Haemodynamics in children with a Fontan circulation: effects of afterload reduction

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Chapter 1

General introduction and outline thesis

Background

The Fontan operation is a palliative procedure performed in patients with a univentricular heart to improve survival and quality of life. A univentricular heart defect is defined by the presence of only one functional ventricle (Single Ventricle; SV). The incidence lies between 0.08-0.4 per 1000 live births (1). A variety of underlying heart defects can lead to a functional SV. Most SV patients can be divided by either having a functional left or right ventricle (Figure 1).

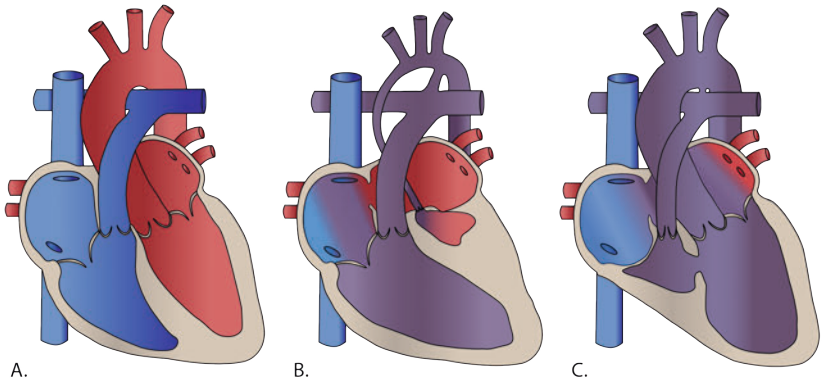

Figure 1. Anatomy of a normal heart **(A)**, an example of a univentricular heart with a functional right ventricle **(B)**, an example of a univentricular heart with a functional left ventricle **(C).**

In a univentricular heart circulation, the pulmonary and systemic circulations are in parallel rather than in series, resulting in chronic cyanosis due to mixing of saturated and desaturated blood, and volume overload of the SV as it receives and ejects blood from both circulations. Without intervention patients will suffer from early morbidity and mortality (2).

In 1971, Fontan and Baudet described a concept of a palliative procedure by which the systemic and pulmonary circulations are separated by directing the systemic venous return directly into the pulmonary arteries, bypassing the heart. As a result, the pulmonary circulation is connected in series with the systemic circulation, leading to reduced volume overload and higher oxygen saturations (3). Originally, the right atrium was directly connected to the pulmonary arteries with an atriopulmonary connection (APC). Over the past decades, however, Fontan palliation has evolved towards the development of staging of the operation, with first the creation of a partial cavopulmonary connection by connecting the superior caval vein to the pulmonary arteries (a bidirectional Glenn procedure) at about 3-6 months of age,

followed by the construction of a total cavopulmonary connection (TCPC, Fontan tunnel) by connecting the inferior caval vein to the pulmonary arteries through an intra-atrial lateral tunnel (ILT) or an extracardiac conduit (ECC), a conduit bypassing the atrium completely, usually performed between 18 months and 4 years of age (1). A fenestration, a small hole between the ILT or ECC and right atrium, can be included in the Fontan tunnel to allow a right-to-left shunt, allowing the circulation to adjust to the TCPC during the direct post-operative period after which it can be closed percutaneously by a device. In addition to these stages, some patients require an additional 'first' stage to palliation. In patients with unobstructed pulmonary blood flow a pulmonary artery banding may be used to prevent pulmonary overcirculation. In patients with severe pulmonary stenosis a systemic to pulmonary artery shunt may be necessary to improve cyanosis. An example of a staged Fontan palliation is shown in Figure 2.

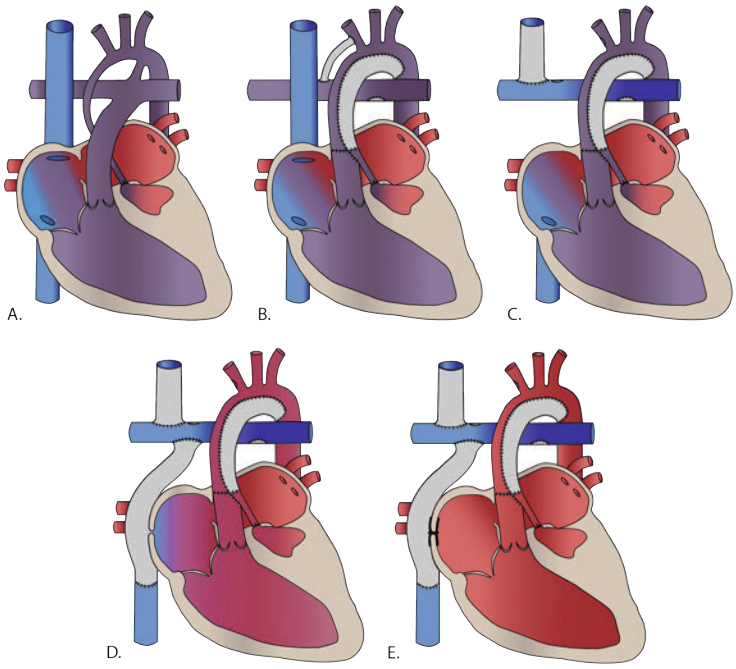

Figure 2. Example of a staged Fontan palliation in a heart with hypoplastic left heart syndrome (HLHS). **(A)** Heart with HLHS. **(B)** Norwood procedure: aortic reconstruction with insertion of a patch, a Damus-Kaye-Stansel (DKS) procedure to join the pulmonary artery and the aorta, an atrial septectomy, and placement of a systemic to pulmonary artery shunt for pulmonary blood flow. **(C)** Partial cavopulmonary connection with a bidirectional Glenn anastomosis; and removal of the systemic to pulmonary artery shunt. **(D)** Total cavopulmonary connection with an extracardiac conduit with the inclusion of a small fenestration from the conduit to the right atrium. **(E)** Fenestration closure by a device.

When the Fontan palliation is completed, the circulation lacks a ventricle that pumps the blood into the pulmonary arteries. Therefore, venous pressure is necessary to overcome pulmonary vascular resistance and drive blood through the pulmonary vascular bed (Figure 3*).* Any increase in pulmonary vascular resistance will lead to increased central venous pressure and results in venous congestion and decreased cardiac output (4). Furthermore, transpulmonary flow is also dependent on atrial pressure, as an increase of atrial pressure will lead to a decrease of the transpulmonary gradient and reduced pulmonary flow. The result is a preload dependent circulation where there is a delicate balance between systemic and pulmonary resistance and a critical fluid balance (5).

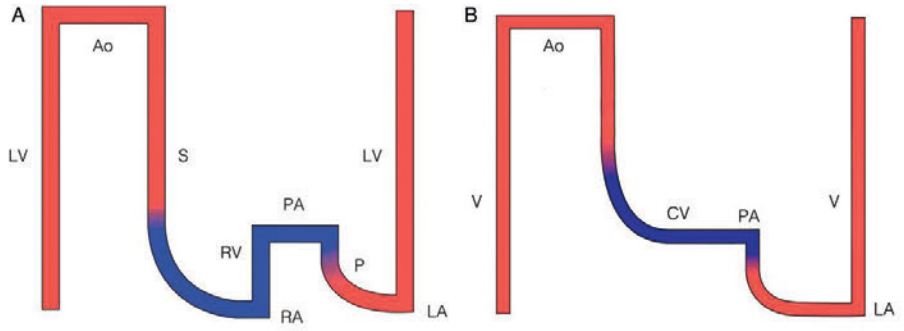

Figure 3. Scheme of pressures (vertical axis) in the normal circulation **(A)** and the Fontan circulation **(B)**. **(A)** Normal biventricular circulation: the pulmonary circulation (P) is connected in series to the systemic circulation (S). The compliance of the right ventricle (RV) ensures that the right atrial (RA) pressure remains lower than the left atrial (LA) pressure and delivers the driving force to the blood to overcome pulmonary impedance. **(B)** Fontan circuit: the caval veins are directly connected to the pulmonary arteries (PA); systemic venous pressures (CV) are markedly elevated. Ao, aorta; CV, caval veins; LA, left atrium; LV, left ventricle; PA, pulmonary artery; RV, right ventricle; V, single ventricle. Line thickness reflects output, and colour reflects oxygen saturation. This figure has been reproduced and reprinted from Gewillig and Brown (4). Copyright © 2016, BMJ Publishing Group Ltd and the British Cardiovascular Society. This is an Open Access article distributed in accordance with the Creative Commons Attribution Non-Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

