Vascular function in adults with cyanotic congenital heart disease

Tarp, Julie Bjerre; Clausen, Peter; Celermajer, David; Christoffersen, Christina; Jensen, Annette Schophuus; Sorensen, Keld; Sillesen, Henrik; Estensen, Mette-Elise; Nagy, Edit; Holstein-Rathlou, Niels-Henrik; Engstrom, Thomas; Feldt-Rasmussen, Bo; Sondergaard, Lars

Published in:
IJC Heart and Vasculature

DOI:
10.1016/j.ijcha.2020.100632

Publication date:
2020

Document version
Publisher's PDF, also known as Version of record

IJC Heart & Vasculature 30 (2020) 100632

Contents lists available at ScienceDirect

IJC Heart & Vasculature

journal homepage: www.journals.elsevier.com/ijc-heart-and-vasculature

Vascular function in adults with cyanotic congenital heart disease

Julie Bjerre Tarp [a,b,*,1], Peter Clausen [c,1], David Celermajer [d,1], Christina Christoffersen [b,e,1], Annette Schophuus Jensen [a,1], Keld Sørensen [f,1], Henrik Sillesen [g,1], Mette-Elise Estensen [h,1], Edit Nagy [i,1], Niels-Henrik Holstein-Rathlou [b,1], Thomas Engstrøm [a,j,1], Bo Feldt-Rasmussen [c,1], Lars Søndergaard [a,1]

[a] Department of Cardiology, Rigshospitalet, University of Copenhagen, Denmark
[b] Department of Biomedical Science, University of Copenhagen, Denmark
[c] Department of Nephrology, Rigshospitalet, University of Copenhagen, Denmark
[d] Department of Cardiology, Royal Prince Alfred Hospital, Sydney, Australia
[e] Department of Biochemistry, Rigshospitalet and Bispebjerg Hospital, University Hospital of Copenhagen, Denmark
[f] Department of Medicine, Aalborg University Hospital (Farsoe), Denmark
[g] Department of Vascular Surgery, Rigshospitalet, University of Copenhagen, Denmark
[h] Department of Cardiology, Rikshospitalet, University of Oslo, Norway
[i] Heart and Vascular Theme, Karolinska University Hospital, Stockholm, Sweden
[j] Department of Cardiology, University of Lund, Sweden

ARTICLE INFO

Article history:
Received 18 May 2020
Received in revised form 20 August 2020
Accepted 27 August 2020
Available online 15 September 2020

Keywords:
Cyanotic congenital heart disease
Atherosclerosis
Endothelial function
Lipoproteins

ABSTRACT

Background: Patients with cyanotic congenital heart disease (CCHD) may have a low burden of atherosclerosis. Endothelial dysfunction is an early stage of atherosclerosis and endothelial function is previously studied in smaller CCHD groups with different techniques and variable results. We aimed to examine endothelial function and carotid atherosclerosis in a larger group of CCHD patients.
Methods: This multicentre study assessed endothelial function in adults with CCHD and controls by measuring the dilatory response of the brachial artery to post-ischemic hyperaemia (endothelium-dependent flow-mediated-vasodilatation (FMD)), and to nitroglycerin (endothelium-independent nitroglycerin-induced dilatation (NID)). Flow was measured at baseline and after ischaemia (reactive hyperaemia). Carotid-intima-media-thickness (CIMT), prevalence of carotid plaque and plaque thickness (cPT-max) were evaluated ultrasonographically. Lipoproteins, inflammatory and vascular markers, including sphingosine-1-phosphate (S1P) were measured.
Results: Forty-five patients with CCHD (median age 50 years) and 45 matched controls (median age 52 years) were included. The patients presented with lower reactive hyperaemia (409 ± 114% vs. 611 ± 248%, $p < 0.0001$), however preserved FMD response compared to controls (106.5 ± 8.3% vs. 106.4 ± 6.1%, $p = 0.95$). In contrast, NID was lower in the patients (110.5 ± 6.1% vs. 115.1 ± 7.4%, $p = 0.053$). There was no difference in CIMT, carotid plaque or cPT-max. The patients presented with lower high-density-lipoprotein cholesterol, and higher level of inflammatory markers and S1P.
Conclusion: Adults with CCHD had preserved FMD in the brachial artery, but impaired NID response and lower reactive hyperaemia than controls. The preserved FMD and the comparable prevalence of carotid atherosclerosis indicate that CCHD patients have the same risk of atherosclerosis as controls.

1. Introduction

Patients with cyanotic congenital heart disease (CCHD) are often stated to be at reduced risk of developing clinical manifestations of atherosclerosis, although documentation for this remains scarce [1–4]. A lower risk of atherosclerosis could be related to better risk profile, but also to protective mechanisms induced by cyanosis and biochemical and physical adaptations secondary to chronic hypoxemia [1–5]. Thus, secondary erythrocytosis, thrombocytopenia, hyperbilirubinaemia are thought to alter the atherosclerotic process and to modify the distribution of lipoproteins beneficially and thereby decrease atherosclerotic plaque formation [1–5]. The high-density-lipoprotein (HDL) associated apolipoprotein-M (apoM)-sphingosine-1-phosphate (S1P) complex

* Corresponding author at: Department of Cardiology 2012, Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen, Denmark.
E-mail address: Julie.bjerre.tarp@regionh.dk (J.B. Tarp).
[1] All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

https://doi.org/10.1016/j.ijcha.2020.100632
2352-9067/

protects the endothelium [6,7] and low S1P levels have been shown to link to an increased risk of atherosclerosis [8,9]. As it is synthesized by the erythrocytes, CCHD patients with secondary erythrocytosis may have elevated S1P levels and thus a relatively protected vasculature.

