.3+/-0.3	n = 115 controls, 9.3+/-0.3 years	No significant differences in RHI. Pubertal status is a main predictor of RHI; positive correlation	10 months of 3 × 40 minutes/week decreased DBP and elicited discrete cardiac adaptations,

10-year-old children. Larsen et al (21)			years (10-16 years)		between Tanner stages and RHI.	suggesting intense exercise classes can have effects on cardiovascular health.
Endothelial Function in Children and Adolescents Is Mainly Influenced by Age, Sex and Physical Activity-An Analysis of Reactive Hyperemic Peripheral Artery Tonometry. Mueller et al (22)	2017	Randomised controlled study, Leipzig School Project followed over 5-year period.	n=931 RHI measurements in 445 students, age 10-17 years (baseline 11.66±0.93). n=247: 60 minutes physical exercise (PE) daily (intervention group).	n=181: 2 units of 45 minutes PE weekly (control group).	Main influential factors were age, gender and PE. RHI was higher in the intervention group by 0.09 [-0.05, 0.23]. Increase RHI from 1.53±0.42 in the youngest to 1.96±0.59 in the oldest students. This increase adjusted by age and sex was estimated as 0.11 [0.08, 0.14] per year.	If RH-PAT is used in research in adolescents, the shown age- and sex-dependence of RHI have to be taken in account.
Reactive hyperaemia index and detection of endothelial dysfunction in children with familial hypercholesterolaemia (FH). Jehlicka et al(23)	2015	Conference abstract	n=24 with FH, 13.9+/-2 years. Biochemical markers of endothelial function were assessed.	n=17 healthy controls, 15.2+/-2.2 years	Significantly lower RHI in FH group (1.63+/-0.50 and 2.03+/-0.54; $p<0.05$). Lower RHI and elevated E-selectin in children with FH.	Possible relationship of ED in children with FH, highlighting the importance of early detection of ED when the atherosclerotic process is still reversible.

Supplementary Table 1: Endo-PAT 2000 in paediatric patients with metabolic syndrome (24 studies) including obesity, type 2 diabetes (T2D) and hypercholesterolemia. Reactive hyperemia index (RHI), augmentation index (AIx) (vascular stiffness), freeze-dried strawberry powder (FDSP), non-alcoholic fatty liver disease (NAFLD), endothelial dysfunction (ED), overweight (OW), familial hypercholesterolaemia (FH), waist circumference (WC), C-type natriuretic peptide (CNP), Laser-Doppler Flowmetry (LDF).

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PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			Page
Title	1	Identify the report as a systematic review.	2
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	2
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	2
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	6
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	5
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	6
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	6
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	6
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	6
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	6
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	6
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	Tables
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	5
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	5
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	7
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	7
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	N/A
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	N/A
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	6
Certainty	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	6

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
assessment			
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Flow diagram
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	6
Study characteristics	17	Cite each included study and present its characteristics.	Tables 1
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Nil
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Tables 1-6
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	N/A
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	N/A
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	N/A
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	N/A
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	N/A
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	N/A
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	16
	23b	Discuss any limitations of the evidence included in the review.	17
	23c	Discuss any limitations of the review processes used.	17
	23d	Discuss implications of the results for practice, policy, and future research.	20
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	No response
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	Prospero – no response
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	N/A
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	Nil
Competing interests	26	Declare any competing interests of review authors.	Nil
Availability of data, code and	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	With authors

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
other materials			

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71
For more information, visit: http://www.prisma-statement.org/

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SEARCH PLANNING FORM

Use this form to identify/clarify the key concepts and the scope of your research topic

DATE RECEIVED: 08/02/2021 DATE SEARCH COMPLETED: 03/03/2021

YOUR RESEARCH TOPIC

Paediatric arterial tonometry on children with any condition or healthy children.

PICO AND SEARCH TERMS

Patient/Population and/or problem	Exposure	Outcomes	Study type	Search limits
Under 16's	**Peripheral Arterial tonometry**		**All studies**	**Under 16 only** **From 2015 to current**

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SOURCES TO SEARCH

Resources	Number of results	Date searched
Cochrane	30	03/03/2021
Embase	123	03/03/2021
Medline EBCSO	56	03/03/2021
Pubmed	61	03/03/2021
Cinahl	20	03/03/2021
Total	**290**	
Total after deduplication	**158**	

SEARCH STRATEGY

Population	exp adolescent/ or exp child/ OR exp pediatrics/ OR (ado* or child* or preschool* or schoolboy* or schoolgirl* or boy or boys or girl or girls or teen* or toddler*).ab,ti,tw. OR (infant or infants or baby or babies or "pre-school").ab,ti,tw. OR (pediatric* or paediatric*).ab,ti,tw.
Intervention	exp *peripheral arterial tonometry/ OR (peripheral adj3 tonometry).ab,ti,tw. OR exp peripheral arterial tonometry/ OR (PAT adj2 (analys* or test*)).ab,ti,tw. OR endopat.ab,ti,tw.
Outcomes	
Date filter	2015 to current

BMJ Open

Endo Peripheral Arterial Tonometry (Endo-PAT 2000) use in Paediatric Patients – a systematic review.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2022-062098.R1
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Date Submitted by the Author:	26-Aug-2022
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Primary Subject Heading:	Paediatrics
Secondary Subject Heading:	Diabetes and endocrinology, Evidence based practice, Nutrition and metabolism, Sports and exercise medicine, Medical management
Keywords:	PAEDIATRICS, Community child health < PAEDIATRICS, Paediatric endocrinology < PAEDIATRICS, EDUCATION & TRAINING (see Medical Education & Training), Change management < HEALTH SERVICES ADMINISTRATION & MANAGEMENT, Organisational development < HEALTH SERVICES ADMINISTRATION & MANAGEMENT

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Title page

Endo Peripheral Arterial Tonometry (Endo-PAT 2000) use in Paediatric Patients – a systematic review.

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Key words: Endo-PAT 2000, peripheral artery tonometry, Endothelial dysfunction, paediatric diabetes mellitus, chronic diseases

Word count: 3881

Abstract:

Objectives: Endo Peripheral Artery Tonometry (EndoPAT-2000) is a non-invasive technology for measuring endothelial dysfunction (ED). The reactive hyperaemia index (RHI) is resulted and is low when ED is present. We aim to synthesise the literature on paediatric ED that utilised Endo-PAT analysis.

Design:

A comprehensive systematic review was conducted from January 2015 to March 2021. The databases included Cochrane, MEDLINE EBSCO, EMBASE (Ovid), PUBMED and CINAHL EBSCO. Exclusion criteria were: 1. If a study used a different device for example. 2. If the study had no results. Inclusion criteria were: 1. Published in the English; 2. More than 50% of study subjects were in the paediatric age range; 3. Data relevant to paediatric age range children could be extrapolated from all data, where not all study subjects were children.

Results:

Following the removal of duplicates, 156 articles were initially identified . Following exclusion, 50 articles were included for review. We have subdivided these papers into different systems for ease of reference and have reported our findings in 6 tables: 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.

Conclusions:

A number of papers using Endo-PAT for children with various chronic diseases have evidence of ED. However, in many cases, there has only been a single cohort study using Endo-PAT. Further studies are required to validate these findings and to help characterise the cardiovascular risk profile of children with chronic disease. Further studies are also required that will characterise more completely the cardiovascular risk profile of these children.

Consensus on other vascular risk markers that could be included in future studies is ideal and if accomplished, this would facilitate meta-analyses of studies of relatively rare conditions.

Strengths and limitations:

- Comprehensive systematic review to synthesise the literature on endothelial dysfunction using Endo-PAT in paediatric patients..
- All study types were reviewed and even the studies without results but were relevant were included in our discussion.
- In many cases, there has only been a single cohort study using Endo-PAT for a particular disease
- Separate paediatric results were obtained where possible from studies with combined adult and paediatric data; however, some papers were of poor quality and had limited results available
- Only papers from January 2015 to March 2021 were included in our review.

Introduction:

Endothelial dysfunction (ED) is an early predictor of cardiovascular disease(1). ED occurs when the endothelium loses its ability to promote vasodilation, fibrinolysis and anti-aggregation(2). It can be caused by oxidative stress with loss of vaso-active or inflammatory homeostasis within the body's vascular system. It may be secondary to mechanical stimuli, for example increased intraluminal pressure within the blood vessel or metabolic factors such as hormones (oestrogen's vasodilation

action)(3). Damaged endothelium can release a cascade of substances which pose a risk of thrombosis, inflammation and ultimately atherosclerosis(4).

ED in paediatric populations has been associated with several conditions including type 1 diabetes (T1D), type 2 diabetes (T2D), renal impairment, obesity and metabolic syndrome(5-8). ED can progress to atherosclerosis which is a chronic condition that poses severe risk of certain diseases including coronary artery disease, stroke and peripheral arterial disease.

Improving glucose control can protect endothelial function. Persistent high sugars can impair endothelial function via oxidative stress and production of free-radicals(2). Diabetic microangiopathy can result in the outcomes of retinopathy, neuropathy and peripheral vascular neuropathy. Subclinical evidence of these complications can be seen in paediatric patients, especially in those with poor glycaemic control. In patients with T2D, obesity and metabolic syndrome, insulin resistance is one of the most importance factors contributing to ED(8). Metabolic syndrome is a pro-inflammatory state where dyslipidaemia, hyperuricemia, and hypertension occur and can predispose to ED(9). Unfortunately, there have been reports of T2D paediatric patients diagnosed with microangiopathic complications, particularly nephropathy(10). This early endothelial damage can be linked with increased morbidity and mortality(11). New onset diabetes after transplantation (NODAT) is characterised by insulin resistance and T2D(12).

In recent decades, the number of childhood cancer survivors is increasing(13). Treatments utilized such as haematopoietic stem cell transplantation have increased risk of cardiovascular disease(14, 15). Following chemotherapy, radiotherapy, immunosuppressive treatments the risk of insulin resistance has been noted(16). With advances in treating malignant paediatric conditions there are long term complications emerging in survivors. High dose chemotherapy including anthracyclines, alkylating agents and vinca alkaloids may disrupt the substances on the surface of the endothelium and impair its ability to dilate and constrict. Moreover, total body radiation poses a risk by damaging the elastic matrix. Heart disease in long-term cancer survivors is 5-10 times higher than their siblings(16).

Endo-PAT 2000:

Endo Peripheral Artery Tonometry (Endo-PAT 2000) is a non-invasive technology for measuring ED developed by Itamar Ltd. Non-invasive pneumatic probes which are placed on the both index fingers, which continuously records pulse wave amplitude. A blood pressure cuff is inflated to occlude blood flow and response after deflation is recorded. The reactive hyperaemic index (RHI) is resulted following this mini-ischemic stress to the vessel. The pulse wave amplitude (PWA) is measured and computes a RHI result automatically. RHI is calculated as the ratio of average PWA divided by the average amplitude during the equilibration period. To compensate for any systemic changes, this ratio is normalized to a concurrent signal from the contralateral finger.

Numerous studies in both adult and paediatric literature reveal Endo-PAT's excellent reproducibility and reliability(17, 18). In ED, the RHI is low and pulse amplitude is high. PAT also provides results on the peripheral augmentation index (PAT-AIx). Bonetti et al report a RHI of <1.35 as indicative of coronary ED in adults(19). However, there is no reported RHI cut off value in paediatric patients. Endo-PAT can be used at the patient's bedside, without extensive training required of the operator.

Prior to Endo-PAT, ED had been assessed by flow-mediated vasodilation (FMD). 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 cuff and is highly dependent on nitric oxide (NO) bioavailability. ED is identified by less vasodilatation (reduced FMD) of the brachial artery. FMD is technically challenging to perform, user-dependent and requires training. FMD results macro blood vessel reactivity whereas Endo-PAT results micro, which may account for the challenges in comparing the two techniques. Endo-PAT is easier to set up, is automated and less user-dependent. Wilk et al reported that RHI correlated with FMD ($r = 0.35$, $P < 0.01$)(20) however there are other studies who have not reported a correlation between the two techniques.

Objective:

The aim of this study is to conduct a systematic review to synthesise the literature on the use of Endo-PAT 2000 in paediatric populations in assessing the risk of ED in chronic diseases.

Methods:

A comprehensive systematic review was conducted to identify publications that investigated Endo-PAT 2000. All papers published from January 2015 to March 2021 in paediatric populations age birth to 16 years of age were analysed. PRIMSA study design was used.

The following scientific databases were searched: The Cochrane Database, MEDLINE EBSCO, EMBASE (Ovid), PUBMED and CINAHL EBSCO. The search was limited by to English studies. The search was limited by type of subjects (human), date (2015 to March 2021) and included all study types. Snowballing method was used. Authors of joint adult and paediatric papers were contacted by email to obtain separate paediatric data.

The database search was repeated several times using the combinations of keywords, MeSH terms and filters (child: birth-16 years). The following MeSH terms or key words were used for searching: Peripheral arterial tonometry, PAT test, endopat, adolescent, ado*, child, paediatric, pediatric, preschool, schoolboy, schoolgirl, boy, girl, teen, toddler, infant, baby.

Exclusion criteria were: 1. If a study used a different device for example 'Watch-PAT;' 2. If the study had no results. Inclusion criteria were: 1. Published in the English; 2. More than 50% of study subjects were in the paediatric age range; 3. Data relevant to paediatric age range children could be extrapolated from all data, where not all study subjects were children. A child was defined as up to 16 years, and this is consistent with PubMed's definition of a child. Where data relevant to children could not be extrapolated from the whole dataset, the study authors were contacted for additional information prior to study inclusion or exclusion.

Patient and public involvement:

No patient involved.

Data collection and analysis:

A total of 290 articles were obtained via the online database search (*Figure 1*: flow diagram). Following removal of duplicates, 158 articles remained. The second screening was conducted by 'Rayyan- systematic review software.' Two further duplicate articles were removed, with 156 remaining for review.

Two independent authors separately performed a blind screen on the 156 abstracts. 65 articles were initially excluded based on title or abstract: 37 adult studies, 18 'PAT' did not represent peripheral arterial tonometry (e.g. prism adaptation test, psychosocial assessment tool), 6 Watch-PAT, 2 sleep studies and 2 had no results available.

The remaining 91 articles were analysed viewing full text articles for further information. A further 20 were excluded as they did not fit inclusion criteria or have results to report. Some of these articles that included Endo-PAT 2000 in paediatrics did not have results for the systematic review but had conclusions that were relevant to the paper were referenced in the results section.

Twenty-eight authors of studies including both adults and paediatric patients were contacted twice by email to gather separate information on the paediatric participants. Twenty authors did not reply and were thus excluded. Eight authors replied: three providing results, four unable to give separate paediatric data and one author's research was on adult patients so was excluded. Three of the articles whose authors replied with data were included in our review. Four studies were obtained via snow balling searching.

A total of 50 articles were included in our results and are represented in tables 1-6. For each eligible study the following data was reported: author, year of publication, design of the study, population studied, control group (if available), RHI results.

Title, lead author	Year	Study design	Population: n=sample size, age; mean ± SD or median	Control group: n=sample size, age; mean ± SD or median	Results: RHI reported. If RHI not specified, we reported p/r values	Outcomes

			(range), [F/M]	**(range), [F/M]**		
Adolescents and young adults with type 1 diabetes display a high prevalence of endothelial dysfunction. Scaramuzza et al (21)	2015	Cohort prospective observational study. Results at baseline and after a 1-year follow-up	n=73 T1D adolescents, diagnosed > 1 year, 16.2 +/- 3.5 years, [F/M 25/48]	No controls.	56 (76.7%) had ED, with lower mean RHI scores (1.26 ± 0.22 versus 2.24 ± 0.48, p < 0.0001). More with ED had abnormal cardiac autonomic tests (p = 0.02) and were more sedentary. After 1 year follow-up in 64/73 patients, 81.8% had ED, despite some improvement in HbA1c.	T1D adolescents had evidence of ED. Good metabolic control (HbA1c ≤7.5%) and regular physical activity might be protective. ED progression despite some improvement to HbA1c.
Alpha-Lipoic Acid and Antioxidant Diet Help to Improve Endothelial Dysfunction in Adolescents with Type 1 Diabetes: A Pilot Trial. Scaramuzza et al (22)	2015	Double-blind, randomized controlled trial – snow balling. Results at baseline and after follow-up	n=71 T1D patients, followed for at least 1 year, age 16.3 ± 3.4 years, [F/M 29/42]. (a) antioxidant diet 10.000 ORAC + alpha-lipoic acid; (b) antioxidant diet 10.000 ORAC + placebo;	(c) controls .	3 double-blind study arms: (a) antioxidant diet 10,000 ORAC + lipoic acid: RHI 1.40 ± 0.68 vs 1.72 ± 0.66 (P<0.05) (baseline vs after 6 months). (b) antioxidant diet 10,000 ORAC + placebo: RHI 1.39 ± 0.41 vs 1.58 ± 0.40 (P>0.05) (c) Controls: RHI 1.58 ± 0.64 vs 1.54 ± 0.42 (P>0.05).	Improved RHI with alpha-lipoic acid in T1D patients.