Although the Fontan operation has improved survival for patients born with a functional SV in recent decades, it is only palliative, and patients still suffer from reduced life expectancy and significant morbidities (6, 7). More than half of the patients experience at least one complication within the first 20 years, including arrhythmias, thromboembolic events, and heart failure (6-8). Furthermore, the

chronically elevated central venous pressure and low cardiac output will eventually cause the Fontan circulation to fail, which in a late stage can manifest itself as ascites, protein losing enteropathy or plastic bronchitis. Unfortunately, a clear therapeutic strategy to improve the Fontan circulation, lower the burden of morbidities and prevent Fontan failure is still lacking.

Since heart failure, with or without preserved systolic ventricular function, is one of the causes of late mortality in Fontan patients, treatment strategies have focussed on prevention or treatment of ventricular dysfunction (9-11). Angiotensin converting enzyme (ACE) inhibitors are effective in reducing mortality and improving exercise performance in patients with a biventricular heart and mild to severe systolic heart failure (12-19). Despite the lack of evidence of its efficacy in Fontan patients (10, 11) many Fontan patients are currently treated on a routine basis with ACE inhibitors, including those without overt systolic ventricular dysfunction. Furthermore, ACE inhibition has several side effects, e.g., it may lead to orthostatic hypotension (20, 21). In Fontan patients these effects may even be more deleterious. Despite its widespread use there is question about the effectiveness of afterload reduction in Fontan patients as the systemic circulation is highly preload dependent and the critical bottleneck of the circulation is not the ventricle, but the pulmonary vasculature. Since many Fontan patients are treated with ACE inhibitors it is important to understand the efficacy and hemodynamic effects of afterload reduction in these patients. Furthermore, the effect of ACE inhibition on the orthostatic response in Fontan patients has not yet been investigated.

To understand the possible beneficial and detrimental effects of ACE inhibition in Fontan patients detailed evaluation of the hemodynamic mechanisms of the Fontan circulation is needed which may also lead to better understanding of the evolution of the failing Fontan circulation. Challenging the Fontan circulation by exercise stress testing or by acute fluid loading or depletion may lead to new insights.

During exercise, the cardiovascular system is maximally challenged; any restrictions in the cardiovascular system will therefore lead to reduced exercise performance. For good exercise performance, an adequate increase of cardiac output is required, which in turn necessitates an adequate increase in stroke volume and heart rate. In Fontan patients, one of the main limiting factors of exercise performance is the inability to adequately increase stroke volume; the absence of a sub-pulmonary ventricle results in an inability to augment venous return (22). However, there

are also other factors that influence stroke volume and heart rate and may contribute to limited exercise performance in Fontan patients, such as chronotropic incompetence, diastolic and systolic ventricular function, and arterial stiffness (23-28).

Besides exercise stress testing, acute fluid challenges can be used to further investigate the Fontan circulation, which is particularly interesting because the fluid balance is more critical because of the lack of a ventricle for the pulmonary circulation it. In Fontan patients a fluid bolus may result in an increased preload and subsequent increase in cardiac output, but it may also lead to an increase of end-diastolic pressure, which may lead to a decrease of the transpulmonary gradient and pulmonary blood flow, resulting in a decrease of cardiac output (29). Moreover, in Fontan patients the response to acute fluid depletion may also differ from the response in patients or healthy individuals with a biventricular circulation. Acute fluid depletion occurs during orthostatic stress and when it occurs, activation of the sympathetic nervous system and concurrent vagal withdrawal is required to increase heart rate, systemic vasoconstriction, and venous return to maintain adequate blood pressure and cerebral blood flow (30, 31). However, in Fontan patients, several of these mechanisms, such as the cardiac autonomic nervous system (ANS) activity, has been described to be impaired (26, 32-35).

Aim

The aim of this thesis is to obtain better insight into cardiovascular and hemodynamic properties of the Fontan circulation in a homogeneous group of paediatric patients with univentricular hearts, and to evaluate the effects of ACE inhibition in this group. This is performed by detailed echocardiographic assessment of cardiovascular function parameters, evaluation of hemodynamic effects of acute fluid loading and depletion, ANS activity, and exercise tests.

Lastly, as little is known about plastic bronchitis, a severe complication of the Fontan circulation, the literature of plastic bronchitis in Fontan patients was systematically reviewed to evaluate the characteristics, survival, and management.

Outline of thesis

In **chapter 2**, the research design and data collection of the studies in chapter 4,5,6, and 7 is described in detail. In **chapter 3**, we conducted a study in healthy children and adolescents to evaluate the normal maturation and provide normative values of cardiac sympathetic and parasympathetic ANS activity. To be able to have a large sample covering the age range of 0.5 to 20 years, data from 5 different

cohorts were combined in this study. In the following three chapters, 4,5, and 6, we evaluated the haemodynamics and limitations of the Fontan circulation in a homogeneous group of paediatric Fontan patients with an extracardiac conduit and moderate to good systolic ventricular function. In **chapter 4**, we evaluated the Fontan circulation by comparing various non-invasively measured cardiovascular parameters between Fontan patients and healthy controls and determined the influence of these parameters on exercise limitation in Fontan patients. In **chapter 5** we further investigated the Fontan circulation by studying the difference in hemodynamic response of paediatric Fontan patients to a fluid challenge by passive leg raising compared to healthy controls. Passive leg raising is a non-invasive and reversible manoeuvre that is reliable in predicting the responsiveness of a fluid bolus. Furthermore, in **chapter 6**, we further evaluated hemodynamic mechanisms of the Fontan circulation by non-invasively examining the cardiovascular response to orthostatic stress, induced by head-up tilt testing, between paediatric Fontan patients and healthy paediatric controls. Besides evaluating the orthostatic stress response of Fontan patients, we also investigated the effect of a three-month enalapril treatment on the orthostatic stress response in paediatric Fontan patients in **chapter 6**. In **chapter 7**, following on from the previous chapter, we investigated the effects of ACE inhibition by examining the short-term effects of a three-month enalapril treatment on exercise capacity, vascular and ventricular function in paediatric Fontan patients with moderate to good systolic ventricular function. In **chapter 8**, we systematically reviewed the literature for all case reports and series of SV patients with plastic bronchitis to give insight into the characteristics, survival, and treatment management of these patients. **Chapter 9** summarizes the main findings of our studies, discusses these in view of present literature and provides future perspectives. Finally, in **chapter 10** a Dutch summary of our work is provided.

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Chapter 2

Research design and data collection

sAFE trial

The sAFE-trial, full-title: "ACE inhibition in Fontan patients: its effect on body fluid regulation", was performed at the Leiden University Medical Centre (LUMC) between July 2017 and October 2019. Data collection took place at different outpatient clinics of the departments of paediatrics, neurology, and cardiology. The study was approved by the Medical Ethics Committee of the LUMC (P 17.045) and by the Central Committee on research involving human subjects in the Netherlands (CCMO), as it concerned a prospective intervention study in children. Furthermore, the study was registered in the Dutch Trial registry (NL6415; NTR6591).