The endothelium is a major regulator of vascular homeostasis, maintaining an antithrombotic milieu and the balance between vasodilatation and vasoconstriction [10]. Damage to the endothelium promotes the entry of circulating inflammatory cells which leads to lipid deposition and fibrosis [11]. Endothelial dysfunction is therefore considered an early stage of atherosclerosis [10,12,13]. If CCHD patients are at reduced risk of atherosclerosis, endothelial dysfunction would be a rarer phenomenon than otherwise expected. Likewise, atherosclerotic markers in the carotid artery, e.g. increased intima media thickness (CIMT) would be less evident.

The present study aimed to investigate the conduit arteries in patients with CCHD for early markers of atherosclerosis, i.e. vascular function in the brachial artery and intimal lesions in the carotid artery.

2. Methods

2.1. Study participants

Clinically stable adults with CCHD followed at The University Hospitals in Copenhagen and Aarhus, (Denmark), Lund and Stockholm (Sweden), Oslo (Norway), and at The Royal Prince Alfred Hospital in Sydney (Australia), were invited to participate.

CCHD was defined as congenital heart lesions with significant right-to-left shunting and systemic oxygen saturation below 92% at rest and/or below 87% during exercise [14]. Healthy controls were drafted from advertisements in Danish newspapers, and from two local recruitment homepages (www.sundhed.dk and www.forsøgspersoner.dk). One to one matching was performed for age (±five years), gender, smoking status (current smoker, former smoker, or lifelong non-smoker) and body mass index (BMI) (<25 kg/m^2, 25 to 29 kg/m^2, ≥30 kg/m^2). None of the participants were ongoing or recent pregnant, or had any symptoms of recent infections.

The Nordic patients and all the controls were examined in Copenhagen, whereas the remaining patients were tested in Sydney.

2.2. Clinical measures and risk factors

Resting heart rate, transcutaneous oxygen saturation, weight and height were recorded. Patients on antihypertensive medications and patients with abnormal twenty-four-hour blood pressure measurements (mean systolic blood pressure > 140 mmHg or a mean diastolic pressure > 90 mmHg) were defined as having hypertension. Risk factors for atherosclerotic cardiovascular disease (CVD), including hypercholesterolemia, hypertension, previous myocardial infarction, and stroke for the patients and all the participants parents and siblings, were recorded. The subjects were classified as current smokers or as non-smokers, and the number of pack-years registered.

The Framingham Risk Score [15] was calculated for all participants from the following parameters: sex, age, diabetes mellitus, smoking, blood pressure, total-cholesterol and HDL-cholesterol and subjects were subsequently divided according to their derived 10 years risk of developing CVD into three subgroups: low (<10%), medium (10–19%) and high risk (>20%) groups [15].

2.3. Assessment of vascular function

Brachial artery dilatory responses to post-ischemic reactive hyperaemia (endothelium-dependent flow-mediated vasodilatation (FMD)), and to nitroglycerin (NTG) (endothelium-independent nitroglycerin-induced vasodilatation (NID)) were used as measures of vascular function [16]. Assessments were performed with an Acuson 128XP/10™, (USA) ultrasound system in Denmark and a GE Vivid E9 ultrasound device in Australia. A video recorder was connected to the ultrasound system and to a frame grabber and an artificial neural network wall detection software (Vessel Image Analysis (VIA)) [17]. The anterior and posterior walls were identified and tracked automatically within a user-defined region of interest. The arterial diameter was initially measured at rest (mean over 30 s - baseline diameter 1). A pneumatic tourniquet placed below the antecubital fossa was then inflated for 4.5 min to a pressure of 250–300 mmHg distal to the scanned segment of the artery and then rapidly deflated to induce reactive hyperaemia. The arterial diameter was measured continuously for the first five minutes immediately after cuff release and the maximal diameter was recorded. Ten minutes later a second baseline diameter was measured (mean of 30 s -baseline diameter 2). NTG (400 μg) was given sublingually, and the arterial diameter measured continuously for ten minutes and the maximal diameter acquired. FMD and NID were automatically calculated as the maximal flow and NTG induced arterial diameters as a percentage of the baseline diameters -1 and 2, respectively.

Flow velocities were obtained from the centre of the artery with pulsed-wave Doppler at a 70° angle. The velocity time integral (VTI), i.e. the area under the velocity/time curve was measured as the mean of four cycles at rest and within the first 60 s after cuff release. Flow was calculated by multiplying VTI by the heart rate by the vessel cross-sectional area ($\pi \times r^2$). Post-ischemic reactive hyperaemia was calculated as the maximal flow in percentage of baseline flow.

Participants were asked to avoid caffeine containing liquids and smoking on the study day. Pulmonary vasodilators (advanced therapy (AT)), i.e. endothelin receptor antagonists (ERA) and phosphodiesterase-5-inhibitors (PDE-5-inhibitor) either as monotherapy or combined were paused 12 h before the assessment. As NTG is considered contraindicated in patients treated with PDE-5-inhibitors, these subjects were excluded from the NTG challenge.

2.4. Carotid ultrasound scanning

B-mode ultrasound scans were performed as previously described [18]. CIMT was measured as the mean CIMT on the far wall in 1 cm lengths of the common carotid artery either immediately proximal to the bifurcation or where measured thickest [18,19]. Carotid plaques were defined as previous described [19]. An experienced vascular surgeon re-evaluated the plaque and maximum plaque thickness (cPT-max) if identified.