Effect of metformin on endothelial function in overweight adolescents with type 1 diabetes (T1D). Nadeau et al(23)	2016	Conference abstract. Endo-PAT scores at baseline and 13 weeks.	Total n=70 overweight T1D patients. n= 41 on metformin (up to 2000 mg/day), 12-19 years (mean 15.8)	n=29 placebo group.	Mean baseline RHI 1.8 +/- 0.6 in metformin group and 1.7 +/- 0.6 placebo group. At 13 weeks, no significant change from baseline RHI (+0.1 in metformin vs. -0.0 in placebo, P = 0.08). There was some improvement in endothelial function among males.	No significant RHI change with metformin overall but some improvement in overweight T1D males.
Assessment of biomarkers of inflammation and premature atherosclerosis in adolescents with type-1 diabetes mellitus. Babar et al (24)	2019	Cross-sectional study	T1D adolescents ≥12 years. Two groups based on different HbA1c ranges. (a) HbA1c ≥9.5% (n=25)	(b) HbA1c ≤8.5% (n=27).	PAT results were not significantly different between the groups. Pearson correlation showed a significant direct relationship between rising HbA1c and PAT (p=0.03, r=0.31).	Suboptimal glycemic control (rising HbA1c) causes early atherosclerosis.
Improvements in peripheral vascular function with vitamin D treatment in deficient adolescents with type 1 diabetes. Deda et al **(25)**	2018	Research article – snow balling. Tested at two different time points.	n=21 T1D patients followed for ~2 years. 25-OH-Vit. D levels < 37.5 nmol/L. Age 15.7 ± 1.4 years, [F/M 19/12]	Controls: matched age, sex and T1D.	After 4.8 ± 1.3 months of Vit. D supplementation RHI improved: 1.83 ± 0.42 vs 2.02 ± 0.68 (P<0.05).	Vit. D supplementation associated with improvement to endothelial function and reduced urinary inflammatory

						markers.
Non-alcoholic Fatty Liver Disease in Hispanic Youth with Dysglycemia: Risk for Subclinical Atherosclerosis? Bacha et al (26)	2017	Cross-sectional study	n=23 overweight/ obese with NAFLD, age 15.2 ± 0.5 years. n=12 prediabetes, n=11 T2D, [F/M 13/10]	n=13 overweight/ obese without NAFLD, age 15.7 ± 0.4 years. n=8 pre-diabetes, n=5 T2D, [F/M 3/10]	NAFLD group had lower RHI (1.4 ± 0.05 vs 1.7 ± 0.09, p= 0.002), greater AIx (-6.0 ± 1.6 vs -12.0 ± 2.1, P = 0.03). Hepatic fat is inversely related to RHI (r = -0.49, P = 0.002) and positively related to AIx (r = 0.45, P = 0.006).	Hepatic fat and AST/ALT levels inversely related to RHI. If dysglycemia, NAFLD is associated with worse ED.
Endothelial function in youth: A Biomarker modulated by adiposity-related insulin resistance. Tomsa et al (27)	2016	Cross sectional study	Total n = 60. n=25 obese without DM, n=19 obese with impaired glucose tolerance, n=16 obese T2D but HB1Ac < 8%. Age 15.5 (0.2), [F/M 37/23]	n=21 normal weight, age 15.5 (0.2), [F/M 9/12]	RHI inversely related to % body fat (r = -0.29, P = .008), total (r = -0.37, P = .004), subcutaneous (r = -0.39, P = .003), and visceral abdominal fat (r = -0.26, P = .04). AIx at heart rate 75 bpm was higher (worse) in the lower RHI groups (P = .04).	Childhood obesity is associated with ED (lower RHI and higher AIx). RHI lower in obese and T2D. RHI negatively related with percentage body fat, WC, Leptin, TNF-alpha, blood glucose.
Circulating fibroblast growth factor-21 (FGF-21): A biomarker of subclinical atherosclerosis in obese youth with non-alcoholic fatty liver disease (NAFLD)? Bacha et al (28)	2017	Conference abstract	Obese adolescents with NAFLD, 15.4+/-0.3 years. n=13 normal glucose tolerance,	Control group: no NAFLD. No difference in age/gender between groups.	Lower RHI in NAFLD group and higher AIx-75. FGF-21 concentrations were related to RHI (r=-0.33,	Increased FGF-21 in obese adolescents with NAFLD and associated with

			n=19 prediabetes, n=16 T2D patients		p=0.03) and AIx (r=0.45, p=0.02).	insulin sensitivity and ED. FGF-21 may constitute a biomarker ED.
Assessment of Microvascular Function in Children and Adolescents with Diabetes and Obesity. Kochummen et al(29)	2019	Cross-sectional study	DM group. n=33 T1D with normal weight. n=8 obese T2D, age 12.7 (3.8) years, [F/M 25/16]	n=17 obese, non-DM children (normal BGL, BP and lipid profile), 12.8 (2.7) years, [F/M 9/8]	For every 1% increase in HbA1C, RHI decreased by 0.097 (P = 0.01). RHI of DM group with HbA1C <10% (1.70 ± 0.58) versus those with ≥10% (1.21 ± 0.19) (p= 0.02).	Poorly-controlled DM (HbA1C ≥ 10%) had lower RHI. RHI negatively related with HbA1C. RHI similar between obese and normal weight with T1D. Similar between T1D and T2D.
Free Vitamin D: Relationship to Insulin Sensitivity and Vascular Health in Youth. Bacha et al (30)	2019	Cross-sectional study. Comparison across tertiles of free 25(OH)D concentrations	n=79, age 15.4 ± 0.2 years, [F/M 45/34]. n=30 overweight. n=31 overweight with prediabetes	n=18 normal weight and normal glucose tolerance.	The lowest tertile group had lower RHI (1.42 ± 0.06, 1.54 ± 0.06, and 1.77 ± 0.09, P = 0.002), compared with the second and third tertiles.	Youth with low free 25(OH)D or BioD concentrations have lower insulin sensitivity and worse endothelial function.
Urine Albumin-to-Creatinine Ratio (UACR): A Marker of Early Endothelial Dysfunction in Youth. Bartz et al(31)	2015	Control study. Fasting UACR analysed.	n=25 overweight (OW) with normal glucose tolerance, 15.6 ± 0.2 years, [F/M	n=13 normal weight, 16.3 ± 0.4, [F/M 7/6].	Normal weight group RHI 1.84 ± 0.1. OW with normal glucose tolerance 1.56 ± 0.1. OW with prediabetes	UACR is an early marker of endothelial dysfunction in youth,

			17/8]. n=20 OWwith prediabetes, [F/M 11/9].		1.56 ± 0.1 (P = .04). UACR was related to RHI (r = -0.33, p = .01).	independent of glycemia.

Table 1: Total of 11 studies included. Endo-PAT 2000 in paediatric type 1 diabetes mellitus (T1D) patients (5 studies), type 2 diabetes and prediabetes (6 studies). Reactive hyperemia index (RHI), type 1 diabetes mellitus (T1D), type 2 diabetes mellitus (T2D), augmentation index (AIx) (vascular stiffness), endothelial dysfunction (ED), Oxygen radical absorbance capacity units (ORAC), non-alcoholic fatty liver disease (NAFLD), overweight (OW), Urine Albumin-to-Creatinine Ratio (UACR).

Title, lead author	**Year**	**Study design**	**Population: n=sample size, age; mean ± SD or median (range), [F/M]**	**Control group: n=sample size, age; mean ± SD or median (range), [F/M]**	**Results: RHI reported. If RHI not specified, we reported p/r values**	**Outcomes**
Effects of a dietary strawberry powder on parameters of vascular health in adolescent males. Djurica et al (32)	2016	Randomised, double-blind, cross-over study	n=15 OW/obese males, 14-18 years (mean 16). 1-week daily 50g of freeze-dried strawberry powder (FDSP) or control powder. Before/after plasma nitrate/nitrite	10 control powder, 14-18 years (mean 16).	Acute plasma nitrate/nitrite levels increased 1 h after consuming the FDSP (P<0.001). When nitrate levels increased after FDSP intake compared to controls, had an increase in RHI (P=0·014).	Strawberries can provide vascular health benefits to OW/obese adolescent males.

			levels measured.			
Flow-mediated dilation in obese adolescents: Correlation with waist circumference (WC) and systolic blood pressure (SBP). Hussid et al (33)	2018	Case control study	n=20 obese patients, median age 14 years	n=10 normal weight, median age 15 years, paired for gender	No RHI difference between groups. 35% obese group had metabolic syndrome, none in control group. OSA in 86.6% obese and 50% of normal weight group.	Obese group had evidence of ED and metabolic syndrome. Increased WC and SBP seem to be related to this finding.
Improvement of microvascular endothelial dysfunction induced by exercise and diet is associated with microRNA-126 in obese adolescents. Donghui et al (34)	2019	Quasi-randomized study	n=57 obese male adolescents, 12-18 (15.38 ± 2.82) years, [F/M = 0/57], 6-week exercise program with dietary intervention.	n=10 normal weight adolescents, 15.38 ± 2.82 years, [F/M 0/10], maintained sedentary	Obese group RHI 1.43 (0.35) vs controls 1.67 (0.36) ($p< 0.05$). After 6 weeks intervention RHI increased ($p <0.01$) and microRNA-126 decreased ($p<0.01$). miRNA-126 positively correlated with ΔRHI ($r = 0.69$, $p<0.05$).	RHI improved in obese group after exercise and diet interventions. Findings might be related to changes in serum miRNA-126.
Distribution of peripheral arterial stiffness and endothelial function as well as their correlations with cardiovascular	2016	Cross-sectional population-based study, conference abstract	n=94 obese, 7-17 years, used automatic waveform analyser (BP-203RPE-I) and Endo-PAT 2000.	n=452 normal-weight	In normal weight group, RHI increased with age ($r=0.33$, $P<0.01$; $r=0.36$, $P<0.01$). RHI positively correlated with BMI ($r=0.10$, $P=0.018$) but	Brachial-ankle pulse wave velocity (baPWV) and RHI increased along with age; arterial stiffness and endothelial function

risk factors in children and adolescents. Mu et al (35)					negatively correlated with DBP (r=-0.10, P= 0.016). RHI did not differ between genders.	continued to develop in the normal weight group.
Urinary biomarkers as indicator of chronic inflammation and endothelial dysfunction in obese adolescents. Singh et al(36)	2017	Control study, research article	n=63 total. n=14 overweight (OW), n=29 obese, age 13.8 (2.4), [F/M 23/20]	n=20 normal weight (NW), age 13.9 (2), [F/M 8/12]	There were no differences in RHI levels: NW 1.6 (0.1), OW 1.66 (0.1) and obese 1.67(0.1). NW girls RHI 1.9 vs NW boys 1.25.	No significant correlation between RHI and urinary markers. RHI higher in NW female adolescents.
Prevalence of Type D personality in obese adolescents and associated cardiovascular risk. Bruyndonckx et al(37)	2018	Control study, conference abstract	Obese adolescents-no definite numbers	Healthy normal weight children	Positive correlation in obese adolescents between negative affectivity and vascular stiffness (r= 0.28; p= .04)	Obese adolescents have worse cardiovascular risk profile with ED.
Endothelial function and arterial stiffness in obese adolescents - A relation to barorefex function. Czippelova et	2017	Conference abstract	n=22 obese, 15.28 +/- 2.8 years, [F/M 10/12]	n=22 non-obese, 15.98 +/- 2.46 years, [F/M 10/12]	No significant difference in RHI (p = 0.473). Baro-reflex sensitivity was also calculated.	No difference in RHI between groups. Findings require further study.

al(38)						
Obesity in children and adolescents: A relation to endothelial function and arterial stiffness. Czippelova et al(39)	2016	Conference abstract	n=16 obese adolescents,15.22 +/- 2.2 years, [F/M 7/9]	n=16 non-obese, 16.22 +/- 1.5 years, [F/M 7/9]	Significant difference in RHI (p = 0.018) with RHI higher in obese group (1.66 +/- 0.28 vrs 1.4 +/- 0.25).	Less early atherosclerotic changes in obese group which was in contrast to expectations. Findings require further study.
Preclinical vascular alterations in obese adolescents detected by Laser-Doppler Flowmetry technique. Fusco et al (40)	2020	Research article	n=22 obese adolescents, 14.11 +/-2.53, [F/M 13/9]	n=24 normal-weight, 15.2 +/- 1.56, [F/M 11/13]	Similar RHI between obese and non-obese groups (1.80 +/- 0.62 and 1.86 +/- 0.51).	RHI not different between groups. RHI did not correlate with LDF. LFD detected preclinical vascular dysfunction by impaired skin microcirculation.
Impaired endothelial function in adolescents with overweight or obesity measured by peripheral artery tonometry. Pareyn et al (41)	2015	Cross sectional study	n=27 overweight (OW)/obesity, 14.7 (13.0–16.4) years, [F/M 11/16]	n=25 normal weight controls, 15.5 (13.9–16.2) years, [F/M 13/12]	RHI normal weight 1.88 (1.7-2.4) vs OW/ obese 1.5 (1.3-1.9) (P< 0.05). Lower RHI if OW /obese and higher baseline pulse amplitude (p = 0.027 and p < 0.0001). RHI positively	ED and higher baseline pulse amplitude in OW group. First time literature reports significant difference in baseline pulse amplitude

					correlated with age and tanner stage (P< 0.05). RHI negatively correlated with DBP (P< 0.05).	between OW adolescents compared to peers.
C-type natriuretic peptide (CNP) plasma levels and whole blood mRNA expression show different trends in adolescents with different degree of endothelial dysfunction. Del Ry et al(42)	2020	Research article -snow balling	n=16 primary obesity, not DM, age 13.3 (0.5) years, [F/M 8/8].	n=24 normal weight, age 14.3 (0.4) years, [F/M 14/10].	RHI normal weight 2.1 (0) vs obese 1.4 (0) (P< 0.005). RHI negatively associated with CNP and diastolic BP (P< 0.005).	RHI significantly lower in obese group. RHI negatively related with CNP, DBP, fat mass and HbA1C.
C-type natriuretic peptide (CNP) is closely associated to obesity in Caucasian adolescents. Del Ry et al (43)	2016	Research article -snow balling	n=10 overweight, age 12.8 (1.6) years, [F/M 5/5]. n=45 obese, 12.8 (1.6) years, [F/M 19/26]	n=27 normal weight, age 12.8 (1.4) years, [F/M 14/13]	Normal weight group RHI 2.1 (0.2) vs OW 1.6 (0.4) (P< 0.05). Normal weight vs obese group RHI 1.4 (0.3) (P< 0.005). RHI negatively associated with CNP (P< 0.005).	RHI significantly lower in overweight/obese groups. CNP negatively related with RHI.
Arterial Stiffness and Endothelial Function in Young Obese Patients - Vascular Resistance Matters. Czippelova et al (6)	2019	Research article	Author contacted for separate paediatric data. n=16 obese group, age <16 years,	n=15 controls, age <16 years, [F/M 7/8]	RHI control vrs obese groups: 1.320 ± 0.427 and 1.457 ± 0.280. RHI obese girls and boys: 1.410 ± 0.253 and	RHI is influenced by vascular tone and resistance. RHI in obese positively related

			[F/M 7/9]		1.494 ± 0.308. RHI control girls and boys: 1.171 ± 0.210 and 1.436 ± 0.524	with SVR.
Cardiovascular adaptations after 10 months of intense school-based physical training for 8- to 10-year-old children. Larsen et al (44)	2018	Randomised control study	n=93 small-sided games group, 9.3+/-0.4 years. n=83 circuit strength training group, 9.3+/-0.3 years (10-16 years)	n = 115 controls, 9.3+/-0.3 years	No significant differences in RHI. Pubertal status is a main predictor of RHI; positive correlation between Tanner stages and RHI.	10 months of 3 × 40 minutes/week decreased DBP and elicited discrete cardiac adaptations, suggesting intense exercise classes can have effects on cardiovascular health.

177 Table 2: Endo-PAT 2000 in paediatric patients who are overweight (OW)/obese (14 studies). Reactive
178 hyperemia index (RHI), augmentation index (AIx) (vascular stiffness), freeze-dried strawberry powder (FDSP),
179 endothelial dysfunction (ED), overweight (OW), normal weight (NW), waist circumference (WC), C-type
180 natriuretic peptide (CNP), Laser-Doppler Flowmetry (LDF).