Study population

Fontan patients

Patients between 8 to 18 years of age with a univentricular heart after Fontan palliation who had a regular clinical follow-up at one of the outpatient clinics of the Centre for Congenital Heart Disease Amsterdam-Leiden (CAHAL), consisting of the LUMC and the Amsterdam University Medical Centres (Amsterdam UMC, location Boelelaan and Meibergdreef), were included in the study. Patients with pre-existent ACE-inhibitor use and those who had difficulties to follow instructions or were unable to exercise were excluded from the study.

Patients who were eligible for the study were asked to participate via telephone or in person when they visited one of the outpatient's clinics for a routine clinical follow-up. At least one of the two visits, and most often the follow-up visit, was part of a regular clinical follow-up.

Descriptive characteristics of the patients who participated in the study can be found in Table 1. Because often patients receive ACE-inhibitors when they have systolic ventricular dysfunction, all patients included in this study had a moderate-good systolic ventricular function. Furtzhermore, as we included paediatric patients, all patients had been operated with the latest surgical techniques, and all had received a total cavopulmonary connection with an extra cardiac conduit. As a result of our inclusion criteria, we were able to study a homogenous group of Fontan patients.

Healthy controls

Healthy children between 8 to 18 years of age were recruited to take part in the study to serve as controls. Healthy controls were recruited through advertisement at local schools in the surrounding of the LUMC, advertisement at the local science

day of the LUMC and posters in the CORPUS museum in Oegstgeest. Medication use or chronic diseases were exclusion criteria. Descriptive characteristics of the healthy controls are depicted in Table 1.

Informed consent

Written informed consent was obtained from all participants or their parents or legal guardians.

Table 1. Descriptive characteristics

Characteristics	Fontan patients (N=36)	Controls (N=35)
Age (years)	14.0 [12.7-16.6]	12.8 [11.1-15.5]
Males (N,%)	23 (56.1)	18 (43.9)
Height (cm)	163.0 (14.1)	160.8 (13.5)
Weight (kg)	51.4 [42.8-60.3]	46.2 [37.0-56.9]
BSA (m^2)	1.55 (0.3)	1.46 (0.27)
Diagnosis (N,%)		
Tricuspid atresia	9 (25.0)	
Pulmonary atresia	2 (5.6)	
Double inlet left ventricle	5 (13.9)	
Double outlet right ventricle	1 (2.8)	
Hypoplastic left heart syndrome	8 (22.2)	
Unbalanced atrioventricular septum defect	4 (11.1)	
Other	7 (19.4)	
Main ventricle (N;%)		
Left	21 (58.3)	
Right	12 (33.3)	
Indifferent	3 (8.3)	
Age at Glenn operation (years)	0.52 [0.38-0.79]	
Age at Fontan operation (years)	3.2 (0.7)	
Patent Fenestration (N,%)	1 (3.0)	
Pacemaker (N,%)	1 (2.8)	
NT-pro BNP (ng/L)	80.2 [48.0-131.3]	
Cardiac medications (N, %)		
Acetylsalicylic acid	34 (94.4)	
Coumarin derivative	2 (5.9)	
β-blocker	1 (2.8)	
Diuretics	1 (2.8)	

Data expressed as n (%), mean (±SD), and median [Q1-Q3].
BSA= body surface area; NT-pro BNP= N-terminal pro brain natriuretic peptide.

Study design

This study consisted of a cross-sectional and prospective intervention study. For the cross-sectional study, several baseline cardiovascular measurements were compared between Fontan patients and healthy controls. For the prospective intervention study, several cardiovascular measurements measured before and after a three-month enalapril treatment in Fontan patients were compared. Healthy controls were not treated with enalapril.

Figure 1A depicts the timeline of the protocol per visit for the different groups. For both groups, Fontan patients and healthy controls, the visit started at the paediatric outpatient clinic with measuring weight and length, and they were asked about their medication use and if they ever had experienced syncope. Then, electrodes of the VU-AMS monitoring system, measuring electro- (ECG) and impedance cardiogram (ICG), were attached, and connected to the device which the participant could wear with an added belt. Thereafter, participants were relocated to the neurology department for a fluid challenge, which took on average 90 minutes. After the fluid challenge, the in-hospital part of the study was completed for the healthy controls, while Fontan patients usually had a break before going to the paediatric outpatient clinic for a blood test and the cardiology department for an exercise test. As adequate reference values of exercise parameters of healthy children exist, cardiopulmonary exercise tests were not performed in healthy controls.

At the end of hospital visit all participants went home wearing the VU-AMS device, which they could detach after 24 hours from start of the measurement. The device could be sent back the next day by mail using a return envelope which the participants received during their hospital visit.

Fluid challenge:

Figure 1B shows the protocol of the fluid challenge. All participants were placed on a tilt table in supine position to measure baseline parameters. First, a thorough echocardiographic assessment was performed. Then, all devices which would measure different parameters continuously were positioned and connected consecutively. These consisted of an arterial cerebral blood flow registration, pulse oximetry, capnography, and blood pressure measurements by means of the Finapress. The VU-AMS device was already connected at the paediatric outpatient

clinic. When everything was connected, a 4-minute baseline registration took place (first light blue marked period in Figure 1B) and during this period an arterial stiffness and blood pressure measurement was performed via an arteriograph device.

After the baseline measurements had been performed, the participants underwent a 45^{o} passive leg raising test (PLR) and a 60^{o} head-up tilt testing (HUTT) on a mechanical tilt table with safety belts (Figure 2). By means of PLR an easy, safe, and reversible fluid challenge can be given and passive HUTT induces an easily and fast reversible unloading of the central blood volume. There was a period of at least 10 minutes between the two tests, in supine position, to allow the circulation to return to baseline levels. Tilt test could prematurely be terminated in case of imminent syncope or at patients request. After 3 minutes from start of PLR and HUTT all measurements performed in supine position were repeated, as the acute phase has then passed, and the parameters of interest would have been stabilized to the new hemodynamic situation. For the continuously monitored measures, final values were obtained by averaging the 3-7 minutes period from start PLR or HUTT (periods marked in light blue in Figure 1B). At the end of the fluid challenge, after HUTT, the participants rested for at least 5 minutes in supine position.