2.5. Blood and urine measurements

Lipoproteins, haematology and inflammatory markers were determined in both study centres in non-fasting blood samples by local standard laboratory methods and assays. Plasma S1P and apoM were measured as previously reported [20,21], but only done in subjects examined in Denmark. Urine spot samples were analysed for albuminuria, defined as an albumin-creatinine ratio ≥ 30 mg/g.

2.6. Statistics

Throughout, continuous variables were expressed as means ± standard deviations (SD) if normally distributed and otherwise as medians [25–75 percentiles], whereas categorical variables were presented as numbers (percentages). For continuous variables, unpaired comparisons between patients and controls were performed using the Student's *t*-test if normally distributed or the Wilcoxon test if otherwise. The Chi-square or Fisher's exact test was used to compare categorical data as appropriate.

Comparison of FMD and NID between the patients and controls were performed primarily by application of the unpaired Student's *t*-test. As a sensitivity analysis, the same analyses were performed by application of the paired Student's *t*-test.

To identify potential confounding factors, univariable regression analyses were applied for each of the potential confounders (Table 3). Factors with a *p*-value < 0.1 were included in a final multivariable regression model including FMD/NID as outcome, type of participants (patient/control) as independent variable of interest and potential confounders as covariates, including the matching parameters: age, sex, BMI and smoking. Interaction terms between type of participant and other covariates were included in the final model if *p*-value for the interaction terms was below 0.05.

To test if treatment with vasoactive medications were possible confounders, a multivariable regression model was applied with FMD as outcome and AT (either ERA or PDE-5-inhibitor or both), ERA and PDE-5-inhibitor as covariates. Further, a subgroup comparison with unpaired T-test was performed in patients not on AT and their matched controls.

General linear models were applied to assess the associations between cPT-max versus oxygen saturation in rest and proBNP level and further between CIMT versus oxygen saturation in rest and proBNP level and between left ventricular ejection fraction (LVEF) and CIMT.

A *p*-value < 0.05 was considered statistically significant. Statistical analyses were performed with the SAS statistic software version 7.1.

2.7. Ethics

All participants signed a written informed consent form before inclusion. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee. The Danish (protocol number: H-1-2014-035), the Norwegian (protocol number: 2014/2243/REK sør-øst A), the Swedish (protocol number: 2015/424-31) and the Australian (protocol number: HREC/16/RPAH/135, 16-0109) ethic committees all approved the study protocol.

3. Results

Forty-five patients with CCHD (53% women, median age 50 years) and 45 age-, BMI-, -sex, and smoking-matched subjects (53% women, median age 52 years) were included. Thirty-five (78%) of the CCHD patients were on AT; whereas 16 (36%) patients received dual therapy (combination of ERA and PDE-5-inhibitor) and 19 (42%) patients received mono-therapy (either ERA or PDE-5-inhibitor). Baseline characteristics are shown in Table 1.

Table 1
Demographics.

Characteristics	CCHD patients (n = 45)	Controls (n = 45)	*p*-value
Clinical data			
Age (years), median (IQR)	50.0 [47–55]	52.0 [44–57]	0.8
Gender (female), n (%)	24 (53)	24 (53)	1.0
Systolic blood pressure, average 24 h (mmHg), mean (±SD)	114 (±13)	120 (±17)	0.08
Diastolic blood pressure, average 24 h (mmHg), mean (±SD)	69 (±9)	75 (±11)	0.01
Heart rate (bpm), mean (±SD)	76 (±15)	63 (±11)	<0.0001
Oxygen saturation at rest, (%), median (IQR)	84 (81–88)	98.5 (98–99)	<0.0001
Body mass index (kg/m^2), mean (±SD)	23.6 (±4.3)	24.6 (±3.8)	0.3
Congenital heart disease diagnoses			
Ventricular septal defect, n (%)	24 (53)	0	
Atrial septal defect, n (%)	6 (13)	0	
Atrioventricular septal defect, n (%)	3 (7)	0	
Persistent ductus arteriosus n, (%)	1 (2)	0	
Tetralogy of Fallot, n (%)	3 (7)	0	
Univentricular heart, n (%)	3 (7)	0	
Double Outlet Right Ventricle, n (%)	2 (5)	0	
Ebstein anomaly with atrial septal defect, n (%)	1 (2)	0	
Hemitruncus arteriosus, n (%)	1 (2)	0	
Pulmonary arterio-venous malformation, n (%)	1 (2)	0	
Additional diagnoses			
Diabetes mellitus, n (%)	2 (4)	0	0.5
Stroke, n (%)	9 (20)	0	0.003
Transient ischemic attacks, n (%)	2 (4)	0	0.5
Family history of CVD, n (%)	30 (67)	33 (73)	0.6
Current smoker, n (%)	4 (9)	3 (7)	0.7
Pack years, median [IQR]	22 [10–39]	30 [4–35]	0.9
Medications			
Statins, n (%)	2 (4.4)	1 (2.2)	0.57
Advanced therapy for pulmonary arterial hypertension, n (%)	35 (78)	0 (0)	<0.0001
Endothelin receptor antagonist, n (%)	33 (73)	0 (0)	<0.0001
Phosphodiesterase 5 inhibitor, n (%)	18 (40)	0 (0)	<0.0001
Framingham 10-year CVD risk score			0.6
Low (<10%), n (%)	29 (78)	39 (87)	
Moderate (10–19%), n (%)	6 (16)	5 (11)	
High (≥20%), n (%)	2 (6)	1 (2)	
Carotid artery			
Presence of carotid plaque, n (%)	11 (24)	7 (16)	0.27
cPT-max (mm), median [IQR]	2.2 [1.2–2.9]	2.8 [1.8–6.7]	0.16
CIMT (mm), mean (±SD)	0.61 (±0.13)	0.64 (±0.13)	0.29

CCHD = Cyanotic congenital heart disease, bpm = beats per minutes, CVD = cardiovascular disease, CIMT = carotid intima media thickness, cPT-max = maximum plaque thickness.