Title, lead author	**Year**	**Study design**	**Population: n=sample size, age; mean ± SD or median (range), [F/M]**	**Control group: n=sample size, age; mean ± SD or median (range), [F/M]**	**Results: RHI reported. If RHI not specified, we reported p/r values**	**Outcomes**
Nocturnal blood pressure dipping as a marker of endothelial and	2020	Cross-sectional study – author	n=20, 9-19 years (mean 16.5), (7 were age 16 or under). Average	Separated into 2 groups based on nocturnal BP dipping status.	Mean In(RHI) for n=7 (aged 16 and under): 0.529. 22% had ED. Reduced	Isolated nocturnal BP non-dipping is associated with ED and atherosclerotic

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cardiac function in pediatric-onset systemic lupus erythematosus (SLE). Chang et al (7)		contacted for separate paeds data	disease duration 3.2 years (± 2.1). [F/M 17/3]		diastolic BP dipping was associated with poorer endothelial function (r 0.5, p = 0.04).	changes. Potential role for routine ABPM for youth with SLE.
Physiological changes in blood pressure (BP) impact peripheral endothelial function during adolescence. Deda et al (45)	2015	Control study. Assessing association between RHI and known cardiovascular risk factors.	n =90 healthy adolescents to assess normal RHI response, 14.2±1.91 years, [F/M 46/44].	No controls	Mean arterial pressure significantly associated with RHI (p=0.01). Positive correlation RHI and age in females (r=0.33, p<0.02). RHI correlated with pubertal status: males (r=0.411, p=0.03), females (r=0.36, p=0.03).	Physiological changes in BP significantly impact RHI results.
Endothelial Function and Arterial Stiffness Relate to Functional Outcomes in Adolescent and Young Adult Fontan Survivors. Goldstein et al (46)	2016	Cross-sectional prospective observational study	n=60, 8-25 years (mean 13.9±4.1), [F/M 29/31]	No controls	AIx (P<0.05) negatively associated with peak VO2. PAT derived baseline pulse amplitude (P<0.05) negatively associated with minute ventilation to C02 ratio. PAT-AIx (P<0.05) negatively associated with parent-reported Paeds QOL total and physical heath scores.	Worse vascular measures associated with worse functional measures. Increased arterial stiffness and decreased endothelial function are associated with lower aerobic capacity, physical activity, and QOL in Fontan survivors.

Natural history of vascular function in adolescent and young adult Fontan survivors: A longitudinal assessment of endothelial function and arterial stiffness. Goldstein et al (47)	2017	Prospective single-centre longitudinal study, conference abstract. Paired testing at a mean interval of 2.0 +/- 0.2 years of Fontan survivors.	n=50, mean 13.7 +/- 4.2 years, [F/M 23/27]	No controls	Decreases in RHI (0.002 +/- 0.01/yr) were not significant. AIx improved by 0.74 +/- 0.3/yr (p=0.02). Changes RHI and AIx did not correlate with peak VO changes. BMI was a predictor for RHI (R 0.17, p=0.007). Change in resting O2 saturation was the only predictor of change in AIx (R 0.09, p=0.04).	Vascular function does not change uniformly in Fontan survivors. Changes in vascular function do not relate to changes in aerobic capacity but are associated with changes in anthropometric measures and O2 saturation.
Vascular function long term after Kawasaki disease: another piece of the puzzle? Pinto et al (48)	2017	Single-centre prospective study	n=43 Kawasaki patients, age >11 years, diagnosed >5 years ago, with no coronary lesions or any other risk factors for cardiovascular disease.	n= 43 control group of individuals without cardiovascular risk factors.	Kawasaki patients had decreased RHI compared with controls (1.59±0.45 versus 1.98±0.41; p<0.001). AI was similar in both groups (-4.5±7 versus -5±9%; p 0.6).	Children with Kawasaki disease may have long-term sequelae, even when there is no detectable coronary artery involvement in the acute stage of disease.
Endothelial function in children with a history of Henoch Schonlein purpura (HSP). Butbul Aviel et al (49)	2017	Observational prospective study	n=19 with HSP, 13.5 ± 3.9 years, [F/M 8/11]	n=23 healthy children, 12.8 ± 4.5 years, [F/M 7/16]	Mean RHI 1.81 study group and 1.87 control group (p = 0.18). RHI higher in patients who had endothelial function measured >6 years since HSP	This study suggests that HSP causes short term endothelial dysfunction that improves with time.

					diagnosis compared with <6 years (1.98 + 0.74 vs. 1.38 ± 0.43 P = 0.037).	
Reactive hyperaemia index and detection of endothelial dysfunction in children with familial hypercholesterolaemia (FH). Jehlicka et al(50)	2015	Conference abstract	n=24 with FH, 13.9+/-2 years. Biochemical markers of endothelial function were assessed.	n=17 healthy controls, 15.2+/-2.2 years	Significantly lower RHI in FH group (1.63+/-0.50 and 2.03+/-0.54; p<0.05). Lower RHI and elevated E-selectin in children with FH.	Possible relationship of ED in children with FH, highlighting the importance of early detection of ED when the atherosclerotic process is still reversible.

182 Table 3: Endo-PAT 2000 in paediatric patients with cardiac and vascular conditions (7 studies). Reactive
183 hyperemia index (RHI), waist circumference (WC), systolic blood pressure (BP), augmentation index (AI), peak
184 VO (peak 02 consumption), quality of life (QOL), systemic lupus erythematosus (SLE), ambulatory blood
185 pressure monitoring (ABPM), quality of life (QOL), Henoch Schonlein purpura (HSP), familial

Title, lead author	**Year**	**Study design**	**Population: n=sample size, age; mean ± SD or median (range), [F/M]**	**Control group: n=sample size, age; mean ± SD or median (range), [F/M]**	**Results: RHI reported. If RHI not specified, we reported p/r values**	**Outcomes**
Vascular function in asthmatic children and adolescents.	2017	Cross-sectional controlled study	n=19 asthmatic patients, age 13.6 ± 0.6 years. [F/M 0/19]	n=18 controls. 14.9 ± 0.7 years. [F/M 0/18]	LnRHI were similar between groups (p = 0.23). The augmentation index	The increased AIx@75 without changes in LnRHI in asthmatic patients

186 hypercholesterolaemia (FH).

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Augusto et al (51)					(AIx@75%) was significantly higher in the asthmatic group (-7.75 ± 1.7) compared to the control group (-15.25 ± 1.8), p < 0.04.	could mean that an early detection of vascular impairment may precede ED.
The effect of weight loss on endothelial function and sleep disordered breathing (SDB) in obese children. Ysebaert et al(52)	2018	Conference abstract. Reassessed after 6-month weight loss programme.	n=62 obese, age 11-19 (mean 15.8) years, [F/M 20/42]	No controls.	Endo-Pat used. At baseline 39% had SDB. After 6 months, 86% had resolution of earlier diagnosed SDB. All showed significant improvement of endothelial function after programme (p < 0.001). No correlations between presence of SDB and improvement in endothelial function found.	Endothelial function significantly improves after weight loss.
Polysomnographic correlates of endothelial function in children with obstructive sleep apnoea (OSA). Zhang et al (53)	2018	Cross sectional study	n=121 mild OSA, 6.2 ± 1.6 years, [F/M 37/84]. n=127 moderate-severe OSA, 6.0 ± 1.6 years, [F/M 31/96]	n=107 primary snorers (PS), age 6.4 ± 1.8 years, [F/M 37/70]	OSA groups lower RHI than PS (P < 0.001, P = 0.001). RHI positively correlated with age (r = 0.17, P = 0.002), BMI z score (r = 0.14, P = 0.008) and oxygen saturation (r = 0.15, P = 0.006).	Children with OSA are at increased risk for abnormal endothelial function than habitually snoring children.
Endothelial	2020	Cross	n=248 OSAS, age	n=107 primary	OSAS had lower RHI	OSAS have

dysfunction in children with obstructive sleep apnoea syndrome (OSAS). Xu et al(54)		sectional study	3-11 years	snorers (PS). No significant differences in age/gender.	1.1±0.1 vrs 1.2±0.2 (P<0.01). RHI independently correlated with age, gender, obstructive apnoea hypopnea index, oxygen desaturation index (*P*<0.01).	significant ED compared with PS. Frequent arousals due to obstructive respiratory events during sleep may be a candidate risk factor for ED.

Table 4: Endo-PAT 2000 in paediatric patients with respiratory conditions (4 studies). Natural logarithm of RHI (LnRHI), endothelial dysfunction (ED), reactive hyperaemia index (RHI), augmentation index (AIx), heart rate-corrected augmentation index (AIx@75), primary snorers (PS), obstructive sleep apnoea (OSA), obstructive sleep apnoea syndrome (OSAS).

Title, lead author	**Year**	**Study design**	**Population: n=sample size, age; mean ± SD or median (range), [F/M]**	**Control group: n=sample size, age; mean ± SD or median (range), [F/M]**	**Results: RHI reported. If RHI not specified, we reported p/r values**	**Outcomes**
Do self-reported stress and depressive symptoms effect	2018	Longitudinal cohort study. LOOK longi tudinal study	n=203, 7.6 ± 0.3 years, [F/M 111/92].	No controls.	All relationships occurred in the hypothesised direction, but no cross-sectional	Contrast to previous findings in adolescents, little evidence between current or previous psychosocial stress or depression and endothelial function in 16-year-old

endothelial function in healthy youth? The LOOK longitudinal study. Olive et al(55)		, who were followed through to adolescence (16 years).			or prospective evidence of early psychological stress or depression was associated with ED (all p > 0.05).	adolescents.
Cerebrovascular reactivity is associated with peripheral endothelial function (EF) among adolescents. Urback et al(56)	2016	Conference abstract	n=11 with bipolar disorder. EF measured by PAT and cerebrovascular reactivity (CVR) by blood-oxygen-level dependent fMRI.	n=35 healthy controls	EF was positively correlated with CVR in grey matter (r=0.41, p=0.012), and a peak voxel in the left-medial-frontal gyrus (r=0.35, p=0.036).	Breath-hold CVR and peripheral EF are linked, suggesting that vascular function may be a multi-systemic phenotype. EF may be a potential proxy for cerebral blood vessel function with greater accessibility and lower cost than fMRI.
Retinal-vascular photography as a window into the cardiovascular and neurocognitive burden of adolescent bipolar disorder (BD). Naiberg et al (57)	2017	Cross-sectional study, author emailed for separate paeds data-most were teenagers	n=30 with bipolar disorder, 17.97±1.86 years	n=32 healthy controls, 16.00±1.62 years	In BD group, higher endothelial function associated with higher arterio-venular ratio (r=0.375, p=0.041).	Retinal photography may help assessing cardiovascular and neurocognitive burden of BD.
Impact of psychological health on peripheral	2017	Longditudinal 3-year follow-up study.	n=162, 14.5 ± 1 years. [F/M 94/68].	No controls.	Lower peripheral endothelial function was associated with high level of anger	High amounts of negative emotions may have adverse effects on peripheral endothelial function and regulation of the HPA-axis activity. High level of self-

endothelial function and the HPA-axis activity in healthy adolescents. Chen et al(58)		Baseline and three-year follow-up.			($\beta = -0.332$, $p = 0.018$) and disruptive behaviour ($\beta = -0.390$, $p = 0.006$) over three years in males, but not in females, adjusted for covariates.	concept might be protective.

Table 5: Endo-PAT 2000 in paediatric patients with psychiatric conditions (4 studies). Endothelial dysfunction (ED), endothelial function (EF), cerebrovascular reactivity (CVR), bipolar disorder (BD), functional magnetic resonance imaging (fMRI), hypothalamic–pituitary–adrenal HPA.

Title, lead author	**Year**	**Study design**	**Population: n=sample size, age; mean ± SD or median (range), [F/M]**	**Control group: n=sample size, age; mean ± SD or median (range), [F/M]**	**Results: RHI reported. If RHI not specified, we reported p/r values**	**Outcomes**
Vascular endothelial function in inflammatory bowel disease (IBD). Winderman et al(59)	2018	Case-control study	n=16 with IBD (all in clinical remission), age 16.7 +/- 2.6 years, [F/M 8/7]	n=16, age 15.1 +/- 2.8 years, [F/M 7/8]	RHI IBD vs controls 1.66 vs 2.02 (P =0.036). IBD group had a mean RHI within the range associated with VD risk in adults (1.67).	IBD group lower RHI compared with controls. IBD patients may need to be monitored for thromboembolic phenomena.
Endothelial health in childhood acute	2015	Case control study	n=16 ALL survivors, age 8-	n=16 healthy sibling pairs	Both groups similar in cardiovascular risk	Evidence of poorer vascular health in

lymphoid leukaemia (ALL) survivors: pilot evaluation with peripheral artery tonometry. Ruble et al (60)			20 years (12.9+/-0.9), [F/M 8/8].	13.8 (0.9), [F/M 10/6].	measures but survivors had lower RHI (1.54 vs. sibling 1.77; P=0.0474).	cancer survivors.
Microvascular endothelial function in Japanese early adolescents. Odanaka et al (61)	2017	Control study	n=157 healthy adolescents divided by gender. Females n=82, median age 14 (1), 13.7 ± 0.9 years	Males n= 75, median age 14 (2) years	No difference in RHI according to sex: boys and girls 1.85 ±0.6, 1.82 ±0.66 and 1.87± 0.54. RHI was significantly associated with systolic and diastolic BP, and had no correlation with anthropometric parameters and arterial stiffness markers.	RHI among adolescents were similar to those reported in previous studies on children and early adolescents.
Endothelial Dysfunction and the Effect of Arginine and Citrulline Supplementation in Children and Adolescents With Mitochondrial Diseases. Al Jasmi, et al (62)	2020	Case control study	9 participants, age 6-17 years (mean 9.6).	3-15 years (mean 9.4). Baseline endothelial dysfunction was assessed in controls.	Lower RHI with mitochondrial diseases. RHI increased with arginine or citrulline supplementation	Supplementation with NO precursors may improve ED by enhancing NO production. First study to use Endo-PAT methodology in mitochondrial diseases.

Assessment of traditional and non-traditional risk factors for premature atherosclerosis in children with juvenile dermatomysoitis (JDM) and pediatric controls. Wahezi et al (63)	2020	Retrospective controlled study	n=40 JDM, age 6-22 (mean 12.4± 4.1) years, [F/M 28/12]	n=20 controls, age 12.7± 3.9 years, [F/M 14/8]	RHI controls 1.43 [1.2, 1.7] and JDM 1.57 [1.2,1.9]. If controlled for lipoprotein A (atherogenic confounder), JDM patients had 41% RHI increase, thus indicating less ED compared to controls.	Rheumatological childhood disorders may be at increased risk of developing ED, but sociodemographic factors may have a greater role in developing cardiovascular disease.
Vascular Health of Children Conceived via In Vitro Fertilization (IVF). Zhang et al (64)	2019	Cross-sectional pilot study	n=17 IVF children, 10-14 years. Also used carotid ultrasound and pulse wave velocity measurements.	Compared to published norms or to historical Stanford controls	Mean Endo-PAT index in the IVF cohort was 1.66+/-0.52, 71% had abnormal values (<1.9). Mean RHI was not significantly different between IVF and controls.	Children conceived by IVF seem to have evidence of abnormal vascular health.
Endothelial dysfunction in South African youth living with perinatally acquired human immunodeficiency virus (PHIV) on antiretroviral therapy. Mahtab	2020	Case control study	n= 431 PHIV, median 14.1 (12.8, 15.5) years, [F/M 213/218]	n=93 without HIV, median 13.9 (12.1, 15.3) years, [F/M 53/40]	PHIV had higher rates of ED (50% vs 34%; P = .01); relationship persisted after adjusting for age, sex, BMI, high BP and hypercholesterolemia (RR, 1.43; P =0.02). PHIV, CD4 count, viral load and current ART	PHIV appear to have increased risk of ED. These findings have important implications as HIV has increased risk of premature CVD and complications.

et al (65)					class were not associated with ED after adjustment.	
Soluble CD14 (sCD14) is associated with endothelial dysfunction in South African youth on ART. Dirajlal-Fargo et al (66)	2020	Case control study	n=283 perinatally acquired HIV (PHIV), 9-14 years.	n=69 age-matched without HIV	PHIVs had lower RHI despite viral suppression (RHI=1.36 vs 1.52, p<0.01). sCD14 at 24 months correlated with ED (p≤0.04). PHIV with ED, sCD14 was associated with lower RHI (β-0.05, p=0.01).	Higher sCD14 is independently associated with ED in PHIVs.
Role of insulin resistance and hyperandrogenemia in early vascular dysfunction in adolescents with PCOS. Bartz et al (67)	2015	Conference abstract	n=14 PCOS adolescents PCOS (on no treatment).	n=7 non-PCOS. Both groups had similar age, tanner stage, race, glucose tolerance status.	Despite higher peripheral and hepatic insulin resistance with PCOS, RHI is not significantly lower when compared with controls of similar total body and abdominal adiposity.	PCOS has evidence of increased vascular inflammation. Hyperandrogenemia and insulin resistance may play an important role in vascular inflammation.
Endothelial Function in Children and Adolescents Is Mainly Influenced by Age, Sex and Physical Activity-An Analysis of	2017	Randomised controlled study, Leipzig School Project followed over 5-year	n=931 RHI measurements in 445 students, age 10-17 years (baseline 11.66±0.93). n=247: 60 minutes physical	n=181: 2 units of 45 minutes PE weekly (control group).	Higher RHI in the intervention group: 0.09 [-0.05, 0.23]. Increase RHI from 1.53±0.42 in the youngest to 1.96±0.59 in the oldest students. This increase adjusted	If Endo-PAT is used for research in adolescents, age and sex must to be taken in account when reporting RHI results.

Reactive Hyperemic Peripheral Artery Tonometry. Mueller et al (68)		period.	exercise (PE) daily (intervention group).		by age and sex was estimated as 0.11 [0.08, 0.14] per year.	

Table 6: Endo-PAT 2000 in paediatric patients with other miscellaneous paediatric conditions (10 studies). Reactive hyperemia index (RHI), augmentation index (Aix) (vascular stiffness), endothelial dysfunction (ED), inflammatory bowel disease (IBD), acute lymphoid leukaemia (ALL), nitric oxide (NO), perinatally acquired human immunodeficiency virus (PHIV), In Vitro Fertilization (IVF), soluable CD 14 (sCD14), polycystic ovarian syndrome (PCOS), physical exercise (PE).