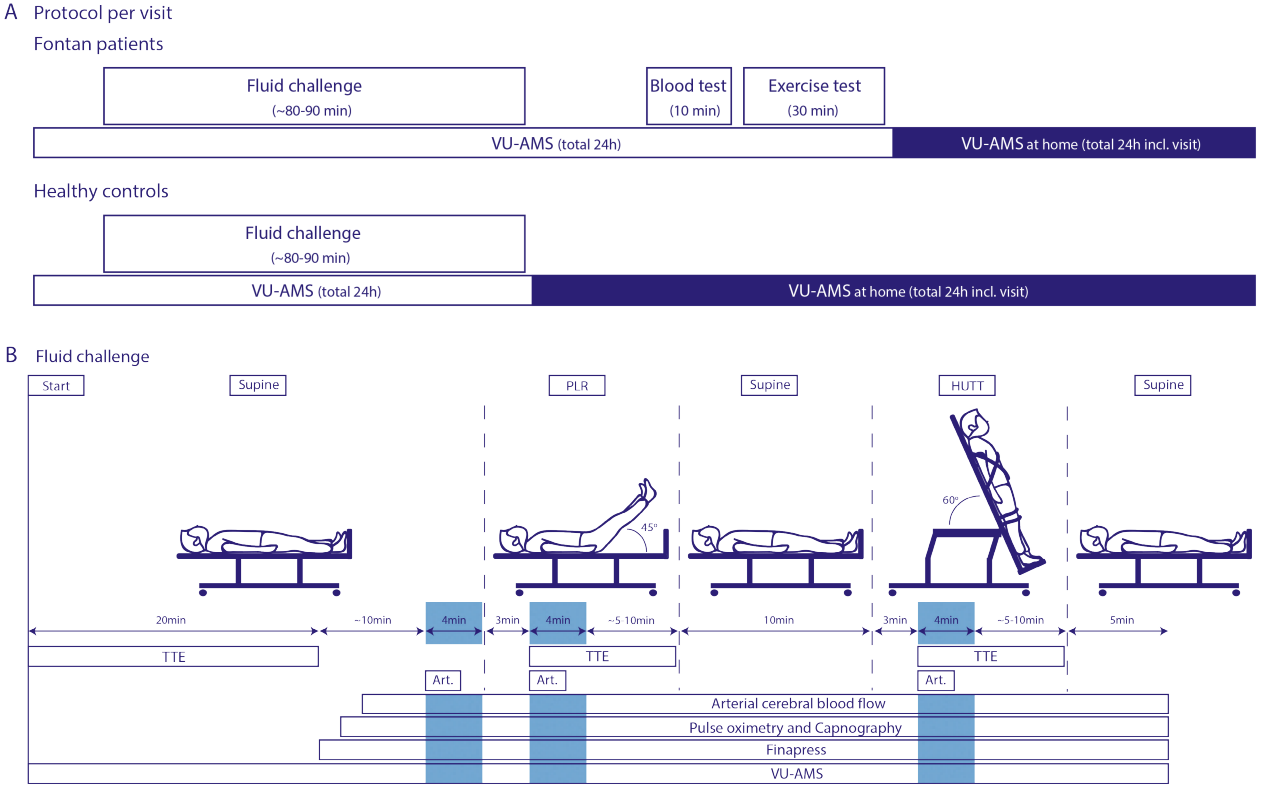

Figure 1. A: Timeline of the protocol per visit and B: Protocol of Fluid challenge. Art.= Arteriograph measurement; HUTT= Head-up tilt table; PLR= passive leg raising; TTE= Transthoracic echocardiogram; VU-AMS= VU University ambulatory monitoring system, recording electro- and impedance cardiogram.

Figure 2. Participant during HUTT

Follow-up and ACE-inhibition

Treatment with enalapril, an angiotensin converting enzyme (ACE) inhibitor, started one day after the first study visit. Treatment started with a dosage of 5mg/day and dosage was further titrated over a period of 2-3 weeks to a targeted dosage of 0.5mg/kg/day or a maximum of 20mg/day. Dosage was titrated according to blood pressure which could be measured by the general practitioners of the patients. When patients experienced side effects or when the systolic blood pressure fell more than 20%, the dosage was lowered. Renal function, measured by creatinine and urea blood levels, was checked after two weeks of treatment with the maximal tolerated dosage. This could also be checked at the general practitioner or at a local hospital near the patient's home.

After three treatment months, all procedures performed at the first study visit were repeated (Figure 1). A period of three months was chosen as studies in adult and paediatric patients had shown beneficial effects of ACE-inhibitors within 12 weeks of treatment (1-5). An overview of the follow-up of Fontan patients during the three-month enalapril treatment period is shown in Figure 3.

Echocardiography

Transthoracic echocardiograms (TTE) were performed on a Vivid S6/S60 (General Electric Healthcare, Norway) and conducted by an experienced technician or a paediatric cardiologist. Images were stored and analysed offline (using EchoPac;

version 203, GE Healthcare) by one researcher and experienced technician, supervised by a paediatric cardiologist. Echocardiography was performed in supine position and during HUTT and PLR. The supine echocardiographic assessment was more extensive than during HUTT or PLR, because for Fontan patients it was also part of a routine clinical follow-up and for healthy controls to evaluate whether they had a structurally and functionally normal heart.

Conventional echocardiography, consisting of pulse wave Doppler or in case of high velocities continuous wave Doppler, 2D echocardiography and M-mode, was performed. In addition, Tissue Doppler Imaging (TDI) and speckle tracking were performed following previously published methods (6, 7). TDI was performed to obtain myocardial velocity curves of the basal part of the systemic ventricles' lateral wall and septum. Strain images were recorded with grayscale images of the four-chamber view (longitudinal analysis) with, preferably, 60-90 frames per second. In Fontan patients, longitudinal global peak strain was conducted if 5 out of 6 segments could be recorded with acceptable curves. Furthermore, if the ventricular septum defect was larger than one segment, strain was conducted from both lateral walls. Variables extracted from the TTE and used for analysis in the sAFE-study project are summarized in Table 2. Averages of three consecutive cardiac cycles were used for analysis, if applicable.

Arteriograph

The Arteriograph (Tensiomed, Budapest, Hungary), an oscillometric device (8), was used to measure blood pressure as well as pulse wave velocity of the aorta (PWVao) and augmentation index of the aorta, both parameters of arterial stiffness. To obtain arterial stiffness measurements, the device performs two consecutive blood pressure measurements with the cuff over-inflated during the second measurement by 35-40mmHg above systolic pressure. The Arteriograph software calculates average values of each measurement and determines accuracy of the measurement with a standard deviation. A measurement is considered accurate when the standard deviation of PWVao <1.0m/s. However automatic calculation is not always possible, for example due to smaller pulse waves and movement which will distort the shape of the wave. As children exhibit smaller pulse waves and HUTT also results in smaller pulse waves, each cardiac cycle had to be analysed one by one using the software. Each measurement was visually checked by one researcher and was considered valid when a reliable value could be calculated with a standard deviation of PWVao <1.0m/s over at least two separate measurements. All measurements were performed with the cuff on the left arm. Arterial stiffness parameters derived from the Arteriograph are summarized in Table 3.

Follow-up Fontan patients

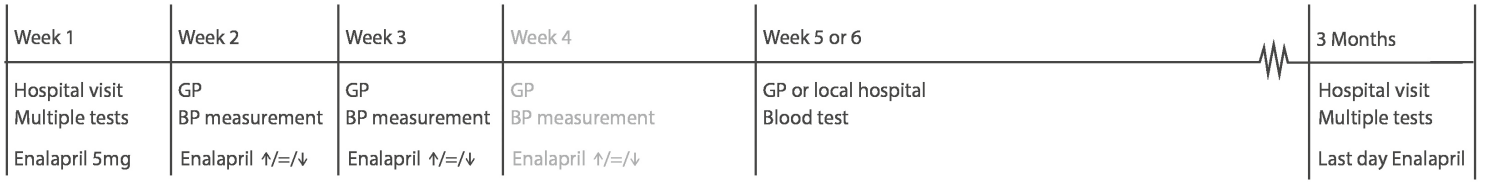

Figure 3. Timeline of Follow-up of Fontan patients. BP= Blood pressure; GP= General practitioner.

Table 2. Echocardiographic parameters

Parameter	Derived from	Parameter definition	Comment
Velocity time integral *VTI (cm)*	PW Doppler across the aortic valve	Equivalent to the area within the pulse wave doppler spectral curve	
Stroke volume index *SVI (ml/beat/m^2)*	Aortic annulus diameter and PW Doppler across the aortic valve	Multiplying the aortic cross-sectional area with VTI divided by the body surface area	
Cardiac index *CI (ml/min/m^2)*	Aortic annulus diameters, PW Doppler across the aortic valve and heart rate	Multiplying SVI with heart rate	
Longitudinal global peak strain *Global strain (%)*	Apical 4-chamber view using speckle tracking strain analysis	Peak from the global time-strain curve of the systemic ventricle	Systolic function parameter, lower value equals worse contraction
Peak myocardial velocities *Peak systolic (S'), peak early (E') and late (A') diastolic velocities (m/s)*	From Tissue Doppler Imaging, curves of the basal part of the systemic ventricles' lateral wall and septum	S' is the maximal velocity during systole, E' and A' are the maximal velocities during the early and late ventricular filling phase	A lower S' equals worse systolic function. A higher E'/A' ratio equals worse diastolic function.
Peak flow velocities *Peak early (E) and late (A) diastolic velocities (m/s)*	PW Doppler across the atrioventricular valve of the systemic ventricle	Maximal velocity during the early and late ventricular filling phase	A higher E/A ratio equals worse diastolic function
E/E' ratio	PW (E) and Tissue Doppler (E' of lateral wall) peak early diastolic velocities	Ratio of pulse wave and tissue doppler peak early diastolic velocities	A Higher E/E' ratio equals worse diastolic function
Peak hepatic flow *(m/s)*	PW Doppler hepatic vein	Maximal antegrade flow	
Inferior vena cava collapsibility index *IVC collapsibility index (%)*	Maximum and minimum diameter of IVC during sniff test, measured by M-mode	Proportional change of the IVC during sniff-test	