3.1. Assessment of vascular function

3.1.1. Endothelium dependent vasodilation

Baseline diameter-1 was similar in patients and controls (4.2 ± 0.8 mm vs. 4.1 ± 0.9 mm, $p = 0.5$), Table 2. There was no significant difference in FMD between patients with CCHD and controls (106.5 ± 8.3% vs. 106.4 ± 6.1%, $p = 0.95$, [95% CI: −3.22 to 3.04%]). There were no associations of any of the included covariates on FMD, Table 3. Specifically, FMD was not associated with AT (ERA or/and PDE-5-inhibitor). In a subgroup analysis with patients not treated with pulmonary vasodilators and controls, FMD was similar in both groups (108.5 ± 11.1% vs 108.0 ± 8.8%, respectively, $p = 0.93$).

3.1.2. Brachial flow analysis

The baseline flow only tended to be higher in the CCHD patients than controls (235 ± 108 ml/min vs 185 ± 128 ml/min, $p = 0.06$), while the increase in flow-velocity was significantly lower in the

Table 2
Vascular parameters.

Vessel characteristic	CCHD patients	Controls	*p*-value
Baseline diameter (mm), mean (±SD)	4.2 ± 0.8	4.1 ± 0.9	0.5
Flow velocity time integral, mean (±SD)	16.0 ± 5.8	13 ± 6.5	<0.05
Baseline flow (mL/min), mean (±SD)	235 ± 108	185 ± 128	0.06
Flow increase, mean (±SD)	409 ± 114	611 ± 248	<0.0001
FMD (%), mean (±SD)	106.5 ± 8.3	106.4 ± 6.1	0.95
NID (%), mean (±SD)	111 ± 6	115 ± 7	0.05

CCHD = cyanotic congenital heart disease, FMD = flow mediated dilatation, NID = nitroglycerin induced dilatation.

CCHD patients than controls (409 ± 114% vs. 611 ± 248%, $p < 0.0001$), Table 2.

The sensitivity analysis confirmed the above-mentioned results in FMD and flow increase.

3.1.3. Endothelium independent vasodilation

Eighteen patients (44% women, median age 49.0 years, IQR: 44.0–53.0 years) and 18 matched controls (44% women, median age 50.0 years, IQR: 39.0–53.0 years) with similar baseline diameter (4.18 ± 0.83 mm vs. 4.26 ± 0.93 mm, $p = 0.8$) were tested after NTG. The NID response was marginally, albeit not significantly, lower in the CCHD patients when unpaired comparison was performed (110.5 ± 6.1% vs. 115.1 ± 7.4%, $p = 0.053$), Table 2. However, the sensitivity analysis with paired comparison of NID revealed a significant difference between the two groups ($p = 0.03$). On univariate analysis, NID was only associated with sex ($p < 0.03$), Table 3.

3.2. Carotid artery analysis

There was no difference in CIMT (0.61 ± 0.13 mm vs. 0.64 ± 0.13 mm, $p = 0.3$), prevalence of plaques (11/45 (24%) vs. 7/45 (16%), $p = 0.27$) or cPT-max (2.2 mm [1.2–2.9 mm] vs. 2.8 mm [1.8–6.7 mm], $p = 0.16$) between patients and controls, Table 1. No associations were found between LVEF and CIMT (-18.75 [CI 95%: −46.1; 8.6], $p = 0.17$). There was no difference in FMD between the subjects with or without a carotid plaque ($p = 0.9$)

No associations were found between cPT-max versus oxygen saturation in rest (0.082 [CI95%: −0.03; 0.19], $p = 0.14$) or between cPT-max versus proBNP (0.0009 [CI95%: −0.005; 0.006], $p = 0.76$) or between CIMT versus oxygen saturation in rest (0.0001 [CI95%: −0.008; 0.008], $p = 0.97$) or between CIMT and proBNP level (0.0002 [CI95%: −0.0001; 0.0005], $p = 0.18$).

3.3. Atherosclerotic, endothelial and inflammatory markers

The patients with CCHD had higher levels of inflammatory markers (CRP: $p = 0.02$, leucocytes: $p = 0.04$, neutrophils: $p = 0.01$, and HsCRP: $p = 0.02$), homocysteine: $p < 0.0001$) as well as of Hb1AC ($p = 0.0001$), Table 4. There were no differences in total-cholesterol or LDL-cholesterol, whereas HDL-cholesterol was significantly lower in the patients (1.36 ± 0.47 mmol/L vs. 1.76 ± 0.45 mmol/L, $p = 0.0003$), Table 4. The patients had higher S1P (0.74 ± 0.48 μmol/L vs. 0.51 ± 0.18 μmol/L, $p = 0.04$) and lower apoM (1.00 ± 0.59 μmol/L vs. 1.39 ± 0.51 μmol/L, $p = 0.02$).