Results:

Endothelial dysfunction in paediatric diabetes mellitus patients (Table 1):

Five studies involve only type 1 diabetes (T1D) patients (*Table 1*). 2/5 studies reported lower RHI results in the T1D group(21, 24). One study which included only adolescent patients, reported RHI negatively correlates with impaired metabolic control and subclinical signs of autonomic neuropathy(21). They concluded that good metabolic control (HbA1c ≤7.5%) and regular physical activity might be protective against ED. One study reports an improved RHI result with an alpha-lipoic acid and antioxidant diet(22). Nadeau et al reported no significant RHI change with metformin overall but some improvement in overweight T1D males(23). Barber et al report suboptimal glycaemic control causes early atherosclerosis(24). One study noted an improvement in RHI post vitamin D supplementation in T1D patients with vitamin D deficiency(25).

6 studies focused on type 2 diabetes (T2D) and impaired glucose tolerance or 'prediabetes.' Tomsa et al note a link between insulin resistance and obesity by utilising Endo-PAT(27). They also noted that RHI is higher if HbA1c is less than 5.5%(27). Two studies compare on Non-alcoholic fatty liver disease (NAFLD), T2D and prediabetes patients(26, 28). If dysglycemia, NAFLD is associated with worse endothelial function. Circulating FGF-21 levels are elevated in obese youth with NAFLD and

are associated ED and therefore may be a biomarker for ED(28). Bartz et al report urine albumin creatinine ratio (UACR) may be an early marker of ED independent of glycemia(31). Endothelial dysfunction may mediate the link between obesity-related insulin resistance and early microalbuminuria.(31). Kochummen *et al* reported a mean RHI in obese adolescents without diabetes was similar to T1D and T2D patients(29). Bacha et al report lower vitamin D concentrations are associated with lower insulin sensitivity and worse endothelial function(30).

Endothelial dysfunction and Obesity (Table 2):

14 studies describe the use of Endo-PAT 2000 in overweight or obese patients (*Table 2*). Studies included measurement of the following parameters: BMI, T1D, T2D, gender, pubertal stage, age, blood pressure values, non-alcoholic fatty liver disease, obstructive sleep apnoea, insulin, plasma glucose levels, inflammatory markers (urinary markers, CNP, micro-RNA-126, E-Selectin). In numerous studies, RHI was significantly lower in obese groups (6, 29, 32-35, 42, 43, 69). ED may mediate the link between obesity-related insulin resistance and early microalbuminuria(31). Exercise and diet control improves glycolipid metabolism(44). Two studies by Czippelova et al did not find a lower RHI in obese groups, but recommended further studies(38, 39). Noma et al (2017) report the beneficial effects of exercise in paediatric patients and is an important message in reducing future endothelial complications(70). Fusco et al noted pre-clinical microvascular changes in obese patients compared to controls using LDF but noted no RHI change(40).

Endothelial dysfunction in cardiac and vascular conditions (Table 3):

7 studies report the use of Endo-PAT and cardiovascular conditions (*Table 3*). Lower RHI is seen with patients with familial hypercholesterolaemia(50). Studies assess ED in patients with systemic lupus erythematosus (SLE) and Henoch Schonlein purpura (HSP)(7, 49, 71). Negishi et al (2016) used Endo-PAT to compare Fontan survivors and healthy controls. The Fontan patients were aged 15 to 32 years. Mean RHI 0.56+ /- 0.26 in Fontan patients and 0.78+ /- 0.31 in controls (p= 0.09). RHI in Fontan patients was associated with diastolic blood pressure, heart rate and haemoglobin A1c

level(72). Endothelial function in Fontan patients was associated with abnormal glucose tolerance and arterial stiffness and therefore concluded that glucose regulation might be a potential target to improve ED in this cohort. Nozaki et al (2018) assessed ED in conduit and resistance arteries and used FMD and Endo-PAT in paediatric patients with repaired coarctation of aorta(73). Adult patients with repaired coarctation of aorta have a high risk of late hypertension.

Endothelial dysfunction in respiratory conditions (Table 4):

4 studies used Endo-PAT in respiratory conditions (*Table 4*). Augusto et al noted an increased augmentation index (AIx) without changes in RHI in asthmatic patients; indicating early detection of vascular impairment may precede ED, and different mechanisms may contribute to the pathogenesis and progression of cardiovascular events in this population(51). One study reported an improvement in sleep disordered breathing post weight loss(52). Also, endothelial function significantly improves after weight loss. Two studies report children with OSA compared to habitual snorers are at increased risk for ED(53, 54). Frequent wakening due to obstructive respiratory events may be a risk factor for ED in OSA.

Endothelial dysfunction and psychological conditions (Table 5):

4 studies report the use of Endo-PAT in psychiatric conditions (*Table 5*). Potential limitations in this area are self-reported methods for detecting psychological distress of children, for example in the LOOK longitudinal study(55). Naiberg et al (2017) utilised retinal vascular photography as a proxy for cerebral microvasculature, and Endo-PAT to assess cardiovascular and neurocognitive burden in adolescents with bipolar disorder (BD)(57). In the BD group, better endothelial function was associated with higher arterio-venular ratio ($r=0.375$, $p=0.041$). Retinal vascular calibre was significantly associated with endothelial function in BD and it has been suggested that it may be used as an assessment tool in this cohort. Olive L.S. (2017) published 'The emerging field of paediatric psycho-cardiology' highlighting the importance of the childhood origins of adult CVD(74). This article highlights that psychological distress can influence CVD risk, directly by physiological change that can negatively impact the integrity of the cardiovascular system.

Endothelial dysfunction and other paediatric conditions (Table 6 – Miscellaneous)

Childhood cancer survivors:

There is evidence of ED in cancer survivors (*Table 6*)(60). Chemotherapy causes cardiomyocyte damage and also negatively affects endothelial function. Broberg et al (2018) utilised Endo-PAT in childhood cancer survivors and noted a lower RHI in this cohort compared to controls(75). Brouwer et al (2013) studied cancer survivor patients after potential cardiovascular toxic treatment (e.g. anthracyclines, platinum) and/or radiotherapy and noted a higher risk of ED compared with sibling controls(76). Broberg et al (2016) identified one-third of cancer survivors (31.2%) compared to 8% of controls ($p= 0.02$) had ED in their study(77). They concluded this may be a useful screening tool of cardiovascular disease in asymptomatic cancer survivor patients. Pao et al (2018) assessed the relationship between blood pressure and ED using Endo-PAT in haematopoietic stem cell transplant recipients. Hypertension on ambulatory blood pressure monitoring ($p= 0.045$) and blunted nocturnal dipping ($p= 0.04$) were associated with a lower Endo-PAT scores(78). Jehlicka et al (2011) used Endo-PAT and noted ALL patients had lower RHI compared to controls (1.57±0.50, 1.96±0.63; $p\leq0.05$)(79).

Autoimmune conditions:

Children with autoimmune diseases may have a high tendency to develop ED which was highlighted in a study using a novel technique(80). Atherosclerosis is an emerging cause of morbidity and mortality in patients with rheumatological conditions such as juvenile idiopathic arthritis, SLE and dermatomyositis. Borenstein-Levin et al assessed a cohort with autoimmune conditions compared to controls: 29% in the study group had ED compared to 6% ($p <0.05$)(80). Chang et al noted nocturnal blood pressure (BP) non-dipping is associated with ED in SLE patients highlighting a potential role for ambulatory BP monitoring in these patients(*Table 3*)(7).

Metabolic diseases:

Yano et al research in Fabry disease patients demonstrated that early diagnosis of ED can help determine the timing of initiating enzyme replacement therapy(81). Utilizing RH-PAT as a screening tool for early renal involvement may be helpful as it may detect abnormalities even prior to microalbuminuria(82). This can provide guidance on enzyme replacement therapy which is required to prevent irreversible progressive renal failure. Al Jasmi et al research in mitochondrial diseases reported that arginine or citrulline supplementation may improve ED, which provides evidence that these amino acids may be therapeutic (*Table 6*)(62).

Inflammatory bowel disease:

One study (*Table 6*) highlights that IBD patients had lower RHI compared with controls(59). Petr et al (2014) provided evidence of increased ED in children with Crohn's disease compared to healthy controls(83). RHI values were significantly lower in the patients with Crohn's than controls ($p < 0.05$).

Infectious diseases:

Dirajlal-Fargo et al used Endo-PAT to assess ED in human immunodeficiency virus (HIV) patients (*Table 6*)(66, 84). Perinatally acquired HIV patients appear to have higher levels of ED (RHI 1.34 (1.20, 1.42) compared with controls (1.52 (1.27, 1.80) ($p<0.01$))(84). The pathogenesis of severe Plasmodium vivax malaria is poorly understood. ED and reduced nitric oxide (NO) bioavailability characterize severe falciparum malaria. Barber et al (2016) identified that endothelial function was impaired in proportion to disease severity. Those with severe vivax malaria, non-severe and healthy controls median RH-PAT index 1.49, 1.73, and 1.97 respectively ($p=0.018$)(85). ED in this cohort was associated with reduced L-arginine bioavailability, which may contribute to microvascular pathogenesis.

Discussion:

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). Only 4 studies are RCTs. The studies cannot be compared for a meta-analysis as most 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.

Strengths of the paper include a comprehensive literature search including contacting authors by email for separate paediatric results in studies with combined adult and paediatric data. All study types were reviewed and even the studies without results but had interesting points were included in our discussion. Also, we do not think that this paediatric Endo-PAT review has been done before.

The potential future role of Endo-PAT for paediatric patients may be an adjunct tool to in screening for cardiovascular risk factors as well other factors such as family history, cholesterol, blood pressure. If atherosclerosis is identified early, it can be halted in its process in certain conditions. There is huge potential for use in diabetic patients. Lower insulin sensitivity poses a risk of diabetic nephropathy(8). Microangiopathic renal damage increases oxygen consumption and increases resistance in the afferent arterioles. Shah et al report T2D patients have greater vascular thickness and stiffness and worse endothelial function compared to obese and lean children(86). This is raising concern that adolescents with T2D are already at risk of developing early onset cardiovascular disease.

Endo-PAT has multiple benefits in obesity (*Table 2*)(6, 34, 39). Berardinelli-Seip syndrome is a rare condition characterized by severe insulin resistance and absence of subcutaneous fat since birth or early childhood. Lipids can deposit in muscle, liver and arterial walls; explaining its clinical complications of diabetes, hepatic injury, hyperlipidaemia and premature atherosclerosis. Fernandes et al (2015) reported 50% with this syndrome had ED (RHI 0.49±0.15)(87). Their results support the risk of ED in this cohort and highlights the necessity of early intervention to avoid cardiovascular complications.

Turner syndrome (TS) patients have increased cardiovascular risk factors which predispose to cardiac and cerebrovascular complications. A literature review concluded that TS have unfavourable cardiometabolic risk factors which predispose them to adverse cardiac and cerebrovascular outcomes in young adulthood(88). It is unclear whether this is secondary to the syndrome itself or from modifiable risk factors such as obesity, hypertension, etc. Moreover, congenital heart disease is a clinical feature in 30% of cases of TS patients. There is a huge emphasis on the importance of regular screening in this cohort and also further research into whether any variables could potentially be altered to reduce the atherosclerosis risk in adulthood. O'Gorman et al (2012) published a case-control study on TS patients(89). This paper excluded any with structural congenital heart disease. Lower RHI scores in TS compared with controls 1.64 (0.34) vs 2.08 (0.32) (P<0.005). Growth hormone may protect endothelial function in TS patients as GH-untreated RHI 1.44 (0.26) versus GH-treated 1.86 (0.28) (p P<0.05). There are countless other paediatric syndromes, that could benefit from ED screening.

Furthermore, in cardiac diseases and post-cardiac surgery Endo-PAT has been proven useful in multiple studies (*Table 3*)(48, 73, 88). Dietz et al (2015) systematic review and metanalysis on peripheral ED in Kawasaki disease, report coronary arterial aneurysms had higher surrogate markers for cardiovascular disease risk(90). This may indicate these patients should be monitored for CVD in adulthood, however significant heterogeneity was noted. Goldstein et al (2016) by using Endo-PAT identified multiple patient and procedural factors for Fontan survivors(91). Some determinants of RHI included prior Norwood procedure, systolic blood pressure, resting heart rate and oxygen saturation.

Targeted intervention of modifiable risk factors may improve long-term vascular health and functional status in Fontan survivors. Further research by Goldstein et al (2015) noted increased arterial stiffness and decreased endothelial function are associated with lower aerobic capacity, quality of life (QOL) and physical activity in adolescent and young adult Fontan survivors(92). 'The LOVE-COARCT study' (Long-term Outcomes and Vascular Evaluation After Successful Coarctation of the Aorta Treatment) compares vascular function in patients with coarctation of the aorta treated with surgery, balloon dilation or stenting and endothelial function was similar among groups(93).

With the rising premature population, Endo-PAT may prove useful in this cohort. Harris et al (2020) assessed cardiovascular outcomes for those born with very low birth weights (VLBW) <1500g. The VLBW cohort (n = 229; 71% of survivors) and term-born controls (n = 100), were assessed at age 26-30 years. The VLBW cohort had lower RHI compared to controls(94). Endo-PAT is also used in haematological conditions. Sivamurthy et al (2009) reported lower RHI in the majority sickle cell disease in a paediatric population (1.53 and 1.71; p value .032). RHI was not normal in children with chronic transfusions or hydroxyurea(95).

Finally, many paediatric autoimmune conditions are linked with ED(7, 80). In SLE patients, ED may occur from impaired clearance of apoptotic cells, oxidative stress, or B cell activation with different circulating autoantibodies(71). Regular ED assessment in SLE patients has been recommended due to risk of subclinical atherosclerosis(71). Moreover, several factors may impact microvascular function in children, for example puberty, which is of particular interest in our paediatric review. Bhangoo et al report improved RHI in correlation with an increase in Tanner stages and postulated that this may be due to sex steroids(96). If Endo-PAT is used in research in adolescents, age, sex and tanner staging must to be taken in account when reporting RHI results(68).

Conclusion:

There are a number of papers in the paediatric literature describing ED at young ages using Endo-PAT. However, in many cases, there has only been a single cohort study using Endo-PAT. Further

studies are required to validate these findings. Additionally, longitudinal studies are required to evaluate how this ED may change as the child ages and their chronic conditions changes. Further studies are also required that will characterise more completely the cardiovascular risk profile of these children with chronic disease. Consensus on other vascular risk markers that could be included in future studies is ideal and if accomplished, this would facilitate meta-analyses of studies of conditions with relatively rare conditions. Paediatricians should start to include an approach to cardiovascular risk assessments in their assessments of young children and adolescents, including but not limited to those with chronic diseases.

Statements and declarations:

a. Authorship contributions:

All authors contributed to the initial search strategy protocol. I deLaunois performed the online database search. J Hayden and G McDonnell separately performed a blind screen of the abstracts and analysed the papers. G McDonnell contacted the authors of joint adult and paediatric papers to obtain separate paediatric data. J Hayden wrote the initial manuscript which was revised by C O'Gorman. All authors reviewed the manuscript prior to submission.

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e. Competing Interest: No competing interests to declare

f. Ethical approval: this was not needed as this study was a systematic review and did not involve human participants.