Table 3. Arterial stiffness parameters

Parameter	Derived from	Parameter definition	Comment
Pulse wave velocity aorta *PWVao (m/s)*	Pulse wave analysis and Jugulum-Symphisis distance	Speed of aortic blood flow: Jug-Sy distance divided by half the time between the direct systolic wave and the late/reflected systolic wave (=return time): $\frac{\text{Jug} - \text{Sy (m)}}{\text{Return time}/2\text{ (s)}}$	Higher value equals a stiffer vessel
Aortic augmentation index *AIXao (%)*	Pulse wave analysis	Blood pulse wave reflection: Difference between the late/reflected (P2) and early (P1) systolic wave pressure, expressed as a percentage of the pulse pressure (PP): $\frac{\text{P2} - \text{P1}}{\text{PP}} \text{ X } 100$	Higher value equals a stiffer vessel; influenced by heart rate

Arterial cerebral blood flow

Transcranial Doppler sonography was performed to assess the mean velocity blood flow of both cerebri media arteries. Sonography was assessed by two 2MHz probes which were attached to an adjusted Marc 500 headframe (Spencer Technology, America; Figure 4) and Doppler signals processed by a non-imaging Doppler-Box with DWL Doppler system and QL software version 3.3 (Compumedics Germany, supplied by VCM medical, Netherlands). Although sonography of both arteries was assessed, the artery with the highest mean velocity was used for analysis (Table 4). Sonography of cerebral arteries is challenging and depends on the echo window of the temporal bone. Due to insufficient echo windows, cerebral blood flow measurements could only be assessed in 19 Fontan patients and 27 controls in the sAFE study.

Pulse oximetry and Capnography

Pulse oximetry was performed with a Nihon Kohden (Japan) finger clip pulse oximeter to measure blood oxygen saturation. Furthermore, capnography was performed by a capONE mainstream CO_2 sensor with a nasal/oral adapter (Figure 4) to measure end-tidal CO_2. Definitions of both parameters are shown in Table 4.

Table 4. Arterial cerebral blood flow measurement, pulse oximetry and capnography

Parameter	Derived from	Parameter definition	Comment
Mean arterial velocity of the artery cerebri media *MAV art. cerebri media (cm/s)*	Transcranial Doppler sonography	Mean velocity of the artery cerebri media.	The artery with the highest mean flow was used for analysis
Oxygen saturation *SpO_2 (%)*	Pulse oximetry	The fraction of oxygen-saturated haemoglobin relative to total haemoglobin in the blood.	
End-tidal CO_2 *$EtCO_2$(mmHg)*	Capnography	The level of carbon dioxide that is released at the end of an exhaled breath.	

Finapress

During the fluid challenge blood pressure was continuously monitored by finger plethysmography with use of a Finometer (Finapress, The Netherlands, Figure 4). Monitoring of blood pressure during tilt test is essential to recognize possible imminent syncope and to abort the tilt test in time before real syncope occurs. As blood pressure is accurately measured by a the arteriograph at baseline, PLR, and HUTT we used the data of blood pressure measurements of the arteriograph for analysis within the sAFE study. By use of Modelflow, the data from the Finometer can be used to calculate stroke volume, cardiac output, and peripheral resistance, among other things. The Modelflow method is, however, a good method for adult patients and has not yet been validated in children. Furthermore, preliminary analysis revealed that the Modelflow method was also not suitable to analyse the altered circulation in Fontan patients. Therefore, data from the Finapress has not been used in the analysis and will not be discussed further.

VU-AMS

The VU Ambulatory Monitoring System (VU-AMS; VU University, Amsterdam, The Netherlands, www.vu-ams.nl) was used to conduct ICG and ECG recordings to assess different cardiac autonomic nervous system activity parameters and respiration (9). Three pregelled Ag/AgCL (Kendal H124SG) spot electrodes on the chest were used to derive a one lead ECG (Figure 5). Thoracic ICG was conducted by introducing a small alternating current (50kHz, 350μA) through the thorax by use of four spot electrodes (same as used for the one lead ECG), two electrodes on the thorax, just above and below the sternum, and the two current electrodes on the back of the thorax, place 3 cm above and below the electrodes from the front of the thorax (Figure 5). The VU-AMS records ECG and ICG by using a sample rate of respectively 1000 and 250Hz.

In this study the 5fs version of the VU-AMS version was used. Furthermore, the VU Data Analysis and Management Software (VU-DAMS; VU-University) was used to analyse the date offline. Ectopic beats and artefacts were removed in the VU-DAMS program after an automated scoring of R-peaks and artefact detection by the software and a final visual check of the ECG. A total of six Fontan patients were excluded for analysis due to an atrial ectopy or a nodal rhythm. In addition, one other patient was also excluded from analysis due to a pacemaker.

Variables extracted from the ECG and ICG recordings and used for analysis in the sAFE-study project are summarized in Table 5.

Figure 4. Participant with headframe, nasal/oral adapter and Finapress (around wrist and middle finger)

Figure 5. Participant with the VU-AMS electrodes on the thorax.

Blood test

At baseline and after three treatment months plasma N-terminal pro brain natriuretic peptide (NT-pro BNP) levels were assessed to evaluate the effect of enalapril treatment. NT-pro BNP is a non-active prohormone that is released in response to changes in pressure in the heart and can be related to heart failure. Higher NT-pro BNP levels are associated with heart failure and worse physical function.

As ACE inhibition may affect renal function, renal function was assessed at baseline, after two weeks of treatment with the maximal tolerated dosage and at the end of the study after three months of treatment. Renal function was measured by assessing plasma creatinine and urea levels.

Table 5. Parameters derived from VU-AMS

Parameter	Derived from	Parameter definition	Comment
Respiration Sinus Arrhythmia *RSA (ms)*	ECG & ICG	Difference between the longest inter-beat interval during exhalation and the shortest inter-beat interval during inhalation	Measure of cardiac parasympathetic (PNS) control. A higher value equals higher PNS activity
Root mean square of successive differences *RMSSD (ms)*	ECG	Root mean square of successive differences	Measure of cardiac parasympathetic control. A higher value equals higher PNS activity
Standard deviation of the inter-beat intervals *SDNN (ms)*	ECG	Standard deviation of the inter-beat intervals	Measure of cardiac parasympathetic control. A higher value equals higher PNS activity
Low frequency power spectral values *LF (ms^2)*	ECG	Absolute power of the low-frequency band (0.04-0.15Hz)	Both parasympathetic and sympathetic activity contributes to LF power.
High frequency power spectral values *HF (ms^2)*	ECG	Absolute power of the high-frequency band (0.15-0.4Hz)	Parasympathetic activity primarily contributes to HF power.
Low frequency/high frequency ratio *LF:HF*	ECG	Ratio of LF to HF power	Measure of sympathicovagal balance, however, controversial as LF is not a pure measure of sympathetic activity
Pre-ejection period *PEP (ms)*	ECG & ICG	Time difference between the start of electrical depolarization (i.e., Q-wave onset on ECG) and start of left ventricular outflow (i.e., B-point in the ICG signal)	Measure of cardiac sympathetic control. A lower value equals a higher sympathetic activity
Respiration rate *RR (breaths/min)*	ICG	Number of breaths per minute	