4. Discussion

The present study showed that flow-mediated dilatation (FMD), a gold standard for assessment of endothelial function in conduit arteries [13], was preserved in our cohort of adults with CCHD. It also showed that parameters of carotid atherosclerosis (CIMT, plaque burden or cPT-max) were comparable to that seen in matched healthy controls. The normal FMD combined with the similar carotid parameters, indicates that patients with CCHD do not seem to have different conduit artery physiology and pathology, i.e. the same atherosclerotic burden as healthy controls, despite exposed to abnormal levels of pro-atherosclerotic factors, including higher inflammatory markers and HbA1c levels as well as to lower HDL-cholesterol levels.

Previous studies have addressed endothelial function in CCHD patients in different age-groups, with different techniques and with diverging results [5,22–25]. In four studies, brachial artery

Table 3
Univariable and multiple logistic regression analyses.

Variables	Univariable analyses		Multivariable analyses	
	β ± SE	*p*-value	partial r2	*p*-value
FMD				
Type of participant (control)	−0.1 ± 1.6	0.96	1.5 ± 1.7	0.41
Age	−0.0 ± 0.1	0.76	0.0 ± 0.1	0.81
Sex (male)	−2.8 ± 1.5	0.07	−3.0 ± 1.6	0.07
BMI	0.2 ± 0.2	0.41	0.1 ± 0.2	0.65
Smoking (No)	1.8 ± 2.3	0.53	0.8 ± 2.9	0.78
S1P	−1.1 ± 3.1	0.73		
HDL cholesterol	0.3 ± 1.7	0.85		
LDL cholesterol	1.2 ± 0.9	0.17		
Albuminuria	3.3 ± 1.8	0.08	−4.3 ± 2.1	0.04
Baseline vessel diameter	−0.3 ± 1.0	0.73		
NID				
Type of participant (control)	4.6 ± 2.3	0.05	4.6 ± 2.3	0.05
Age	0.1 ± 0.1	0.57	0.1 ± 0.1	0.40
Sex (male)	−5.0 ± 2.3	0.03	−5.2 ± 2.3	0.03
BMI	0.1 ± 0.3	0.84	0.0 ± 0.3	0.99
Smoking (No)	2.5 ± 4.3	0.57	0.2 ± 4.4	0.97
S1P	−0.3 ± 2.9	0.93		
HDL cholesterol	−1.2 ± 3.6	0.74		
LDL cholesterol	1.4 ± 1.2	0.25		
Albuminuria	−0.2 ± 2.8	0.93		
Baseline vessel diameter	−1.3 ± 1.4	0.37		

FMD = flow mediated vasodilatation, BMI = body mass index, S1P: sphingosine-1-phosphate, HDL: high-density-lipoprotein, LDL = low density lipoprotein, Albuminuria: urine-albumin/creatinine > 30 mg/g.

Table 4
Biochemistry.

Parameters	CCHD patients	Control	*p*-value
Haematocrit (%), median (IQR)	0.58 (0.53–0.63)	0.42 (0.38–0.44)	<0.0001
Haemoglobin (mmol/L), median (IQR)	12.0 (11.1–12.9)	8.7 (8.2–9.2)	<0.0001
Platelets ($\times 10^9$/L), mean (±SD)	171.0 (±55.9)	241.3 (±52.9)	<0.0001
CRP (mg/L), median [p25-p75]	2.9 [1.0–5.5]	1.0 [1.0–2.5]	0.02
Leukocytes ($\times 10^9$/L), mean (±SD)	6.77 (±1.56)	6.02 (±1.90)	0.04
Neutrophils ($\times 10^9$/L), mean (±SD)	4.41(±1.23)	3.65 (±1.64)	0.01
Creatinine (μmol/L), mean (±SD)	80.5(±23.5)	77.4 (±14.1)	0.5
ProBNP (pmol/L), mean (±SD)	104.9 (±134.7)	6.6 (±8.9)	<0.0001
Total cholesterol (mmol/L), mean (±SD)	4.91 (±1.16)	5.29 (±0.90)	0.1
LDL cholesterol (mmol/L), mean (±SD)	3.03 (±1.01)	3.2 (±0.78)	0.4
HDL cholesterol (mmol/L), mean (±SD)	1.36 (±0.47)	1.76 (±0.45)	0.0003
Triglycerides (mmol/L), median [IQR]	1.38 [0.85–2.03]	1.0 [0.72–1.44]	0.02
Hb1AC (mmol/mol), mean (±SD)	37.7 (±3.9)	34.2 (±3.8)	0.0001
Homocysteine (μmol/L), mean (±SD)	18.3 (±6.7)	12.6 (±4.6)	<0.0001
HsCRP (mg/L), median [IQR]	2.25 [1.0–4.7]	1.0 [0.6–2.0]	0.02
ApoM (μmol/L), mean (±SD)	1.00 (±0.59)	1.39 (±0.51)	0.02
S1P (μmol/L), mean (±SD)	0.74 (±0.48)	0.51(±0.18)	0.04
Albuminuria, % (n)	42 (19)	7 (3)	0.0001

CCHD = cyanotic congenital heart disease, IQR: interquartile range, SD = standard deviation, LDL = low density lipoprotein, HDL = high density lipoprotein, HbA1C = haemoglobin A1C, apoM = apolipoprotein M, S1P = sphingosine-1-phosphate.

physiology was used to study endothelial function [5,22–24], including one in a paediatric cohort [5]. In the three adult studies available, the patients included were marginally younger, but otherwise comparable to our patients with regard to oxygen saturation, haematocrit and haemoglobin level [22–24]. In one of these studies, FMD was also found to be normal[24] while two studies reported significantly lower FMD in the CCHD patients [22,23]. The reason for this discrepancy is unclear, but in these studies, flow and NID data were not provided. The evaluation of vascular function in the brachial artery not only includes FMD measures, but also data on the post-ischemic hyperaemic response (the stimulus for FMD) and on the vasodilatory NTG response (vascular smooth cell function stimulus). The difference in FMD measures seen between our study and the two studies, which did not include these data, could therefore theoretically relate to differences in the two vasomotor stimuli employed.