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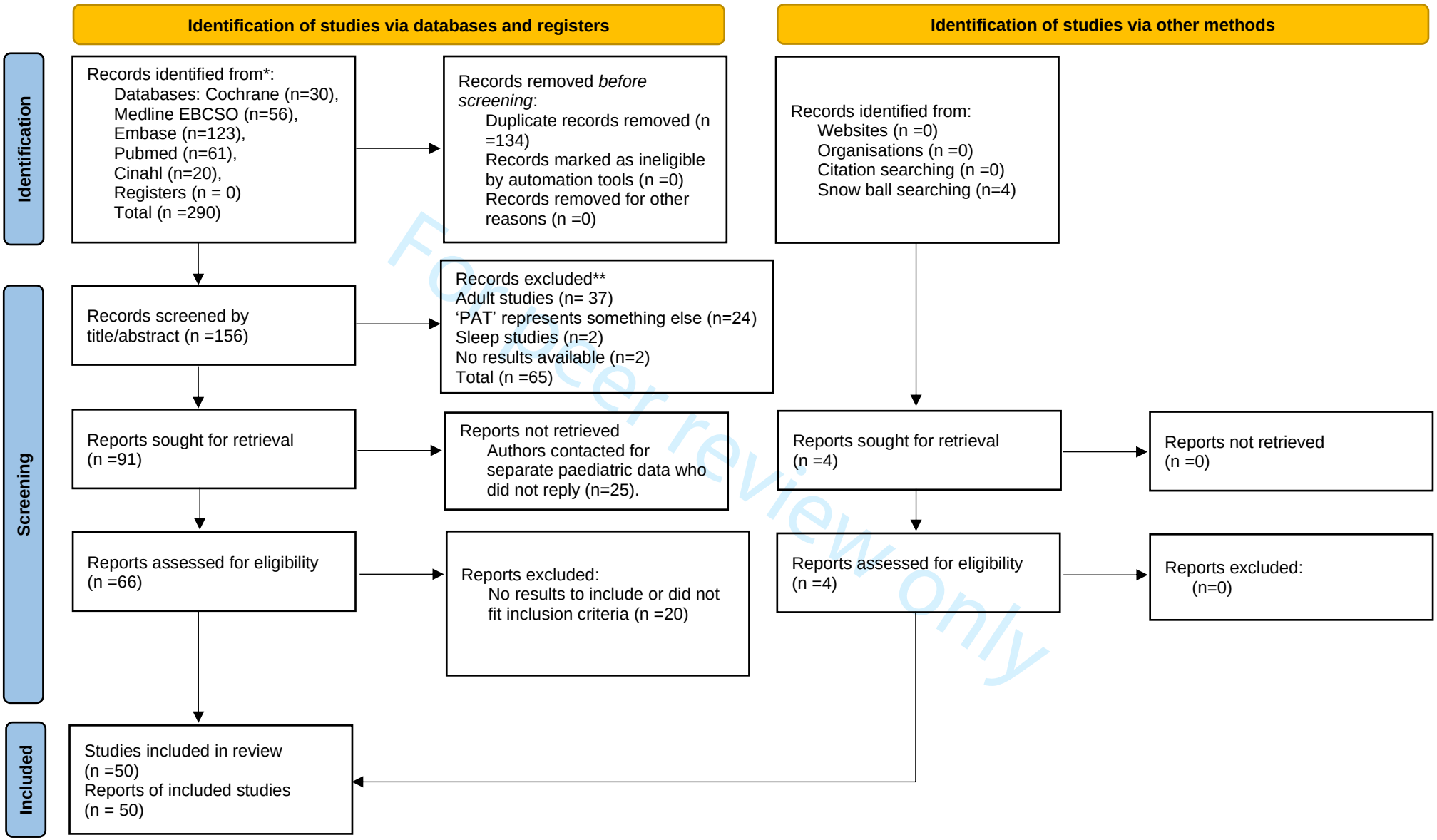
Figure 1: PRISMA 2020 Flow diagram of systematic search for Endo-PAT 2000 in paediatric populations.

PRISMA 2020 flow diagram for new systematic reviews which included searches of databases, registers and other sources



*Consider, if feasible to do so, reporting the number of records identified from each database or register searched (rather than the total number across all databases/registers).
**If automation tools were used, indicate how many records were excluded by a human and how many were excluded by automation tools.

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71. For more information, visit: http://www.prisma-statement.org/

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			Page
Title	1	Identify the report as a systematic review.	2
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	2
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	2
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	6
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	5
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	6
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	6
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	6
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	6
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	6
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	6
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	Tables
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	5
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	5
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	7
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	7
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	N/A
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	N/A
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	6
Certainty	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	6

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
assessment			
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Flow diagram
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	6
Study characteristics	17	Cite each included study and present its characteristics.	Tables 1
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Nil
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Tables 1-6
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	N/A
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	N/A
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	N/A
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	N/A
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	N/A
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	N/A
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	16
	23b	Discuss any limitations of the evidence included in the review.	17
	23c	Discuss any limitations of the review processes used.	17
	23d	Discuss implications of the results for practice, policy, and future research.	20
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	No response
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	Prospero – no response
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	N/A
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	Nil
Competing interests	26	Declare any competing interests of review authors.	Nil
Availability of data, code and	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	With authors

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
other materials			

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71
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BMJ Open

Endo Peripheral Arterial Tonometry (Endo-PAT 2000) use in Paediatric Patients – a systematic review.

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Title page

Endo Peripheral Arterial Tonometry (Endo-PAT 2000) use in Paediatric Patients – a systematic review.

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Key words: Endo-PAT 2000, peripheral artery tonometry, Endothelial dysfunction, paediatric diabetes mellitus, chronic diseases

Word count:

Abstract:

Objectives: Endo Peripheral Artery Tonometry (EndoPAT-2000) is a non-invasive technology for measuring endothelial dysfunction (ED). The reactive hyperaemia index (RHI) is resulted and is low when ED is present. We aim to synthesise the literature on paediatric ED that utilised Endo-PAT analysis.

Design:

A comprehensive systematic review was conducted from January 2015 to March 2021. The databases included Cochrane, MEDLINE EBSCO, EMBASE (Ovid), PUBMED and CINAHL EBSCO. Exclusion criteria were: 1. If a study used a different device for example. 2. If the study had no results. Inclusion criteria were: 1. Published in the English; 2. More than 50% of study subjects were in the paediatric age range; 3. Data relevant to paediatric age range children could be extrapolated from all data, where not all study subjects were children.

Results:

Following the removal of duplicates, 156 articles were initially identified . Following exclusion, 50 articles were included for review. We have subdivided these papers into different systems for ease of reference and have reported our findings in 6 tables: 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.

Conclusions:

A number of papers using Endo-PAT for children with various chronic diseases have evidence of ED. However, in many cases, there has only been a single cohort study using Endo-PAT. Further studies are required to validate these findings and to help characterise the cardiovascular risk profile of children with chronic disease. Further studies are also required that will characterise more completely the cardiovascular risk profile of these children.

Consensus on other vascular risk markers that could be included in future studies is ideal and if accomplished, this would facilitate meta-analyses of studies of relatively rare conditions.

Strengths and limitations:

- Comprehensive systematic review to synthesise the literature on endothelial dysfunction using Endo-PAT in paediatric patients.
- All study types were reviewed and even the studies without results but were relevant were included in our discussion.
- In many cases, there has only been a single cohort study using Endo-PAT for a particular disease
- Separate paediatric results were obtained where possible from studies with combined adult and paediatric data; however, some papers were of poor quality and had limited results available
- Only papers from January 2015 to March 2021 were included in our review.

Introduction:

Endothelial dysfunction (ED) is an early predictor of cardiovascular disease(1). Negative alterations in endothelial physiology, also known as ED, cause the endothelium to lose its ability to promote vasodilation, fibrinolysis and anti-aggregation(2). It is the beginning of atherosclerosis formation which can lead to plaque progression and luminal narrowing(3). There is an imbalance between vasodilation and vasoconstriction, abnormal reactive oxygen species, and nitric oxide (NO) bioavailability(2). ED is a complication of cardiovascular risk factors such as smoking,

hypercholesterolemia, hypertension, hyperglycaemia and family history of premature atherosclerosis. ED can be caused by oxidative stress with loss of vaso-active or inflammatory homeostasis within the body's vascular system. It may be secondary to mechanical stimuli, for example increased intraluminal pressure within the blood vessel or metabolic factors such as hormones (oestrogen's vasodilation action)(4).

Damaged endothelium can release a cascade of substances which pose a risk of thrombosis, inflammation and ultimately atherosclerosis(5). ED in paediatric populations has been associated with several conditions including type 1 diabetes (T1D), type 2 diabetes (T2D), renal impairment, obesity and metabolic syndrome(6-9). In patients with T2D, obesity and metabolic syndrome, insulin resistance is one of the most importance factors contributing to ED(9). Metabolic syndrome is a pro-inflammatory state where dyslipidaemia, hyperuricemia, and hypertension occur and can predispose to ED(10).

ED can progress to atherosclerosis which is a chronic condition that poses severe risk of certain diseases including coronary artery disease, stroke and peripheral arterial disease. If detected early and specific patient modifications are made, the progression to permanent vessel damage may be halted. ED can be detected by invasive techniques assessing the coronary vessels or by non-invasive techniques via the peripheral circulation. The gold standard test would utilise coronary angiography and assess response to vasodilators. However, this is not feasible in practice as a screening tool, especially in paediatrics.

This review highlights the variety of conditions that Endothelial Peripheral Artery Tonometry (Endo-PAT) can be useful in paediatric patients. This systematic review will add to other reviews of endothelial function assessments in paediatric populations as it includes further studies and an increasing variety of paediatric conditions as well(11).

Endo-PAT 2000:

Endo-PAT 2000 is a non-invasive technology for measuring ED developed by Itamar Ltd. Non-invasive pneumatic probes which are placed on the both index fingers, which continuously records

pulse wave amplitude. A blood pressure cuff is inflated to occlude blood flow and response after deflation is recorded. The reactive hyperaemic index (RHI) is resulted following this mini-ischemic stress to the vessel. The pulse wave amplitude (PWA) is measured and computes a RHI result automatically. RHI is calculated as the ratio of average PWA divided by the average amplitude during the equilibration period. To compensate for any systemic changes, this ratio is normalized to a concurrent signal from the contralateral finger.

Numerous studies in both adult and paediatric literature reveal Endo-PAT's excellent reproducibility and reliability(12-14). However, RHI has limitations as a reliable method for defining ED, especially in paediatric patients due to the metabolic change's children go through throughout childhood including growth and puberty. There is no RHI cut off value in paediatric patients. In ED, the RHI is low and pulse amplitude is high. PAT also provides results on the peripheral augmentation index (PAT-AIx). Bonetti et al report a RHI of <1.35-1.49 as indicative of coronary ED in adults(14, 15).

Prior to Endo-PAT, ED had been assessed by flow-mediated vasodilation (FMD). 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 cuff and is highly dependent on nitric oxide (NO) bioavailability. ED is identified by less vasodilatation (reduced FMD) of the brachial artery. FMD is technically challenging to perform, user-dependent and requires training. FMD results macro blood vessel reactivity whereas Endo-PAT results micro, which may account for the challenges in comparing the two techniques. Endo-PAT is easier to set up, is automated and less user-dependent. It can be used at the patient's bedside, without extensive training required of the operator. Wilk et al reported that RHI correlated with FMD ($r = 0.35$, $P < 0.01$) however there are other studies who have not reported a correlation between the two techniques(16).

Objective:

A systematic review was conducted on the use of Endo-PAT 2000 in paediatric populations in assessing the risk of ED, with the aim of synthesising the literature, to determine a cohort of paediatric patients at high risk of ED and who may benefit from screening.

Methods:

A comprehensive systematic review was conducted to identify publications that investigated Endo-PAT 2000. All papers published from January 2015 to March 2021 in paediatric populations age birth to 16 years of age were analysed. PRIMSA study design was used.

The following scientific databases were searched: The Cochrane Database, MEDLINE EBSCO, EMBASE (Ovid), PUBMED and CINAHL EBSCO. The search was limited by to English studies. The search was limited by type of subjects (human), date (2015 to March 2021) and included all study types. Snowballing method was used. Authors of joint adult and paediatric papers were contacted by email to obtain separate paediatric data.

The database search was repeated several times using the combinations of keywords, MeSH terms and filters (child: birth-16 years). The following MeSH terms or key words were used for searching: Peripheral arterial tonometry, PAT test, endopat, adolescent, ado*, child, paediatric, pediatric, preschool, schoolboy, schoolgirl, boy, girl, teen, toddler, infant, baby.

Exclusion criteria were: 1. If a study used a different device for example 'Watch-PAT;' 2. If the study had no results. Inclusion criteria were: 1. Published in the English; 2. More than 50% of study subjects were in the paediatric age range; 3. Data relevant to paediatric age range children could be extrapolated from all data, where not all study subjects were children. A child was defined as up to 16 years, and this is consistent with PubMed's definition of a child. Where data relevant to children could not be extrapolated from the whole dataset, the study authors were contacted for additional information prior to study inclusion or exclusion.

Patient and public involvement:

No patient involved.

Data collection and analysis:

A total of 290 articles were obtained via the online database search (*Figure 1*: flow diagram). Following removal of duplicates, 158 articles remained. The second screening was conducted by 'Rayyan- systematic review software.' Two further duplicate articles were removed, with 156 remaining for review.

Two independent authors separately performed a blind screen on the 156 abstracts. 65 articles were initially excluded based on title or abstract: 37 adult studies, 18 'PAT' did not represent peripheral arterial tonometry (e.g. prism adaptation test, psychosocial assessment tool), 6 Watch-PAT, 2 sleep studies and 2 had no results available.

The remaining 91 articles were analysed viewing full text articles for further information. A further 20 were excluded as they did not fit inclusion criteria or have results to report. Some of these articles that included Endo-PAT 2000 in paediatrics did not have results for the systematic review but had conclusions that were relevant to the paper were referenced in the results section.

Twenty-eight authors of studies including both adults and paediatric patients were contacted twice by email to gather separate information on the paediatric participants. Twenty authors did not reply and were thus excluded. Eight authors replied: three providing results, four unable to give separate paediatric data and one author's research was on adult patients so was excluded. Three of the articles whose authors replied with data were included in our review. Four studies were obtained via snow balling searching.

A total of 50 articles were included in our results and are represented in tables 1-6. For each eligible study the following data was reported: author, year of publication, design of the study, population studied, control group (if available), RHI results.

Title, lead author	Year	Study design	Population: n=sample size, age; mean ± SD or median (range), [F/M]	Control group: n=sample size, age; mean ± SD or median (range), [F/M]	Results: RHI reported. If RHI not specified, we reported p/r values	Outcomes
Adolescents and young adults with	2015	Cohort prospective	n=73 T1D adolescents,	No controls.	56 (76.7%) had ED, with lower mean RHI	T1D adolescents had evidence of

type 1 diabetes display a high prevalence of endothelial dysfunction. Scaramuzza et al (17)		observational study. Results at baseline and after a 1-year follow-up	diagnosed > 1 year, 16.2 +/- 3.5 years, [F/M 25/48]		scores (1.26 ± 0.22 versus 2.24 ± 0.48, p < 0.0001). More with ED had abnormal cardiac autonomic tests (p = 0.02) and were more sedentary. After 1 year follow-up in 64/73 patients, 81.8% had ED, despite some improvement in HbA1c.	ED. Good metabolic control (HbA1c ≤7.5%) and regular physical activity might be protective. ED progression despite some improvement to HbA1c.
Alpha-Lipoic Acid and Antioxidant Diet Help to Improve Endothelial Dysfunction in Adolescents with Type 1 Diabetes: A Pilot Trial. Scaramuzza et al (18)	2015	Double-blind, randomized controlled trial – snow balling. Results at baseline and after follow-up	n=71 T1D patients, followed for at least 1 year, age 16.3 ± 3.4 years, [F/M 29/42]. (a) antioxidant diet 10.000 ORAC + alpha-lipoic acid; (b) antioxidant diet 10.000 ORAC + placebo;	(c) controls	3 double-blind study arms: (a) antioxidant diet 10,000 ORAC + lipoic acid: RHI 1.40 ± 0.68 vs 1.72 ± 0.66 (P<0.05) (baseline vs after 6 months). (b) antioxidant diet 10,000 ORAC + placebo: RHI 1.39 ± 0.41 vs 1.58 ± 0.40 (P>0.05). (c) Controls: RHI 1.58 ± 0.64 vs 1.54 ± 0.42 (P>0.05).	Improved RHI with alpha-lipoic acid in T1D patients.
Effect of metformin on endothelial function in overweight adolescents with type 1 diabetes (T1D). Nadeau et al(19)	2016	Conference abstract. Endo-PAT scores at baseline and 13 weeks.	Total n=70 overweight T1D patients. n= 41 on metformin (up to 2000 mg/day), 12-19 years (mean 15.8)	n=29 placebo group.	Mean baseline RHI 1.8 +/- 0.6 in metformin group and 1.7 +/- 0.6 placebo group. At 13 weeks, no significant change from baseline RHI (+0.1 in metformin vs. -0.0 in placebo, P = 0.08). Some improvement in endothelial function in males.	No significant RHI change with metformin overall but some improvement in overweight T1D males.
Assessment of biomarkers of inflammation and premature atherosclerosis in adolescents with type-1 diabetes mellitus. Babar et al (20)	2019	Cross-sectional study	T1D adolescents ≥12 years. Two groups based on different HbA1c ranges. (a) HbA1c ≥9.5% (n=25)	(b) HbA1c ≤8.5% (n=27).	PAT results were not significantly different between the groups. Pearson correlation showed a significant direct relationship between rising HbA1c and PAT (p=0.03, r=0.31).	Suboptimal glycemic control (rising HbA1c) causes early atherosclerosis.
Improvements in peripheral vascular function with vitamin D treatment in deficient adolescents with type 1 diabetes. Deda et al **(21)**	2018	Research article – snow balling. Tested at two different time points.	n=21 T1D patients followed for ~2 years. 25-OH-Vit. D levels < 37.5 nmol/L. Age 15.7 ± 1.4 years, [F/M 19/12]	Controls: matched age, sex and T1D.	After 4.8 ± 1.3 months of Vit. D supplementation RHI improved: 1.83 ± 0.42 vs 2.02 ± 0.68 (P<0.05).	Vit. D supplementation associated with improvement to endothelial function and reduced urinary inflammatory markers.
Non-alcoholic Fatty Liver Disease in Hispanic Youth with Dysglycemia:	2017	Cross-sectional study	n=23 overweight/ obese with NAFLD, age 15.2 ± 0.5 years.	n=13 overweight/ obese without NAFLD, age	NAFLD group had lower RHI (1.4 ± 0.05 vs 1.7 ± 0.09, p= 0.002).. Hepatic fat is	Hepatic fat and AST/ALT levels inversely related to RHI. If