Cardiopulmonary exercise test

Cardiopulmonary exercise tests were performed in upright position on a bicycle ergometer (Jaeger ER 900; Viasys Healthcare, Höchberg, Germany) with breath-by-breath analysis using a flowmeter (Triple V volume transducer) and computerized gas analyser (Jaeger Oxycon Champion, Viasys Healthcare or Carefusion Vyntus, Vyaire Medical) (Figure 6). Breath-by-breath minute ventilation (VE), oxygen uptake (VO_2), carbon dioxide production (VCO_2) and respiratory exchange ratio (RER,

defined as VCO_2/VO_2 ratio) were conducted per 10 seconds. Furthermore, heart rate was continuously monitored through a twelve lead ECG and blood pressure was measured every two minutes. Starting wattage and workload increment per minute were determined by the physician and by the age of the patient. For Fontan patients who underwent the exercise test at baseline and after three months of treatment, the same starting wattage and workload increment per minute was used for both tests. During the test, the patients had to maintain a pedalling rate between 60-65 revolutions per minute. Patients were encouraged by the technician, physician, and researcher to exercise until exhaustion. The test was ended by the patient in case of discomfort or by the supervising physician in case of ECG changes, an excessive breathing pattern or otherwise. After cessation of the test, the patient was instructed to pedal at <40 revolutions per minute with no resistance for at least two minutes. The ECG was detached when the heart rate had returned to resting values.

Variables extracted from the cardiopulmonary exercise test are summarized in Table 6. A test was considered maximally performed when RER was ≥1.0. Maximally exercise parameters were only determined in patients who had an RER ≥1.0, submaximal parameters were assessed in all patients.

Besides absolute values, the percentage of predicted values adjusted for age and sex were calculated, where applicable, using previous published methods (10-12), except for peak oxygen uptake ($VO_{2\,peak}$) which was adjusted for age, sex, weight and height (13) and oxygen pulse for which we used the data of Ten Harkel et al. (10).

Table 6. Parameters derived from cardiopulmonary exercise test

Parameter	Derived from	Parameter definition	Comment
Peak respiratory exchange ratio *RER_{peak}*	Breath-by-breath analyser	Respiratory exchange ratio at peak exercise, calculated by dividing carbon dioxide production by oxygen uptake at peak exercise (VCO_2/VO_2).	An RER of 1.0 is the aerobic threshold. A test was considered maximally performed when RER was ≥1.0.
Maximal exercise parameters			
Peak work rate *WR_{peak} (watt or watt/kg)*	Power meter	Highest work rate achieved and finished (1 minute completed).	
Peak heart rate *HR_{peak} (bpm)*	ECG	Highest heart rate at peak exercise	Measure of chronotropic competence
Heart rate reserve *HR reserve (bpm)*	ECG	Difference between peak heart rate and resting heart rate (measured from baseline ECG laying on the bench before exercise test)	Measure of chronotropic competence
Heart rate recovery at 1 min in % *HR01 percentage (%)*	ECG	Percentage heart rate recovery at 1 minute after exercise in relation to heart rate reserve: $\frac{\text{HR peak exercise} - \text{HR1min. after exercise}}{\text{HR reserve}} x100$	
Peak oxygen uptake *VO_{2peak} (L/min or ml/kg/min)*	Breath-by-breath analyser	Highest measured oxygen consumption at peak exercise	Measure of aerobic fitness
Peak O_2 pulse *(ml/beat)*	Breath-by-breath analyser and ECG	Peak oxygen uptake per heartbeat.	Has been used as a correlation of stroke volume

Table 6. Parameters derived from cardiopulmonary exercise test (continued)

Parameter	Derived from	Parameter definition	Comment
Submaximal exercise parameters			
Respiratory minute to CO_2 production slope *VE/VCO_2 slope*	Breath-by-breath analyser	Slope of the linear regression of ventilatory efficiency (VE) and CO_2 production (VCO2) during the entire period of the test.	Measure of anaerobic fitness A higher slope reflects poor ventilatory efficiency
Oxygen uptake efficiency slope *OUES*	Breath-by-breath analyser	Linear least squares regression of the oxygen uptake (VO_2) on the common logarithm of the ventilatory efficiency (VE) by the equation: VO_2 = a log (VE) + b, where the constant a is the regression coefficient.	Measure of anaerobic fitness
Oxygen uptake efficiency plateau *OUEP*	Breath-by-breath analyser	The 90-sec average of the highest consecutive oxygen uptake efficiency (OUE) values (OUE= ventilatory efficiency divided by oxygen uptake, VE/VO_2)	Measure of anaerobic fitness

Figure 6. Participant performing a cardiopulmonary exercise test

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Chapter 3

Maturation of the Cardiac Autonomic Nervous System Activity in Children and Adolescents

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Abstract

Background

Despite the increasing interest in cardiac autonomic nervous activity, the normal development is not fully understood. The main aim was to determine the maturation of different cardiac sympathetic-(SNS) and parasympathetic nervous system (PNS) activity parameters in healthy patients aged 0.5 to 20 years. A second aim was to determine potential sex differences.

Methods and Results

Five studies covering the 0.5- to 20-year age range provided impedance- and electrocardiography recordings from which heart rate, different PNS-parameters (eg, respiratory sinus arrhythmia) and an SNS-parameter (pre-ejection period) were collected. Age trends were computed in the mean values across 12 age-bins and in the age-specific variances.

Age was associated with changes in mean and variance of all parameters. PNS-activity followed a cubic trend, with an exponential increase from infancy, a plateau phase during middle childhood followed by a decrease to adolescence. SNS-activity showed a more linear trend, with a gradual decrease from infancy to adolescence. Boys had higher SNS-activity at ages 11 to 15 years, while PNS-activity was higher at 5 and 11 to 12 years with the plateau level reached earlier in girls. Interindividual variation was high at all ages. Variance was reasonably stable for SNS- and the log-transformed PNS-parameters.

Conclusion

Cardiac PNS- and SNS-activity in childhood follows different maturational trajectories. Whereas PNS-activity shows a cubic trend with a plateau phase during middle-childhood, SNS-activity shows a linear decrease from 0.5 to 20 years. Despite the large samples used, clinical use of the sex-specific centile and percentile normative values is modest in view of the large individual differences, even within narrow age bands.

What is New?

- To our knowledge, this is the first and largest multi-cohort study simultaneously comparing cardiac sympathetic and parasympathetic activity in 4820 healthy subjects from childhood to late adolescence.
- Impedance cardiography and ECG analysis showed a linear increase in sympathetic activity and a non-linear pattern for parasympathetic activity with an exponential increase from infancy, a plateau phase during middle childhood, followed by a decrease to adolescence.
- Parasympathetic nervous system maturation in boys peaked later than that in girls.

What Are the Clinical Implications?

- Our study provides the longitudinal maturation of sympathetic and parasympathetic activity from childhood to adolescence in healthy children.
- These maturational trajectories may be used as normative for different subjects in whom normal development may be precluded, eg, due to various disease states as cardiopulmonary, metabolic, or musculoskeletal diseases.
- The clinical use of the age-specific centile and percentile values for cardiac autonomic nervous system indices at a single point of the maturational trajectory is modest in view of the large individual differences even within narrow age ranges.

Introduction

The autonomic nervous system (ANS) coordinates bodily functions to ensure homeostasis in response to the external and the internal environment(1). This system can be subdivided into 2 branches; the parasympathetic nervous system (PNS), which prevails during periods of rest, and the sympathetic nervous system (SNS), which prevails when the body is active (1). The effect of ANS activity on the heart rate (HR) can be measured using respiratory sinus arrhythmia (RSA) mainly reflecting PNS activity and the pre-ejection period (PEP) reflecting SNS activity. RSA refers to heart rate variability (HRV) that is closely coupled to inspiratory and expiratory phases of the respiratory cycle and reflects cardiac vagal effects on the sinoatrial node (2). PEP is the time interval between ventricular electrical depolarization and the beginning of ventricular ejection(3, 4). It has been shown to reflect β-adrenergic inotropic drive to the left ventricle through a variety of manipulations of cardiac SNS activity by pharmacological (ant)agonists, exercise, and mental stress (5-22).