Unfortunately, our brachial data were somewhat heterogeneous with a tendency to higher baseline flow velocities, reduced reactive hyperaemic flow velocities, and NID responses that tended to be lower than in controls. The reason for this remains unclear. A high number of patients were on pulmonary vasodilators, which may influence vascular function, although the "post-hoc" analysis did suggest this effect to be absent or small. Some of the observations may suggest that patients with CCHD have more baseline NO release from their endothelium. A more "tonic" NO release would in full extent lead to higher flow, bigger vessels and a lower "vasodilator reserve" to NTG, as there is already more baseline NO secretion. Apart from the non-significant difference in vessel size, this explanation remains possible. If so, the trigger for a higher NO production could be increased wall shear-stress secondary to the erythrocytosis seen in the CCHD patients [26].

The combined observation of preserved FMD, reduced post-ischemic flow velocities and borderline lower NID responses in the CCHD group, may also suggest discordant vasomotor responses in the conduit arteries and in the microcirculation. This is in line with observations from Oechslin et al. They measured forearm blood flow with plethysmography and found lower baseline blood flow and a reduced response to intra-arterial infusion of acetylcholine in CCHD patients compared to controls indicating microvascular endothelial dysfunction. However, the endothelium-independent vasodilatation was preserved as also seen in the present study [25].

Endothelial function measured by FMD varies considerable from study to study. A large meta-analysis demonstrated that FMD ranges between 2.3 and 13.5 % among diseased and asymptomatic populations, as well as an inverse relationship exists between the risk of CVD and FMD [27]. FMD below 4.7% has been found associated with a higher probability of CVD [28]. Four out of five studies assessing FMD among patients with CCHD, including the present study, shows a FMD above 5.0%, hence in the higher end of the FMD spectrum [5,23,24]. This may be interpreted as patients with CCHD have a better endothelial function than patients with a high risk of CVD.

S1P is known to induce angiogenesis and the higher levels seen in the CCHD patients may therefore play a major role for eventual remodelling of the microvasculature [6,20]. S1P activates eNOS and is linked to NO production [29]. However, the increased S1P levels seen in our CCHD patients, were not reflected in an increased FMD and no association between S1P and FMD was found. Interestingly, plasma S1P is transported mainly by apoM-containing HDL particles (70%), and to a minor extend by albumin (30%) [6,7]. S1P-ApoM is the main plasma component that can activate the S1P-receptors on the vessel wall [20,30]. Hence, S1P-albumin alone cannot protect and maintain normal vascular barrier integrity. The increased plasma S1P in the patients with CCHD, may predominantly be bound in the S1P-albumin complex since both the levels of apoM and HDL-cholesterol were low in the patients. A high concentration of S1P in the S1P-albumin complex does not represent a more protected endothelium, and a higher plasma S1P in CCHD patients will maybe not contribute beneficially to endothelium protection.

Previous studies included patients with pulmonary hypertension, but not those on pulmonary vasodilators. In the present study, 78% of the patients were on AT. Since these probably represent the most ill patients, one would have expected detectable endothelial dysfunction if patients with CCHD were prone to develop atherosclerosis. This was not the case. The medications could theoretically affect the measured vascular parameters, although in the opposite direction. ERAs will thus tend to increase resting vessel size, and hence attenuate FMD which is highly related to vessel size [11]. PDE-5-inhibitors block the breakdown of c-GMP, which would enhance the effect of the NO released in response to the reactive hyperaemia. The plasma half time of Bosentan and Sildenafil are approximately five hours, and it there-

fore seemed sufficient to minimize the possible acute effect on FMD by pausing the medication for 12 h prior to the assessment, although this may not change the more long-time effect. These considerations are supported by the lack of difference in baseline diameter of the brachial artery between CCHD patients and controls, by the identical FMD of patients and controls in a subgroup analysis where the patients were not on AT, and lastly by the lack of association between AT and FMD.

The carotid parameters showed no difference in CIMT, prevalence of plaque or cPT-max between patients and controls. This is similar to previously reported data in paediatric patients [5] but in contrast to the only available study in adults with CCHD which found a lower CIMT in the patients [2]. Plaque data have previously not been reported. We not only found similar CIMT in the two groups, but also an equivalent overall plaque burden. The results hereby provide a thorough assessment of the carotid arteries showing the same prevalence of atherosclerosis in patients with CCHD as in matched controls.

Atherosclerotic disease progresses with advancing age. The age difference between the present study (median 50.0 years) and the study by Duffel et al. (mean age 38 years) may partly explain the difference in CIMT between the two patient cohorts, who otherwise may be assumed to present with the same conduit artery physiology and pathology [2].