Risk for Subclinical Atherosclerosis? Bacha et al (22)			n=12 prediabetes, n=11 T2D, [F/M 13/10]	15.7 ± 0.4 years. n=8 pre-diabetes, n=5 T2D, [F/M 3/10]	inversely related to RHI (r = -0.49, P = 0.002).	dysglycemia, NAFLD is associated with worse ED.
Endothelial function in youth: A Biomarker modulated by adiposity-related insulin resistance. Tomsa et al (23)	2016	Cross sectional study	Total n = 60. n=25 obese without DM, n=19 obese with impaired glucose tolerance, n=16 obese T2D but HB1Ac < 8%. Age 15.5 (0.2), [F/M 37/23]	n=21 normal weight, age 15.5 (0.2), [F/M 9/12]	RHI inversely related to % body fat (r = -0.29, P = .008), total (r = -0.37, P = .004), subcutaneous (r = -0.39, P = .003), and visceral abdominal fat (r = -0.26, P = .04).	Childhood obesity is associated with ED (lower RHI). RHI lower in obese and T2D. RHI negatively related with percentage body fat, WC, Leptin, TNF-alpha, blood glucose.
Circulating fibroblast growth factor-21 (FGF-21): A biomarker of subclinical atherosclerosis in obese youth with non-alcoholic fatty liver disease (NAFLD)? Bacha et al (24)	2017	Conference abstract	Obese adolescents with NAFLD, 15.4+/-0.3 years. n=13 normal glucose tolerance, n=19 prediabetes, n=16 T2D patients	Control group: no NAFLD. No difference in age/gender between groups.	Lower RHI in NAFLD group. High FGF-21 concentrations related to RHI (r=-0.33, p=0.03).	Increased FGF-21 in obese adolescents with NAFLD associated with insulin sensitivity and ED. FGF-21 may constitute a biomarker ED.
Assessment of Microvascular Function in Children and Adolescents with Diabetes and Obesity. Kochummen et al(25)	2019	Cross-sectional study	DM group. n=33 T1D with normal weight. n=8 obese T2D, age 12.7 (3.8) years, [F/M 25/16]	n=17 obese, non-DM children (normal BGL, BP and lipid profile), 12.8 (2.7) years, [F/M 9/8]	For every 1% increase in HbA1C, RHI decreased by 0.097 (P = 0.01). RHI of DM group with HbA1C <10% (1.70 ± 0.58) versus those with ≥10% (1.21 ± 0.19) (p= 0.02).	Poorly-controlled DM (HbA1C ≥ 10%) had lower RHI. RHI negatively related with HbA1C. RHI similar between obese and normal weight with T1D. Similar between T1D and T2D.
Free Vitamin D: Relationship to Insulin Sensitivity and Vascular Health in Youth. Bacha et al (26)	2019	Cross-sectional study. Comparison across tertiles of free 25(OH)D concentrations	n=79, age 15.4 ± 0.2 years, [F/M 45/34]. n=30 overweight. n=31 overweight with prediabetes	n=18 normal weight and normal glucose tolerance.	The lowest tertile group had lower RHI (1.42 ± 0.06, 1.54 ± 0.06, and 1.77 ± 0.09, P = 0.002), compared with the second and third tertiles.	Youth with low free 25(OH)D or BioD concentrations have lower insulin sensitivity and worse endothelial function.
Urine Albumin-to-Creatinine Ratio (UACR): A Marker of Early Endothelial Dysfunction in Youth. Bartz et al(27)	2015	Control study. Fasting UACR analysed.	n=25 overweight (OW) with normal glucose tolerance, 15.6 ± 0.2 years, [F/M 17/8]. n=20 OWwith prediabetes, [F/M 11/9].	n=13 normal weight, 16.3 ± 0.4, [F/M 7/6].	Normal weight group RHI 1.84 ± 0.1. OW with normal glucose tolerance 1.56 ± 0.1. OW with prediabetes 1.56 ± 0.1 (P = .04). UACR was related to RHI (r = -0.33, p = .01).	UACR is an early marker of endothelial dysfunction in youth, independent of glycemia.

170 Table 1: Total of 11 studies included. Endo-PAT 2000 in paediatric type 1 diabetes mellitus (T1D) patients (5

171 studies), type 2 diabetes and prediabetes (6 studies). Reactive hyperemia index (RHI), type 1 diabetes mellitus

(T1D), type 2 diabetes mellitus (T2D),, endothelial dysfunction (ED), Oxygen radical absorbance capacity units (ORAC), non-alcoholic fatty liver disease (NAFLD), overweight (OW), Urine Albumin-to-Creatinine Ratio (UACR).

Title, lead author	Year	Study design	Population: n=sample size, age; mean ± SD or median (range), [F/M]	Control group: n=sample size, age; mean ± SD or median (range), [F/M]	Results: RHI reported. If RHI not specified, we reported p/r values	Outcomes
Effects of a dietary strawberry powder on parameters of vascular health in adolescent males. Djurica et al (28)	2016	Randomised, double-blind, cross-over study	n=15 OW/obese males, 14-18 years (mean 16). 1-week daily 50g freeze-dried strawberry powder (FDSP) Before/after nitrate/nitrite levels measured.	n=10 control powder, 14-18 years (mean 16).	Acute plasma nitrate/nitrite levels increased 1 h after consuming the FDSP (P<0.001). When nitrate levels increased after FDSP intake compared to controls, had an increase in RHI (P=0·014).	Strawberries can provide vascular health benefits to OW/obese adolescent males.
Flow-mediated dilation in obese adolescents: Correlation with waist circumference (WC) and systolic blood pressure (SBP). Hussid et al (29)	2018	Case control study	n=20 obese patients, median age 14 years	n=10 normal weight, median age 15 years, paired for gender	No RHI difference between groups. 35% obese group had metabolic syndrome, none in control group. OSA in 86.6% obese and 50% of normal weight group.	Obese group had evidence of ED and metabolic syndrome. Increased WC and SBP seem to be related to this finding.
Improvement of microvascular endothelial dysfunction induced by exercise and diet is associated with microRNA-126 in obese adolescents. Donghui et al (30)	2019	Quasi-randomized study	n=57 obese male adolescents, 12-18 (15.38 ± 2.82) years, [F/M = 0/57], 6-week exercise program with dietary intervention.	n=10 normal weight adolescents, 15.38 ± 2.82 years, [F/M 0/10], maintained sedentary	Obese group RHI 1.43 (0.35) vs controls 1.67 (0.36) (p< 0.05). After 6 weeks RHI increased (p <0.01) and microRNA-126 decreased (p<0.01). miRNA-126 positively correlated with ΔRHI (r = 0.69, p<0.05).	RHI improved in obese group after exercise and diet interventions. Findings might be related to changes in serum miRNA-126.
Distribution of peripheral arterial stiffness and endothelial function as well as their correlations with cardiovascular risk factors in children and adolescents. Mu et al (31)	2016	Cross-sectional population-based study, conference abstract	n=94 obese, 7-17 years, used automatic waveform analyser (BP-203RPE-I) and Endo-PAT 2000.	n=452 normal-weight	In normal weight group, RHI increased with age (r=0.33, P<0.01; r=0.36, P<0.01). RHI positively correlated with BMI (r=0.10, P=0.018) but negatively with DBP (r=-0.10, P= 0.016).	RHI increased along with age. Arterial stiffness and endothelial function continued to develop in the normal weight group.
Urinary biomarkers as indicator of chronic inflammation and	2017	Control study, research	n=63 total. n=14 overweight (OW), n=29 obese, age	n=20 normal weight (NW), age 13.9 (2),	There were no differences in RHI levels: NW 1.6 (0.1),	No significant correlation between RHI

endothelial dysfunction in obese adolescents. Singh et al(32)		article	13.8 (2.4), [F/M 23/20]	[F/M 8/12]	OW 1.66 (0.1) and obese 1.67(0.1). NW girls RHI 1.9 vs NW boys 1.25.	and urinary markers. RHI higher in NW female adolescents.
Prevalence of Type D personality in obese adolescents and associated cardiovascular risk. Bruyndonckx et al(33)	2018	Control study, conference abstract	Obese adolescents- no definite numbers	Healthy normal weight children	Positive correlation in obese adolescents between negative affectivity and vascular stiffness (r= 0.28; p= .04)	Obese adolescents have worse cardiovascular risk profile with ED.
Endothelial function and arterial stiffness in obese adolescents - A relation to barorefex function. Czippelova et al(34)	2017	Conference abstract	n=22 obese, 15.28 +/- 2.8 years, [F/M 10/12]	n=22 non-obese, 15.98 +/- 2.46 years, [F/M 10/12]	No significant difference in RHI (p = 0.473). Baro-reflex sensitivity was also calculated.	No difference in RHI between groups. Findings require further study.
Obesity in children and adolescents: A relation to endothelial function and arterial stiffness. Czippelova et al(35)	2016	Conference abstract	n=16 obese adolescents,15.22 +/- 2.2 years, [F/M 7/9]	n=16 non-obese, 16.22 +/- 1.5 years, [F/M 7/9]	Significant difference in RHI (p = 0.018) with RHI higher in obese group (1.66 +/- 0.28 vrs 1.4 +/- 0.25).	Less early atherosclerotic changes in obese group; in contrast to expectations. Findings require further study.
Preclinical vascular alterations in obese adolescents detected by Laser-Doppler Flowmetry technique. Fusco et al (36)	2020	Research article	n=22 obese adolescents, 14.11 +/-2.53, [F/M 13/9]	n=24 normal-weight, 15.2 +/- 1.56, [F/M 11/13]	Similar RHI between obese and non-obese groups (1.80 +/- 0.62 and 1.86 +/- 0.51).	RHI did not differ between groups. RHI did not correlate with LDF.
Impaired endothelial function in adolescents with overweight or obesity measured by peripheral artery tonometry. Pareyn et al (37)	2015	Cross sectional study	n=27 overweight (OW)/obesity, 14.7 (13.0–16.4) years, [F/M 11/16]	n=25 normal weight controls, 15.5 (13.9–16.2) years, [F/M 13/12]	RHI normal weight 1.88 (1.7-2.4) vs OW/ obese 1.5 (1.3-1.9) (p< 0.05). Lower RHI if OW/obese (p = 0.027). RHI positively correlated with age and tanner stage (P< 0.05).	ED and higher baseline pulse amplitude in OW group.
C-type natriuretic peptide (CNP) plasma levels and whole blood mRNA expression show different trends in adolescents with different degree of endothelial dysfunction. Del Ry et al(38)	2020	Research article - snow balling	n=16 primary obesity, not DM, age 13.3 (0.5) years, [F/M 8/8].	n=24 normal weight, age 14.3 (0.4) years, [F/M 14/10].	RHI normal weight 2.1 (0) vs obese 1.4 (0) (P< 0.005). RHI negatively associated with CNP and diastolic BP (P< 0.005).	RHI significantly lower in obese group. RHI negatively related with CNP, DBP, fat mass and HbA1C.
C-type natriuretic peptide (CNP) is closely associated to obesity in Caucasian adolescents. Del Ry et al (39)	2016	Research article - snow balling	n=10 overweight, age 12.8 (1.6) years, [F/M 5/5]. n=45 obese, 12.8 (1.6) years, [F/M 19/26]	n=27 normal weight, age 12.8 (1.4) years, [F/M 14/13]	Normal weight group RHI 2.1 (0.2) vs OW 1.6 (0.4) (P< 0.05). Normal weight vs obese group RHI 1.4 (0.3) (P< 0.005). RHI negatively associated with CNP (P< 0.005).	RHI lower in overweight/ obese groups. CNP negatively related with RHI.
Arterial Stiffness and Endothelial Function in Young Obese Patients -	2019	Research article	Author contacted for separate paediatric data.	n=15 controls, age <16 years, [F/M 7/8]	RHI control vrs obese groups: 1.320 ± 0.427 and 1.457 ± 0.280. RHI	RHI is influenced by vascular tone

Vascular Resistance Matters. Czippelova et al (7)			n=16 obese group, age <16 years, [F/M 7/9]		obese girls and boys: 1.410 ± 0.253 and 1.494 ± 0.308. RHI control girls and boys: 1.171 ± 0.210 and 1.436 ± 0.524	and resistance. RHI in obese positively related with SVR.
Cardiovascular adaptations after 10 months of intense school-based physical training for 8- to 10-year-old children. Larsen et al (40)	2018	Randomised control study	n=93 small-sided games group, 9.3+/-0.4 years. n=83 circuit strength training group, 9.3+/-0.3 years (10-16 years)	n = 115 controls, 9.3+/-0.3 years	No significant differences in RHI. Pubertal status is a main predictor of RHI; positive correlation between Tanner stages and RHI.	10 months of regular exercise per week decreased DBP and had effects on cardiovascular health.

177 Table 2: Endo-PAT 2000 in paediatric patients who are overweight (OW)/obese (14 studies). Reactive

178 hyperemia index (RHI), freeze-dried strawberry powder (FDSP), endothelial dysfunction (ED), overweight

179 (OW), normal weight (NW), waist circumference (WC), C-type natriuretic peptide (CNP), Laser-Doppler

180 Flowmetry (LDF).

Title, lead author	**Year**	**Study design**	**Population: n=sample size, age; mean ± SD or median (range), [F/M]**	**Control group: n=sample size, age; mean ± SD or median (range), [F/M]**	**Results: RHI reported. If RHI not specified, we reported p/r values**	**Outcomes**
Nocturnal blood pressure dipping as a marker of endothelial and cardiac function in pediatric-onset systemic lupus erythematosus (SLE). Chang et al (8)	2020	Cross-sectional study – author contacted for separate paeds data	n=20, 9-19 years (mean 16.5), (7 were age 16 or under). Average disease duration 3.2 years (± 2.1). [F/M 17/3]	Separated into 2 groups based on nocturnal BP dipping status.	Mean RHI for n=7 (aged 16/under): 0.529. 22% had ED. Reduced diastolic BP dipping was associated with poorer endothelial function (r 0.5, p = 0.04).	Isolated nocturnal BP non-dipping is associated with ED and atherosclerotic changes. Potential role for routine ABPM for youth with SLE.
Physiological changes in blood pressure (BP) impact peripheral endothelial function during adolescence. Deda et al (41)	2015	Control study. Assessing association between RHI and known cardiovascular risk factors.	n =90 healthy adolescents to assess normal RHI response, 14.2±1.91 years, [F/M 46/44].	No controls	Mean arterial pressure significantly associated with RHI (p=0.01). Positive correlation RHI and age in females (r=0.33, p<0.02). RHI correlated with pubertal status: males (r=0.411, p=0.03), females (r=0.36, p=0.03).	Physiological changes in BP significantly impact RHI results.
Endothelial Function and Arterial Stiffness Relate to Functional Outcomes in Adolescent and Young Adult	2016	Cross-sectional prospective observational study	n=60, 8-25 years (mean 13.9±4.1), [F/M 29/31]	No controls	PAT derived baseline pulse amplitude (P<0.05) negatively associated with minute ventilation to C02 ratio. RHI 1.2 (0.2–4.8).	Worse vascular measures associated with worse functional measures. Increased arterial stiffness and decreased endothelial function are associated with lower

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Fontan Survivors. Goldstein et al (42)						aerobic capacity, physical activity, and QOL in Fontan survivors.
Natural history of vascular function in adolescent and young adult Fontan survivors: A longitudinal ass essment of endothelial function and arterial stiffness. Goldstein et al (43)	2017	Prospective single-centre longit udinal study, conference abstract. Paired testing at a mean interval of 2.0 +/- 0.2 years of Fontan survivors.	n=50, mean 13.7 +/- 4.2 years, [F/M 23/27]	No controls	Decreases in RHI (0.002 +/- 0.01/yr) were not significant. BMI was a predictor for RHI (R 0.17, p=0.007).	Vascular function does not change uniformly in Fontan survivors. Changes in vascular function do not relate to changes in aerobic capacity but are associated with changes in anthropometric measures and O2 saturation.
Vascular function long term after Kawasaki disease: another piece of the puzzle? Pinto et al (44)	2017	Single-centre prospective study	n=43 Kawasaki patients, age >11 years, diagnosed >5 years ago, with no coronary lesions or any other risk factors for cardiovascular disease.	n= 43 control group of individuals without cardiovascular risk factors.	Kawasaki patients had decreased RHI compared with controls (1.59±0.45 versus 1.98±0.41; p<0.001).	Children with Kawasaki disease may have long-term sequelae, even when there is no detectable coronary artery involvement in the acute stage of disease.
Endothelial function in children with a history of Henoch Schonlein purpura (HSP). Butbul Aviel et al (45)	2017	Observation al prospective study	n=19 with HSP, 13.5 ± 3.9 years, [F/M 8/11]	n=23 healthy children, 12.8 ± 4.5 years, [F/M 7/16]	Mean RHI 1.81 study group and 1.87 control group (p = 0.18). RHI higher in patients who had endothelial function measured >6 years since HSP diagnosis compared with <6 years (1.98 + 0.74 vs. 1.38 ± 0.43 P = 0.037).	This study suggests that HSP causes short term endothelial dysfunction that improves with time.
Reactive hyperaemia index and detection of endothelial dysfunction in children with familial hypercholesterola emia (FH). Jehlicka et al(46)	2015	Conference abstract	n=24 with FH, 13.9+/-2 years. Biochemical markers of endothelial function were assessed.	n=17 healthy controls, 15.2+/-2.2 years	Significantly lower RHI in FH group (1.63+/-0.50 and 2.03+/-0.54; p<0.05). Lower RHI and elevated E-selectin in children with FH.	Possible relationship of ED in children with FH, highlighting the importance of early detection of ED when the atherosclerotic process is still reversible.