Because of its immediate relevance for cardiovascular disease and mortality in adults the interest in cardiac ANS activity has grown exponentially(23). The field further expanded with increasing evidence for cardiac ANS activity as a biomarker for healthy development of mental and physical functioning in children(24-26). Although many studies addressed the maturation of the cardiac ANS from birth to adolescence using RSA or PEP (24, 27-39), most used short follow-up times in a single developmental period (27, 29-33, 35-37, 39) and relatively small samples of <500 children (24, 27-37, 39), often selected to be at high risk for or diagnosed with psychopathology (27, 31-33, 36, 38, 39). Moreover, studies *simultaneously* comparing PNS- and SNS-activity, with PEP and RSA levels, in healthy children across the childhood and adolescent age ranges are rare (27, 31, 32). This is unfortunate, as knowledge of the normal development of the cardiac ANS in childhood is essential if we want to use ANS activity to discriminate between health and disease.

Results of various studies, using different HRV parameters, have suggested substantial maturational changes in cardiac PNS activity (40-43). Most of these studies investigated the SD of the inter-beat interval of normal sinus beats (SDNN) or the root mean square of successive differences between normal sinus beats (RMSSD), both HRV parameters reflecting PNS activity in short-term recordings (44). A clear and steep increase in HRV in the breathing frequency range is seen in the first months of life (33), congruent with an increase in the amount of myelinated fibres of the vagus nerve(45). HRV then further increases rapidly in early childhood and levels

off in late childhood, reaching its peak in adolescence (28, 40, 42, 43, 46). Effects of sex on the maturation of HRV in children have not been consistent across studies. Higher HRV values in boys have been found in some studies (29, 41, 47-49), while others have reported minimal or no differences (24, 28, 32, 34, 39, 43, 46, 50, 51). In contrast to PNS maturation, the developmental trajectory across childhood and adolescence has been less frequently studied. The few studies so far showed that PEP increases from infancy to late adolescence with an inconsistent sex difference across the those studies (27, 30, 32, 35).

An increase or decrease in individual differences of ANS activity can arise from age-specific hormonal (52) and brain connectivity changes (53) relevant to ANS functioning. In addition, differential exposure across childhood to ANS affecting factors like psychosocial stress and lifestyle behaviours may act to increase the variance in ANS activity between individuals (54, 55). Many of the previous studies have implicitly assumed homogeneity of variance across childhood, when comparing means between age-groups, whereas this assumption has rarely been tested explicitly.

The main aim of the present study was to investigate changes in PEP, RSA, RMSSD and SDNN from ages 6 months to 20 years to evaluate normal maturation of cardiac PNS and SNS activity. A second aim was to determine if there were sex differences in maturation of cardiac ANS activity. The results of this study can provide normative values for the maturation of cardiac PNS and SNS activity in childhood, which would have substantial clinical use.

Methods

Study Populations

In this study we combined data from participants of 5 different cohorts and different ages. All study cohorts had received ethical approval. In all studies written informed consent was obtained from all participants and/or parent(s)/guardian(s) where appropriate. The data that support the findings of this study are available from the corresponding author upon reasonable request. Methods of cardiac ANS measurement were identical in all 5 cohorts with all studies using the same ambulatory impedance cardiography (ICG) and electrocardiography recording device for data collection. Data collection of every cohort is described separately below.

1. NTR (Netherlands Twin Register) is a large cohort consisting of multiples and their families from the Netherlands, enrolled in ongoing longitudinal survey

studies (56). From this cohort, twin pairs aged between 16 to 18 years as well as their siblings aged between 12 to 25 years were invited to participate in a study investigating determinants of adolescent exercise behaviour (4). A total of 549 healthy participants completed combined ICG and electrocardiography during a 4-minute sitting baseline measurement. A random selection of one member per family yielded 285 non-related participants for a sensitivity analysis testing the potential effects of clustering within family.

2. The FemNAT-CD (Neurobiology and Treatment of Adolescent Female Conduct Disorder) study is a European project aimed at understanding the neurobiological and sex differences of conduct disorder (57). The study therefore investigates autonomic nervous system activity in boys and girls with conduct disorder and in normally developing boys and girls. Participants were recruited in England, Germany, Switzerland, Spain, Greece, Hungary and the Netherlands. A control group of 753 normally developing boys and girls between the ages of 9 to 18 years completed ICG and electrocardiography recording during a 5-minute baseline assessment in a sitting position.

3. MINDS (Mother-Infant Neurodevelopment Study) is a Dutch longitudinal study that is aimed at investigating factors of influence on emotional and behavioural problems in children (58). Primiparas women between ages 17 to 25 years with uncomplicated pregnancies were recruited to participate. To obtain enough variance in children's early behavioural problems, the study oversampled women from a high-risk background (i.e., due to the presence of ≥1 risk factors, eg, maternal psychopathology, substance use, and social adversity). A total of 86 healthy infants from low-risk and 54 healthy infants from high-risk mothers completed ICG and electrocardiography baseline measurements at the age of 6 months during a 2-minute relaxing movie while lying on a blanket. Given that published data from the MINDS-study showed that there were no differences in baseline measurements of PEP and RSA between the low- and high-risk groups(58), we found it justified to include the data from all 140 children.

4. Nederend et al. performed a study to improve stroke volume assessment from a spot-electrode based impedance-cardiogram in a paediatric population of both healthy children and children with a corrected congenital heart disease between ages 1 to 18 years (3). Here we use the former group of 117 healthy controls only. They completed the combined ICG and electrocardiography recording during a 4-minute sitting baseline measurement.

5. The ABCD (Amsterdam Born Children and their Development) study is a Dutch prospective birth cohort study examining the association between prenatal and early-life influences on later health (59). The study population included 8266 pregnant women. At follow-up, an electrocardiography and ICG during a 7-minute sitting baseline measurement was completed in 3083 children at ages 5 to 7 years (ABCD 1 cohort) and 962 children at 11 to 12 years (ABCD 2 cohort). From these, 784 children completed the measurement at both follow-up points, allowing us to compute temporal stability of the PEP and RSA across a 5-year period. To avoid double use of the same children and introduce within-subject correlation, only the second measurements for these 784 children were used for determination of the maturational effects across all age groups. Omission of their data had no effect on the ANS outcomes in the remaining ABCD 1 cohort. ANOVA for the HR, PEP and RSA between the initial group of 3083 children at the age of 5 to 7 years versus the remaining 2299 children showed no significant differences (HR mean 91.8 versus 91.8 bpm, P=0.909; PEP mean 73.4 versus 73.3 ms, P=0.639; RSA mean 108.2 versus 108.1 ms, P=0.916).

Age Grouping

All subjects from the previously mentioned cohorts were divided in 12 age groups, aged 6 months, 1 to 4, 5, 6, 7 to 10, 11, 12, 13 to 15, 16, 17, 18, and ≥19 years. This age grouping provided an optimal balance of having a sufficient sample size in each age group, while maintaining a good representation of the maturational trajectory across the entire 0.5- to 20-year age span.

Data Collection

For all studies the following data were collected per individual subject: date of measurement , age, sex, and the mean HR, heart period (HP), PEP, RSA, RMSSD and SDNN across the entire baseline measurement period of the study.