5. Limitations

We did not match women for day of menstrual cycle and only asked the participants to avoid caffeine containing liquids and smoking on day of examination. This might have some impact on the results but are unlikely to affect the group comparisons or the conclusions drawn. Investigations were performed in two different centres (eight patients in Sydney and 37 patients and all controls in Denmark). Both centres used the same protocol and the assessments were performed by trained staff. This set-up potentially introduces some variability [13]. Finally, 78% of the patients were on AT which, as eluded to above, could introduce some bias.

6. Conclusion

Brachial artery FMD is preserved in adults with CCHD. Increased resting flow velocities and attenuated reactive hyperaemia may reflect functional remodelling and perhaps endothelial dysfunction in the microvasculature. Although the CCHD patients presented with higher levels of inflammatory markers and lower HDL-cholesterol levels, no difference in the prevalence or degree of carotid atherosclerosis was found. The results indicate that patients with CCHD may have a similar risk of developing atherosclerosis as the background population.

Declaration of Competing Interest

Henrik Hedegaard Sillesen has received research grant from Philips Ultrasound and consulting fees from Bayer A/S, Denmark, and Novo Nordisk, Denmark.

All other authors: None

Acknowledgements

We would like to thank the patients and controls for participating in the study. Andreas Haltorp, Bente Culmsee and Cathrine Larsen for skilful contribution. This work was supported by the Research Foundation of the Heart Centre, Rigshospitalet, and The Danish Heart Association [grant numbers 14-R97-A5000-26026, 15-R98-A5054-26033, 16-R99-A5076-26038]. Henrik Hedegaard Sillesen has received research grant from Philips Ultrasound and consulting fees from Bayer A/S, Denmark, and Novo Nordisk, Denmark.

References

[1] A. Fyfe, J.K. Perloff, K. Niwa, J.S. Child, P.D. Miner, Cyanotic congenital heart disease and coronary artery atherogenesis, Am. J. Cardiol. 96 (2005) 283–290.
[2] M.G.J. Duffels, K.M. Mulder, M.D. Trip, E. de Groot, J. Gort, A.P.J. van Dijk, E.S. Hoendermis, L. Daliento, A.H. Zwinderman, R.M.F. Berger, B.J.M. Mulder, Atherosclerosis in patients with cyanotic congenital heart disease, Circ. J. 74 (2010) 1436–1441.
[3] G. Giannakoulas, K. Dimopoulos, R. Engel, O. Goktekin, Z. Kucukdurmaz, M.A. Vatankulu, E. Bedard, G.P. Diller, M. Papaphylactou, D.P. Francis, C. Di Mario, M. A. Gatzoulis, Burden of coronary artery disease in adults with congenital heart disease and its relation to congenital and traditional heart risk factors, Am. J. Cardiol. 103 (2009) 1445–1450.
[4] J.B. Tarp, A.S. Jensen, T. Engstrøm, N.-H. Holstein-Rathlou, L. Søndergaard, Cyanotic congenital heart disease and atherosclerosis, Heart Br. Card. Soc. 103 (2017) 897–900.
[5] M. Ciftel, A. Simşek, O. Turan, F. Kardelen, G. Akçurin, H. Ertuğ, Endothelial dysfunction and atherosclerosis in children with irreversible pulmonary hypertension due to congenital heart disease, Ann. Pediatr. Cardiol. 5 (2012) 160–164.
[6] M.-J. Lee, S. Thangada, K.P. Claffey, N. Ancellin, C.H. Liu, M. Kluk, M. Volpi, R.I. Sha'afi, T. Hla, vascular endothelial cell adherens junction assembly and morphogenesis induced by sphingosine-1-phosphate, Cell. 99 (1999) 301–312.
[7] A. Borup, P.M. Christensen, L.B. Nielsen, C. Christoffersen, Apolipoprotein M in lipid metabolism and cardiometabolic diseases, Curr. Opin. Lipidol. 26 (2015) 48–55.
[8] I. Soltau, E. Mudersbach, M. Geissen, E. Schwedhelm, M.S. Winkler, M. Geffken, S. Peine, G. Schoen, E.S. Debus, A. Larena-Avellaneda, G. Daum, Serum-sphingosine-1-phosphate concentrations are inversely associated with atherosclerotic diseases in humans, PLOS ONE 11 (2016) e0168302.
[9] M. Bot, P.P. Van Veldhoven, S.C.A. de Jager, J. Johnson, N. Nijstad, P.J. Van Santbrink, M.M. Westra, G. Van Der Hoeven, M.J. Gijbels, C. Müller-Tidow, G. Varga, U.J.F. Tietge, J. Kuiper, T.J.C. Van Berkel, J.-R. Nofer, I. Bot, E.A.L. Biessen, Hematopoietic sphingosine 1-phosphate lyase deficiency decreases atherosclerotic lesion development in LDL-receptor deficient mice, PLoS ONE 8 (2013) e63360.
[10] A. Lerman, Endothelial function: cardiac events, Circulation 111 (2005) 363–368.
[11] D.S. Celermajer, K.E. Sorensen, V. Gooch, D. Spiegelhalter, O. Miller, I. Sullivan, J. Llooy, J. Deanfield, Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis, Lancet Lond. 340 (1992) 1111–1115.
[12] D.S. Celermajer, K.E. Sorensen, C. Bull, J. Robinson, J.E. Deanfield, Endothelium-dependent dilation in the systemic arteries of asymptomatic subjects relates to coronary risk factors and their interaction, J. Am. Coll. Cardiol. 24 (1994) 1468–1474.
[13] J.E. Deanfield, J.P. Halcox, T.J. Rabelink, Endothelial function and dysfunction: testing and clinical relevance, Circulation 115 (2007) 1285–1295.
[14] C.S. Broberg, M. Ujita, S. Prasad, W. Li, M. Rubens, B.E. Bax, S.J. Davidson, B. Bouzas, J.S.R. Gibbs, J. Burman, M.A. Gatzoulis, Pulmonary arterial thrombosis in eisenmenger syndrome is associated with biventricular dysfunction and decreased pulmonary flow velocity, J. Am. Coll. Cardiol. 50 (2007) 634–642.
[15] Framingham Heart Study, Cardiovascular disease (10 year risk) [Internet]. Fram. Heart Study. Available from: https://www.framinghamheartstudy.org/risk-functions/cardiovascular-disease/10-year-risk.php.
[16] P. Clausen, B. Feldt-Rasmussen, J. Iversen, M. Lange, I. Eidemak, S. Strandgaard, Flow-associated dilatory capacity of the brachial artery is intact in early autosomal dominant polycystic kidney disease, Am. J. Nephrol. 26 (2006) 335–339.
[17] J.S. Sidhu, V.R. Newey, D.K. Nassiri, J.-C. Kaski, A rapid and reproducible on line automated technique to determine endothelial function, Heart Br. Card. Soc. 88 (2002) 289–292.
[18] H. Sillesen, P. Muntendam, A. Adourian, R. Entrekin, M. Garcia, E. Falk, V. Fuster, Carotid plaque burden as a measure of subclinical atherosclerosis: comparison with other tests for subclinical arterial disease in the High Risk Plaque BioImage study, JACC Cardiovasc. Imaging 5 (2012) 681–689.
[19] P.-J. Touboul, M.G. Hennerici, S. Meairs, H. Adams, P. Amarenco, N. Bornstein, L. Csiba, M. Desvarieux, S. Ebrahim, R. Hernandez Hernandez, M. Jaff, S. Kownator, T. Naqvi, P. Prati, T. Rundek, M. Sitzer, U. Schminke, J.-C. Tardif, A. Taylor, E. Vicaut, K.S. Woo, Mannheim carotid intima-media thickness and plaque consensus (2004-2006-2011), in: An update on behalf of the advisory board of the 3rd, 4th and 5th watching the risk symposia, at the 13th, 15th and 20th European Stroke Conferences, Mannheim, Germany, 2004, Brussels, Belgium, 2006, and Hamburg, Germany, 2011. Cerebrovasc Dis Basel Switz, vol. 34, 2012, pp. 290–296.
[20] C. Christoffersen, H. Obinata, S.B. Kumaraswamy, S. Galvani, J. Ahnström, M. Sevvana, C. Egerer-Sieber, Y.A. Muller, T. Hla, L.B. Nielsen, B. Dahlbäck, Endothelium-protective sphingosine-1-phosphate provided by HDL-associated apolipoprotein M, Proc. Natl. Acad. Sci. USA 108 (2011) 9613–9618.