Table 3: Endo-PAT 2000 in paediatric patients with cardiac and vascular conditions (7 studies). Reactive hyperemia index (RHI), waist circumference (WC), systolic blood pressure (BP),, peak VO (peak 02 consumption), quality of life (QOL), systemic lupus erythematosus (SLE), ambulatory blood pressure monitoring (ABPM), quality of life (QOL), Henoch Schonlein purpura (HSP), familial hypercholesterolaemia (FH).

Title, lead author	Year	Study design	Population: n=sample size, age; mean ± SD or median (range), [F/M]	Control group: n=sample size, age; mean ± SD or median (range), [F/M]	Results: RHI reported. If RHI not specified, we reported p/r values	Outcomes
Vascular function in asthmatic children and adolescents. Augusto et al (47)	2017	Cross-sectional controlled study	n=19 asthmatic patients, age 13.6 ± 0.6 years. [F/M 0/19]	n=18 controls. 14.9 ± 0.7 years. [F/M 0/18]	RHI were similar between groups (p = 0.23). Asthmatic group RHI did not correlate with the different variables.	The increased AIx@75 without changes in RHI in asthmatic patients could mean that an early detection of vascular impairment may precede ED.
The effect of weight loss on endothelial function and sleep disordered breathing (SDB) in obese children. Ysebaert et al (48)	2018	Conference abstract. Baseline and reassessed after 6-month weight loss programme.	n=62 obese, age 11-19 (mean 15.8) years, [F/M 20/42]	No controls.	Baseline: 39% had SDB. After 6 months: 86% had resolution of earlier diagnosed SDB. All had significant improvement of endothelial function after programme (p < 0.001). No correlations between SDB and improvement in endothelial function found.	Endothelial function significantly improves after weight loss.
Polysomnographic correlates of endothelial function in children with obstructive sleep apnoea (OSA). Zhang et al (49)	2018	Cross sectional study	n=121 mild OSA, 6.2 ± 1.6 years, [F/M 37/84]. n=127 moderate-severe OSA, 6.0 ± 1.6 years, [F/M 31/96]	n=107 primary snorers (PS), age 6.4 ± 1.8 years, [F/M 37/70]	OSA groups lower RHI than PS (P < 0.001, P = 0.001). RHI positively correlated with age (r = 0.17, P = 0.002), BMI z score (r = 0.14, P = 0.008) and oxygen saturation (r = 0.15, P = 0.006).	Children with OSA are at increased risk for abnormal endothelial function than habitually snoring children.
Endothelial dysfunction in children with obstructive sleep apnoea syndrome (OSAS). Xu et al(50)	2020	Cross sectional study	n=248 OSAS, age 3-11 years	n=107 primary snorers (PS). No significant differences in age/gender.	OSAS had lower RHI 1.1±0.1 vrs 1.2±0.2 (P<0.01). RHI independently correlated with age, gender, obstructive apnoea hypopnea index, oxygen desaturation index (*P*<0.01).	OSAS have significant ED compared with PS. Frequent arousals due to obstructive respiratory events during sleep may be a candidate risk factor for ED.

Table 4: Endo-PAT 2000 in paediatric patients with respiratory conditions (4 studies). Endothelial dysfunction (ED), reactive hyperaemia index (RHI), heart rate-corrected augmentation index (AIx@75), primary snorers (PS), obstructive sleep apnoea (OSA), obstructive sleep apnoea syndrome (OSAS).

Title, lead author	Year	Study design	Population: n=sample size, age; mean ± SD or median (range), [F/M]	Control group: n=sample size, age; mean ± SD or median (range), [F/M]	Results: RHI reported. If RHI not specified, we reported p/r values	Outcomes
Do self-reported stress and depressive symptoms effect endothelial function in healthy youth? The LOOK longitudinal study. Olive et al(51)	2018	Longitudinal cohort study. LOOK longitudinal study, who were followed through to adolescence (16 years).	n=203, 7.6 ± 0.3 years, [F/M 111/92].	No controls.	All relationships occurred in the hypothesised direction, but no cross-sectional or prospective evidence of early psychological stress or depression was associated with ED (all $p > 0.05$).	Contrast to previous findings in adolescents, little evidence between current or previous psychosocial stress or depression and endothelial function in 16-year-old adolescents.
Cerebrovascular reactivity is associated with peripheral endothelial function (EF) among adolescents. Urback et al(52)	2016	Conference abstract	n=11 with bipolar disorder. EF measured by PAT and cerebrovascular reactivity (CVR) by blood-oxygen-level dependent fMRI.	n=35 healthy controls	EF was positively correlated with CVR in grey matter (r=0.41, p=0.012), and a peak voxel in the left-medial-frontal gyrus (r=0.35, p=0.036).	Breath-hold CVR and peripheral EF are linked, suggesting that vascular function may be a multi-systemic phenotype. EF may be a potential proxy for cerebral blood vessel function with greater accessibility and lower cost than fMRI.
Retinal-vascular photography as a window into the cardiovascular and neurocognitive burden of adolescent bipolar disorder (BD). Naiberg et al (53)	2017	Cross-sectional study, author emailed for separate paeds data-most were teenagers	n=30 with bipolar disorder, 17.97±1.86 years	n=32 healthy controls, 16.00±1.62 years	In BD group, higher endothelial function associated with higher arterio-venular ratio (r=0.375, p=0.041).	Retinal photography may help assessing cardiovascular and neurocognitive burden of BD.
Impact of psychological health on peripheral endothelial function and the HPA-axis activity in healthy adolescents. Chen et al(54)	2017	Longditudinal 3-year follow-up study. Baseline and three-year follow-up.	n=162, 14.5 ± 1 years. [F/M 94/68].	No controls.	Lower peripheral endothelial function was associated with high level of anger ($\beta = -0.332$, $p = 0.018$) and disruptive behaviour ($\beta = -0.390$, $p = 0.006$) over three years in males, but not in females, adjusted for covariates.	High amounts of negative emotions may have adverse effects on peripheral endothelial function and regulation of the HPA-axis activity. High level of self-concept might be protective.

196 Table 5: Endo-PAT 2000 in paediatric patients with psychiatric conditions (4 studies). Endothelial dysfunction

197 (ED), endothelial function (EF), cerebrovascular reactivity (CVR), bipolar disorder (BD), functional magnetic

198 resonance imaging (fMRI), hypothalamic–pituitary–adrenal HPA.

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Title, lead author	Year	Study design	Population: n=sample size, age; mean ± SD or median (range), [F/M]	Control group: n=sample size, age; mean ± SD or median (range), [F/M]	Results: RHI reported. If RHI not specified, we reported p/r values	Outcomes
Vascular endothelial function in inflammatory bowel disease (IBD). Winderman et al(55)	2018	Case-control study	n=16 with IBD (all in clinical remission), age 16.7 +/- 2.6 years, [F/M 8/7]	n=16, age 15.1 +/- 2.8 years, [F/M 7/8]	RHI IBD vs controls 1.66 vs 2.02 (P =0.036). IBD group had a mean RHI within the range associated with VD risk in adults (1.67).	IBD group lower RHI compared with controls. IBD patients may need to be monitored for thromboembolic phenomena.
Endothelial health in childhood acute lymphoid leukaemia (ALL) survivors: pilot evaluation with peripheral artery tonometry. Ruble et al (56)	2015	Case control study	n=16 ALL survivors, age 8-20 years (12.9+/-0.9), [F/M 8/8].	n=16 healthy sibling pairs 13.8 (0.9), [F/M 10/6].	Both groups similar in cardiovascular risk measures but survivors had lower RHI (1.54 vs. sibling 1.77; P=0.0474).	Evidence of poorer vascular health in cancer survivors.
Microvascular endothelial function in Japanese early adolescents. Odanaka et al (57)	2017	Control study	n=157 healthy adolescents divided by gender. Females n=82, median age 14 (1), 13.7 ± 0.9 years	Males n= 75, median age 14 (2) years	No difference in RHI according to sex: boys and girls 1.85 ±0.6, 1.82 ±0.66 and 1.87± 0.54. RHI was significantly associated with systolic and diastolic BP, and had no correlation with anthropometric parameters and arterial stiffness markers.	RHI among adolescents were similar to those reported in previous studies on children and early adolescents.
Endothelial Dysfunction and the Effect of Arginine and Citrulline Supplementation in Children and Adolescents With Mitochondrial Diseases. Al Jasmi, et al (58)	2020	Case control study	9 participants, age 6-17 years (mean 9.6).	3-15 years (mean 9.4). Baseline endothelial dysfunction was assessed in controls.	Lower RHI with mitochondrial diseases. RHI increased with arginine or citrulline supplementation	Supplementation with NO precursors may improve ED by enhancing NO production. First study to use Endo-PAT methodology in mitochondrial diseases.
Assessment of traditional and non-traditional risk factors for premature atherosclerosis in children with juvenile dermatomysoitis (JDM) and pediatric controls. Wahezi et al (59)	2020	Retrospective controlled study	n=40 JDM, age 6-22 (mean 12.4± 4.1) years, [F/M 28/12]	n=20 controls, age 12.7± 3.9 years, [F/M 14/8]	RHI controls 1.43 [1.2, 1.7] and JDM 1.57 [1.2,1.9]. If controlled for lipoprotein A (atherogenic confounder), JDM patients had 41% RHI increase, thus indicating less ED compared to controls.	Rheumatological childhood disorders may be at increased risk of developing ED, but sociodemographic factors may have a greater role in developing cardiovascular disease.
Vascular Health of Children Conceived via	2019	Cross-sectional pilot study	n=17 IVF children, 10-14 years. Also used	Compared to published norms or to	Mean Endo-PAT index in the IVF cohort was 1.66+/-0.52, 71% had	Children conceived by IVF seem to have evidence of abnormal

In Vitro Fertilization (IVF). Zhang et al (60)			carotid ultrasound and pulse wave velocity measurements.	historical Stanford controls	abnormal values (<1.9). Mean RHI was not significantly different between IVF and controls.	vascular health.
Endothelial dysfunction in South African youth living with perinatally acquired human immunodeficiency virus (PHIV) on antiretroviral therapy. Mahtab et al (61)	2020	Case control study	n= 431 PHIV, median 14.1 (12.8, 15.5) years, [F/M 213/218]	n=93 without HIV, median 13.9 (12.1, 15.3) years, [F/M 53/40]	PHIV had higher rates of ED (50% vs 34%; P = .01); relationship persisted after adjusting for age, sex, BMI, high BP, high cholesterol (RR, 1.43; P =0.02). PHIV, CD4 count, viral load and current ART class were not associated with ED after adjustment.	PHIV appear to have increased risk of ED. These findings have important implications as HIV has increased risk of premature CVD and complications.
Soluble CD14 (sCD14) is associated with endothelial dysfunction in South African youth on ART. Dirajlal-Fargo et al (62)	2020	Case control study	n=283 perinatally acquired HIV (PHIV), 9-14 years.	n=69 age-matched without HIV	PHIVs had lower RHI despite viral suppression (RHI=1.36 vs 1.52, p<0.01). sCD14 at 24 months correlated with ED (p≤0.04). PHIV with ED, sCD14 was associated with lower RHI (β-0.05, p=0.01).	Higher sCD14 is independently associated with ED in PHIVs.
Role of insulin resistance and hyperandrogenemia in early vascular dysfunction in adolescents with PCOS. Bartz et al (63)	2015	Conference abstract	n=14 PCOS adolescents PCOS (on no treatment).	n=7 non-PCOS. Both groups had similar age, tanner stage, race, glucose tolerance status.	Despite higher peripheral and hepatic insulin resistance with PCOS, RHI is not significantly lower when compared with controls of similar total body and abdominal adiposity.	PCOS has evidence of increased vascular inflammation. Hyperandrogenemia and insulin resistance may play an important role in vascular inflammation.
Endothelial Function in Children and Adolescents Is Mainly Influenced by Age, Sex and Physical Activity-An Analysis of Reactive Hyperemic Peripheral Artery Tonometry. Mueller et al (64)	2017	Randomised controlled study, Leipzig School Project followed over 5-year period.	n=931 RHI measurements in 445 students, age 10-17 years (baseline 11.66±0.93). n=247: 60 minutes physical exercise (PE) daily (intervention group).	n=181: 2 units of 45 minutes PE weekly (control group).	Higher RHI in the intervention group: 0.09 [-0.05, 0.23]. Increase RHI from 1.53±0.42 in the youngest to 1.96±0.59 in the oldest students. This increase adjusted by age and sex was estimated as 0.11 [0.08, 0.14] per year.	If Endo-PAT is used for research in adolescents, age and sex must to be taken in account when reporting RHI results.

201 Table 6: Endo-PAT 2000 in paediatric patients with other miscellaneous paediatric conditions (10 studies).

202 Reactive hyperemia index (RHI), endothelial dysfunction (ED), inflammatory bowel disease (IBD), acute

203 lymphoid leukaemia (ALL), nitric oxide (NO), perinatally acquired human immunodeficiency virus (PHIV),

204 In Vitro Fertilization (IVF), soluable CD 14 (sCD14), polycystic ovarian syndrome (PCOS), physical exercise

205 (PE).

Results:

Endothelial dysfunction in paediatric diabetes mellitus patients (Table 1):

Five studies involve only type 1 diabetes (T1D) patients (*Table 1*). 2/5 studies reported lower RHI results in the T1D group(17, 20). One study which included only adolescent patients, reported RHI negatively correlates with impaired metabolic control and subclinical signs of autonomic neuropathy(17). They concluded that good metabolic control (HbA1c ≤7.5%) and regular physical activity might be protective against ED. One study reports an improved RHI result with an alpha-lipoic acid and antioxidant diet(18). Nadeau et al reported no significant RHI change with metformin overall but some improvement in overweight T1D males(19). Barber et al report suboptimal glycaemic control causes early atherosclerosis(20). One study noted an improvement in RHI post vitamin D supplementation in T1D patients with vitamin D deficiency(21).

6 studies focused on type 2 diabetes (T2D) and impaired glucose tolerance or 'prediabetes.' Tomsa et al note a link between insulin resistance and obesity by utilising Endo-PAT(23). They also noted that RHI is higher if HbA1c is less than 5.5%(23). Two studies compare on Non-alcoholic fatty liver disease (NAFLD), T2D and prediabetes patients(22, 24). If dysglycemia, NAFLD is associated with worse endothelial function. Circulating FGF-21 levels are elevated in obese youth with NAFLD and are associated ED and therefore may be a biomarker for ED(24). Bartz et al report urine albumin creatinine ratio (UACR) may be an early marker of ED independent of glycemia(27). Endothelial dysfunction may mediate the link between obesity-related insulin resistance and early microalbuminuria.(27). Kochummen *et al* reported a mean RHI in obese adolescents without diabetes was similar to T1D and T2D patients(25). One study noted an improvement in RHI post vitamin D supplementation in T1D patients with vitamin D deficiency(21). Another study noted lower vitamin D concentrations are associated with lower insulin sensitivity and worse endothelial function (26).

Endothelial dysfunction and Obesity (Table 2):

14 studies describe the use of Endo-PAT 2000 in overweight or obese patients (*Table 2*). Studies included measurement of the following parameters: BMI, T1D, T2D, gender, pubertal stage, age, blood pressure values, non-alcoholic fatty liver disease, obstructive sleep apnoea (OSA), insulin, plasma glucose levels, inflammatory markers (urinary markers, CNP, micro-RNA-126, E-Selectin). In numerous studies, RHI was significantly lower in obese groups (7, 25, 28-31, 38, 39, 65). ED may mediate the link between obesity-related insulin resistance and early microalbuminuria(27). Exercise and diet control improves glycolipid metabolism(40). Two studies by Czippelova et al did not find a lower RHI in obese groups, but recommended further studies(34, 35). Noma et al (2017) report the beneficial effects of exercise in paediatric patients and is an important message in reducing future endothelial complications(66). Fusco et al noted pre-clinical microvascular changes in obese patients compared to controls using LDF but noted no RHI change(36).

Endothelial dysfunction in cardiac and vascular conditions (Table 3):

7 studies report the use of Endo-PAT and cardiovascular conditions (*Table 3*). Lower RHI is seen with patients with familial hypercholesterolaemia(46). Studies assess ED in patients with systemic lupus erythematosus (SLE) and Henoch Schonlein purpura (HSP)(8, 45, 67). Negishi et al (2016) used Endo-PAT to compare Fontan survivors and healthy controls. The Fontan patients were aged 15 to 32 years. Mean RHI 0.56+ /- 0.26 in Fontan patients and 0.78+ /- 0.31 in controls (p= 0.09). RHI in Fontan patients was associated with diastolic blood pressure, heart rate and haemoglobin A1c level(68). Endothelial function in Fontan patients was associated with abnormal glucose tolerance and arterial stiffness and therefore concluded that glucose regulation might be a potential target to improve ED in this cohort. Nozaki et al (2018) assessed ED in conduit and resistance arteries and used FMD and Endo-PAT in paediatric patients with repaired coarctation of aorta(69).