[21] M.H. Bosteen, B. Dahlbäck, L.B. Nielsen, C. Christoffersen, Protein unfolding allows use of commercial antibodies in an apolipoprotein M sandwich ELISA, J. Lipid Res. 56 (2015) 754–759.
[22] R.L. Cordina, S. Nakhla, S. O'Meagher, J. Leaney, S. Graham, D.S. Celermajer, Widespread endotheliopathy in adults with cyanotic congenital heart disease, Cardiol. Young. 1–9 (2014).
[23] O. Trojnarska, L. Szczepaniak-Chicheł, M. Gabriel, A. Bartczak-Rutkowska, J. Rupa-Matysek, A. Tykarski, S. Grajek, Arterial stiffness and arterial function in adult cyanotic patients with congenital heart disease, J. Cardiol. 70 (2017) 62–67.
[24] C.M. Pedersen, M.R. Schmidt, B. Mortensen, H. Contractor, H.E. Bøtker, R.K. Kharbanda, K.E. Sørensen, Preserved flow-mediated dilation in adults with cyanotic congenital heart disease, Pediatr. Cardiol. 30 (2009) 965–970.
[25] E. Oechslin, W. Kiowski, R. Schindler, A. Bernheim, B. Julius, H.P. Brunner-La Rocca, Systemic endothelial dysfunction in adults with cyanotic congenital heart disease, Circulation 112 (2005) 1106–1112.
[26] R. Wells, Syndromes of Hyperviscosity, N. Engl. J. Med. 283 (1970) 183–186.
[27] R.T. Ras, M.T. Streppel, R. Draijer, P.L. Zock, Flow-mediated dilation and cardiovascular risk prediction: a systematic review with meta-analysis, Int. J. Cardiol. 168 (2013) 344–351.
[28] T. Yoshida, H. Kawano, S. Miyamoto, T. Motoyama, H. Fukushima, N. Hirai, H. Ogawa, Prognostic value of flow-mediated dilation of the brachial artery in patients with cardiovascular disease, Intern. Med. Tokyo Jpn. 45 (2006) 575–579.
[29] J. Igarashi, T. Michel, S1P and eNOS regulation, Biochim. Biophys. Acta 1781 (2008) 489–495.
[30] B.A. Wilkerson, G.D. Grass, S.B. Wing, W.S. Argraves, K.M. Argraves, Sphingosine 1-phosphate (S1P) carrier-dependent regulation of endothelial barrier, J. Biol. Chem. 287 (2012) 44645–44653.