Endothelial dysfunction in respiratory conditions (Table 4):

4 studies used Endo-PAT in respiratory conditions (*Table 4*). Augusto et al noted an increased augmentation index (AIx) without changes in RHI in asthmatic patients (47). One study reported an improvement in sleep disordered breathing post weight loss and also, endothelial function

significantly improved after weight loss(48). Two studies report children with OSA compared to habitual snorers are at increased risk for ED(49, 50). Frequent wakening due to obstructive respiratory events may be a risk factor for ED in OSA.

Endothelial dysfunction and psychological conditions (Table 5):

4 studies report the use of Endo-PAT in psychiatric conditions (*Table 5*). Potential limitations in this area are self-reported methods for detecting psychological distress of children, for example in the LOOK longitudinal study(51). Naiberg et al (2017) utilised retinal vascular photography as a proxy for cerebral microvasculature, and Endo-PAT to assess cardiovascular and neurocognitive burden in adolescents with bipolar disorder (BD)(53). In the BD group, better endothelial function was associated with higher arterio-venular ratio (r=0.375, p=0.041). Olive L.S. (2017) published 'The emerging field of paediatric psycho-cardiology' highlighting the importance of the childhood origins of adult CVD(70). This article highlights that psychological distress can influence CVD risk, directly by physiological change that can negatively impact the integrity of the cardiovascular system.

Endothelial dysfunction and other paediatric conditions (Table 6 – Miscellaneous)

Childhood cancer survivors:

There is evidence of ED in cancer survivors (*Table 6*)(56). Chemotherapy causes cardiomyocyte damage and also negatively affects endothelial function. Broberg et al (2018) utilised Endo-PAT in childhood cancer survivors and noted a lower RHI in this cohort compared to controls(71). Broberg et al (2016) identified one-third of cancer survivors (31.2%) compared to 8% of controls (p= 0.02) had ED in their study(72). They concluded this may be a useful screening tool of cardiovascular disease in asymptomatic cancer survivor patients. Pao et al (2018) assessed the relationship between blood pressure and ED using Endo-PAT in haematopoietic stem cell transplant recipients. Hypertension on ambulatory blood pressure monitoring (p= 0.045) and blunted nocturnal dipping (p= 0.04) were associated with a lower Endo-PAT scores(73).

Autoimmune conditions:

Children with autoimmune diseases may have a high tendency to develop ED which was highlighted in a study using a novel technique(74). Atherosclerosis is an emerging cause of morbidity and mortality in patients with rheumatological conditions such as juvenile idiopathic arthritis, SLE and dermatomyositis. Borenstein-Levin et al assessed a cohort with autoimmune conditions compared to controls: 29% in the study group had ED compared to 6% ($p < 0.05$)(74). Chang et al noted nocturnal blood pressure (BP) non-dipping is associated with ED in SLE patients highlighting a potential role for ambulatory BP monitoring in these patients(*Table 3*)(8).

Metabolic diseases:

Yano et al research in Fabry disease patients demonstrated that early diagnosis of ED can help determine the timing of initiating enzyme replacement therapy(75). Utilizing RH-PAT as a screening tool for early renal involvement may be helpful as it may detect abnormalities even prior to microalbuminuria(76). This can provide guidance on enzyme replacement therapy which is required to prevent irreversible progressive renal failure. Al Jasmi et al research in mitochondrial diseases reported that arginine or citrulline supplementation may improve ED, which provides evidence that these amino acids may be therapeutic (*Table 6*)(58).

Inflammatory bowel disease:

One study (*Table 6*) highlights that IBD patients had lower RHI compared with controls(55). Petr et al (2014) provided evidence of increased ED in children with Crohn's disease compared to healthy controls(77). RHI values were significantly lower in the patients with Crohn's than controls ($p < 0.05$).

Infectious diseases:

Dirajlal-Fargo et al used Endo-PAT to assess ED in human immunodeficiency virus (HIV) patients (*Table 6*)(62, 78). Perinatally acquired HIV patients appear to have higher levels of ED (RHI 1.34 (1.20, 1.42) compared with controls (1.52 (1.27, 1.80) ($p<0.01$))(78). The pathogenesis of severe

Plasmodium vivax malaria is poorly understood. ED and reduced nitric oxide (NO) bioavailability characterize severe falciparum malaria. Barber et al (2016) identified that endothelial function was impaired in proportion to disease severity. Those with severe vivax malaria, non-severe and healthy controls median RH-PAT index 1.49, 1.73, and 1.97 respectively (p=0.018)(79). ED in this cohort was associated with reduced L-arginine bioavailability, which may contribute to microvascular pathogenesis.

Discussion:

To our knowledge, this study is the first to conduct a rigorous systematic review of published and presented literature on the results of RHI as measured by Endo-PAT 2000 as a measure of endothelial dysfunction in children and adolescents. One of the benefits of RHI as a measure of ED is that it is an easy test to conduct, is well-tolerated by children and adolescents and it can be performed at the point of care.

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). Only 4 studies are RCTs. The studies cannot be compared for a meta-analysis as most are not RCT level research of high enough quality. Therefore, the conclusions drawn from many of these studies are limited. There are also limitations of RHI as reliable method for defining ED. There is no defined RHI cut off value in paediatric populations. Moreover, 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. 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.

Strengths of the paper include a comprehensive literature search including contacting authors by email for separate paediatric results in studies with combined adult and paediatric data. All study types were reviewed and even the studies without results but had interesting points were included in our discussion. Also, we do not think that this paediatric Endo-PAT review has been done before. Our results highlight that Endo-PAT has benefits including point-of-care and ease of conduct of test for assessor.

The potential future role of Endo-PAT for paediatric patients may be an adjunct tool in screening for cardiovascular risk factors. If atherosclerosis is identified early, it can be halted in its process in certain conditions. There is huge potential for its use in diabetic patients. Improving glucose control can protect endothelial function. Persistent high sugars can impair endothelial function via oxidative stress and production of free-radicals(2). Lower insulin sensitivity poses a risk of diabetic nephropathy(9). Microangiopathic renal damage increases oxygen consumption and increases resistance in the afferent arterioles. Shah et al report T2D patients have greater vascular thickness and stiffness and worse endothelial function compared to obese and lean children(80). This is raising concern that adolescents with T2D are already at risk of developing early onset cardiovascular disease.

Diabetic microangiopathy can result in retinopathy, neuropathy and peripheral vascular neuropathy. Subclinical evidence of these complications can be seen in paediatric patients, especially in those with poor glycaemic control. Unfortunately, there have been reports of T2D paediatric patients diagnosed with microangiopathic complications, particularly nephropathy(81). This early endothelial damage can be linked with increased morbidity and mortality(82). Moreover, new onset diabetes after transplantation (NODAT) is characterised by insulin resistance and T2D(83). Endo-PAT has multiple benefits in obesity as it can identify if early ED is present and therefore strategies to reverse or halt this process can be made (*Table 2*)(7, 30, 35).

In recent decades, the number of childhood cancer survivors is increasing(84). Treatments utilized such as haematopoietic stem cell transplantation have increased risk of cardiovascular disease(85, 86). Following chemotherapy, radiotherapy, immunosuppressive treatments the risk of insulin resistance has been noted(87). With advances in treating malignant paediatric conditions there are long term complications emerging in survivors. High dose chemotherapy including anthracyclines, alkylating agents and vinca alkaloids may disrupt the substances on the surface of the endothelium and impair its ability to dilate and constrict. Moreover, total body radiation poses a risk by damaging the elastic matrix. Heart disease in long-term cancer survivors is 5-10 times higher than their siblings(87). Brouwer et al (2013) studied cancer survivor patients after potential cardiovascular toxic treatment (e.g. anthracyclines, platinum) and/or radiotherapy and noted a higher risk of ED compared with sibling controls(88). Jehlicka et al (2011) used Endo-PAT and noted acute lymphoblastic leukaemia (ALL) patients had lower RHI compared to controls (1.57±0.50, 1.96±0.63; $p \leq 0.05$)(89).

Turner syndrome (TS) patients have increased cardiovascular risk factors which predispose to cardiac and cerebrovascular complications(90). A case-control study on TS patients noted a statistically significant increase in RHI in GH-treated girls(91). There are countless other paediatric syndromes with risk of ED that could benefit from screening.

Furthermore, in cardiac diseases and post-cardiac surgery Endo-PAT has been proven useful in multiple studies (*Table 3*)(44, 69, 90). Dietz et al (2015) systematic review and metanalysis on peripheral ED in Kawasaki disease, report coronary arterial aneurysms had higher surrogate markers for cardiovascular disease risk(92). This may indicate these patients should be monitored for CVD in adulthood, however significant heterogeneity was noted. Endo-PAT has been shown to be beneficial postoperatively in Fontan survivors and comparing surgical techniques like in the 'The LOVE-COARCT study' (Long-term Outcomes and Vascular Evaluation After Successful Coarctation of the Aorta Treatment) (93-95).

With the rising premature population, Endo-PAT may prove useful in this cohort. Harris et al (2020) assessed cardiovascular outcomes for those born with very low birth weights (VLBW) <1500g. The

VLBW cohort (n = 229; 71% of survivors) and term-born controls (n = 100), were assessed at age 26-30 years. The VLBW cohort had lower RHI compared to controls(96). Endo-PAT is also used in haematological conditions. Sivamurthy et al (2009) reported lower RHI in the majority sickle cell disease in a paediatric population (1.53 and 1.71; p value .032). RHI was not normal in children with chronic transfusions or hydroxyurea(97).

The psychological studies in our paper raise an interesting link between the vascular system and the psychiatric diagnoses. Retinal vascular calibre was shown to be associated with endothelial function in bipolar disorder patients and it has been suggested that it may be used as an assessment tool in this cohort.

Finally, many paediatric autoimmune conditions are linked with ED(8, 74). In SLE patients, ED may occur from impaired clearance of apoptotic cells, oxidative stress, or B cell activation with different circulating autoantibodies(67). Regular ED assessment in SLE patients has been recommended due to risk of subclinical atherosclerosis(67). Moreover, several factors may impact microvascular function in children, for example puberty, which is of particular interest in our paediatric review. Bhangoo et al report improved RHI in correlation with an increase in Tanner stages and postulated that this may be due to sex steroids(98). If Endo-PAT is used in research in adolescents, age, sex and tanner staging must to be taken in account when reporting RHI results(64).

Conclusion:

There are a number of papers in the paediatric literature describing ED at young ages using Endo-PAT. However, in many cases, there has only been a single cohort study using Endo-PAT. Further studies are required to validate these findings. Additionally, longitudinal studies are required to evaluate how this ED may change as the child ages and their chronic conditions changes. Further studies are also required that will characterise more completely the cardiovascular risk profile of these children with chronic disease. Consensus on other vascular risk markers that could be included in

future studies is ideal and if accomplished, this would facilitate meta-analyses of studies of conditions with relatively rare conditions.

The establishment of a threshold RHI for normal or abnormal would be helpful if it correlated well with clinical outcomes. This might be achieved in the future either by meta-analysis of the literature, if outcomes are measured and reported in a standardised manner, or by the conduct of a prospective longitudinal study that follows RHI in childhood to adulthood along with identification of cardiac outcomes. The latter would by its nature require to be a long-term study and would require a repeated iterative process to establish the threshold of normal for RHI, as a continuous variable. Therefore, a meta-analysis may be preferable. In the short term, a systematic approach to cardiovascular risk assessments should be promoted.

Statements and declarations:

a. Authorship contributions:

All authors contributed to the initial search strategy protocol. I deLaunois performed the online database search. J Hayden and G McDonnell separately performed a blind screen of the abstracts and analysed the papers. G McDonnell contacted the authors of joint adult and paediatric papers to obtain separate paediatric data. J Hayden wrote the initial manuscript which was revised by C O'Gorman. All authors reviewed the manuscript prior to submission.

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d. Data sharing: search technique and data analysis are available from Rayyan software and the corresponding author.

e. Competing Interest: No competing interests to declare

f. Ethical approval: this was not needed as this study was a systematic review and did not involve human participants.

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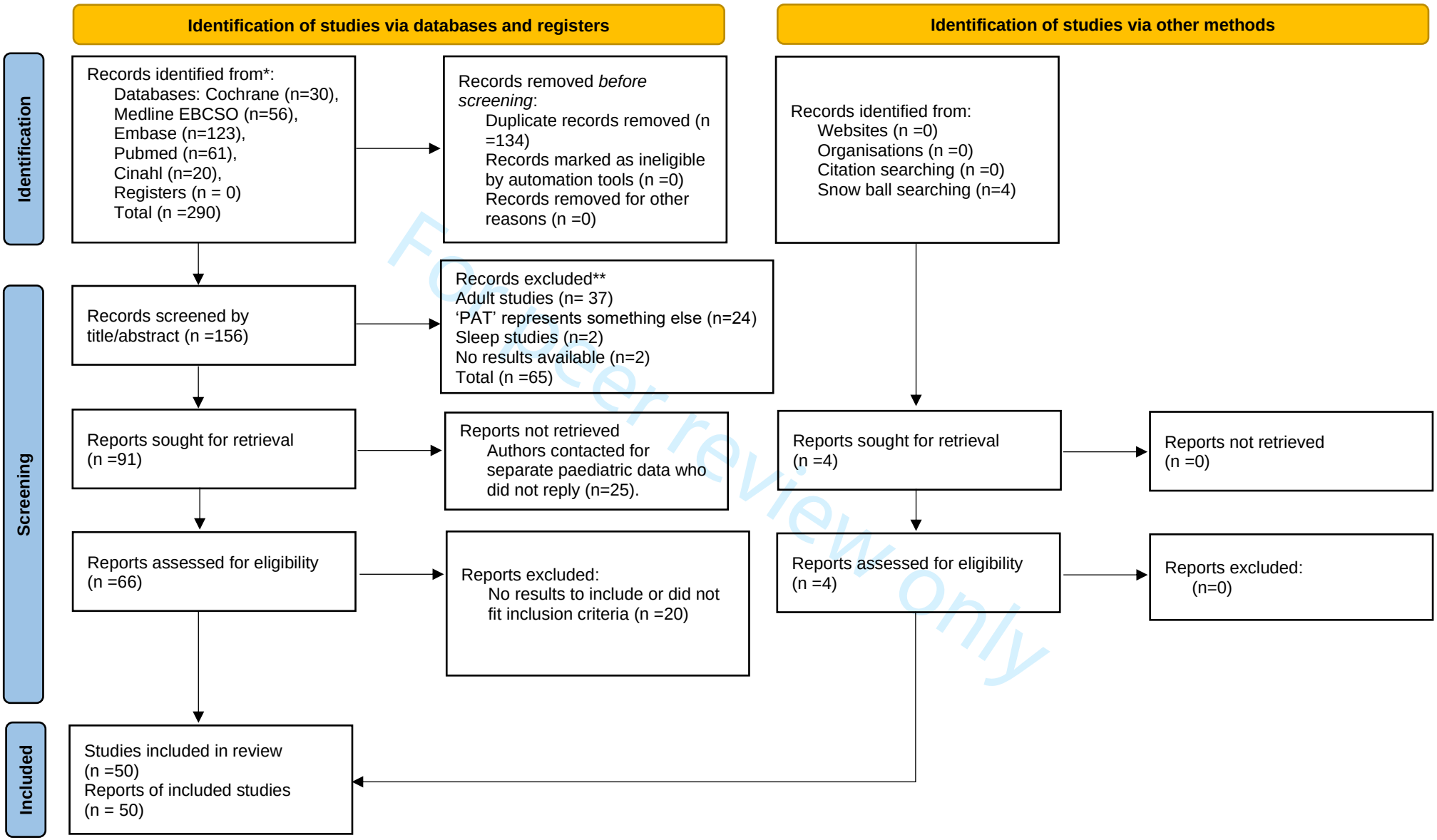
Figure 1: PRISMA 2020 Flow diagram of systematic search for Endo-PAT 2000 in paediatric populations.

PRISMA 2020 flow diagram for new systematic reviews which included searches of databases, registers and other sources



*Consider, if feasible to do so, reporting the number of records identified from each database or register searched (rather than the total number across all databases/registers).
**If automation tools were used, indicate how many records were excluded by a human and how many were excluded by automation tools.

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71. For more information, visit: http://www.prisma-statement.org/

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			Page
Title	1	Identify the report as a systematic review.	2
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	2
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	2
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	6
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	5
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	6
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	6
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	6
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	6
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	6
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	6
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	Tables
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	5
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	5
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	7
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	7
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	N/A
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	N/A
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	6
Certainty	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	6

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
assessment			
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Flow diagram
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	6
Study characteristics	17	Cite each included study and present its characteristics.	Tables 1
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Nil
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Tables 1-6
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	N/A
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	N/A
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	N/A
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	N/A
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	N/A
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	N/A
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	16
	23b	Discuss any limitations of the evidence included in the review.	17
	23c	Discuss any limitations of the review processes used.	17
	23d	Discuss implications of the results for practice, policy, and future research.	20
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	No response
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	Prospero – no response
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	N/A
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	Nil
Competing interests	26	Declare any competing interests of review authors.	Nil
Availability of data, code and	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	With authors

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
other materials			

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