ICG and electrocardiography recording were conducted using the 5fs version of the VU Ambulatory Monitoring System (VU University, Amsterdam, The Netherlands, www.vu-ams.nl). One lead electrocardiography was derived from 3 pre-gelled Ag/AgCl (Kendal H124SG) spot electrodes on the chest. Thoracic impedance (Z) was conducted by introducing a small alternating current (50 kHz, 350 mA) through the thorax, also by the use of spot electrodes(3). The VU Ambulatory Monitoring System records electrocardiography and ICG using a sample rate of respectively 1000 and 250 Hz. The whole electrocardiography/ICG recording of each separate study, ranging from 2 to 7 minutes, was used for analysis. All cohorts used the Vrije Universiteit Data Analysis and Management Software (also designed at the VU

University) offline for data analysis and the analysts were trained by the same team. Ectopic beats and artefacts were removed in the Vrije Universiteit Data Analysis and Management Software program after an automated scoring of R-peaks and artefact detection by the software and a final visual check of the electrocardiography.

PEP, a measure of SNS activity (5-22), is defined as the time interval between the ventricular electrical depolarization (i.e., Q-wave onset in the electrocardiography signal) and the start of left ventricular outflow (i.e., B-point in the ICG signal). The Q-point were checked manually in the Vrije Universiteit Data Analysis and Management Software. The ICG was ensemble averaged and automatically suggested candidate B-points were visually inspected and corrected when needed. This procedure yields a mean intraclass correlation coefficient (ICC) of .75 for PEP across 7 different raters (60) and ICC of .57 with PEP derived from transthoracic echocardiography (3). Increases in SNS activity are reflected in a shorter PEP (5-22).

Furthermore, RSA, a measure of PNS activity(2), was calculated using the peak valley method by combining the respiration signal and the inter-beat-interval time series. RSA is measured by subtracting the shortest inter-beat-interval during inhalation from the longest inter-beat-interval during exhalation. If no shortest or longest inter-beat-interval could be detected, RSA was set to zero for that particular breath. Increases in PNS activity are reflected in a higher RSA value. PNS activity was additionally measured by calculating RMSSD and SDNN(44). SDNN refers to the SD of the inter-beat interval of normal sinus beats and RMSSD to the root mean square of successive differences between normal sinus beats.

For all variables the mean of the whole recording of each included study was used for analysis.

ANS Adjustment

HRV variables are highly correlated to HR (or its inverse, the HP/inter-beat interval) (2, 61). Various previous studies on HRV maturation have resorted to some form of adjustment of HRV variables for HR (43) although the value of such “correction” has been questioned(2). Here, we have taken an empirical approach to this debate and present the results for the HRV measures with and without adjustment for the HP. HR adjusted values were calculated by dividing the mean of RSA, RMSSD, SDNN and PEP with the mean of the HP of the whole recording of each study.

Statistical Analysis

SPSS (IBM, version 25) and R statistics software (R Core Team, 2019(62)) were used for statistical analysis. Within each cohort separately (NTR, FemNAT-CD, MINDS, Nederend, ABCD 1 and 2) outliers were identified by standardized Z-scores >3.8 and removed. Distributional and QQ-plots and assessment of skewness were used to detect deviations from normality, and suggested that a natural log transformation of RSA, RMSSD, SDNN as well as their HP adjusted values were needed for parametric testing. However, to provide interpretable normative values we computed the sex and age stratified values of the median and 2.5th and 97.5th percentiles of the untransformed variables. To test for sex differences in these values (or their appropriate transforms) at each age, we compared boys and girls across all 12 age groups with an age by sex ANOVA. A significant age by sex interaction was followed up by an ANOVA of the sex difference per age group.

We next tested a variety of trends to the developmental trajectory over age (linear, quadratic, cubic, power, exponential, compound, and logistic) and used the R-squared value as a measure of model fit. Linear, quadratic, or cubic trends came out as the best fitting curves for all variables, untransformed, transformed and HP adjusted. The *ggplot 2* package (Wickham, 2016(63)) was used to create scatterplots of all variables per sex (boys, girls) and the *lm* function with sex and –dependent on the best fitting model - age, age^2, and age^3 s predictors were used to create centile as well as 2.5th and 97.5th percentile curves.

To visualize the age-specific variance of the different cardiac ANS parameters, histograms of the SD per age group were produced, separately for boys and girls. To obtain an idea of the sampling distribution of the age-specific SD we performed a 1000-fold resampling of 100% of the sample per age group and added the 95% CI to the histograms. For the HRV parameters, this was done for the SD of the untransformed and the transformed parameters. Using age groups as the independent variable, Levene test provided a formal test of homogeneity of variance in these SDs across age. In the subset of children from the ABCD study who were measured twice across a 5.5-year period, temporal stability of all variables, untransformed, natural log-transformed, and HP-adjusted were computed, using both Pearson correlation and the ICC.

Results

Because of technical failures, artefacts or excessive ectopic beats 266 (Nederend, 9; FemNAT-CD, 25; ABCD first wave, 161; ABCD second wave, 64; MINDS, 2; NTR, 5) children were excluded for analysis. Therefore, a total of 4820 children from 5 different cohorts were included in the study (Table 1). Overall, 47% of the subjects were boys, varying from 33.6% to 53.6% in the individual cohorts. Age distribution of multiple cohorts overlapped. All recordings were performed between October 2008 and February 2018. Table 2 shows the number of subjects per age group and sex for the total study population. Most age groups contained >100 subjects, except for age groups 1 to 4 years (25 subjects) and ≥19 years (73 subjects). In young children from the MINDS-cohort, the thorax impedance signal showed substantial clipping and was deemed not sufficient enough to quantify PEP and RSA in a way that would be comparable with the older children of the remaining cohorts. Therefore, PEP and RSA are missing for age group of 6 months.

Table 1. Characteristics Per Study Cohort

	NTR	FemNat-CD	MINDS	Nederend	ABCD Wave 1	ABCD Wave 2
Total participants (n)	549	753	140	117	2299	962
Boys (%)	261 (47.5%)	253 (33.6%)	75 (53.6%)	61 (52.1%)	1149 (50%)	485 (50.4%)
Age (Y) (±SD)	17.36 (±1.26)	14.76 (±2.48)	0.54 (±0.04)	10.16 (±4.92)	5.74 (±0.50)	11.83 (±0.38)
Period of testing	02-2012 - 08-2013	03-2014 - 02-2018	02-2012 - 02-2015	02-2014 – 04-2016	10-2008 - 12-2010	10-2015 - 03-2016

Table 2. Age and Sex Distribution of the Total Study Population

Age group	Boys (n)	Girls (n)	Total (n)
6, mo	75	65	140
1-4, y	12	13	25
5, y	804	792	1596
6, y	336	337	673
7-10, y	54	86	140
11, y	347	333	680
12, y	194	222	416
13-15, y	116	212	328
16, y	157	187	344
17, y	107	186	293
18, y	55	57	112
≥19, y	27	46	73
Total	2285	2537	4822

Developmental Trajectories in Mean PEP and HRV

The cubic model had overall the highest R-square and best fit according to Akaike Information Criterium for resting HR (R^2, 0.55-0.58), RSA (R^2, 0.07-0.11), RMSSD (R^2, 0.07-0.11) and SDNN (R^2, 0.10-0.13). Whereas the cubic model for PEP had a slightly lower Akaike Information Criterium, we chose the simple linear model as the difference in R^2 was very small (R^2, 0.45-0.54 for Cubic and 0.42-0.53 for Linear). Figures 1 and 2 depict scatterplots of resting HR, PEP, RSA, RMSSD and SDNN with fitted centile curves, separately for boys and girls. We display the raw data for ease of comparison to previous studies whereas statistical testing was always performed on the transformed data that better matched the assumptions of a normal distribution. The logarithmic transformation of the HRV variables did not change the pattern of the trajectories (Figure S1). As illustrated by Figure 1, resting HR declined almost linearly from infancy to middle childhood 17 and reached a plateau towards adolescence. However, PEP increased gradually from infancy to adolescence. The maturation of the HRV parameters showed comparable curves with a clear increase from infancy to middle/late childhood followed by a plateau phase during middle/late childhood and a small decrease to late adolescence (see Figure 2). Moreover, in girls the plateau phase of the HRV parameters was reached earlier compared with boys.

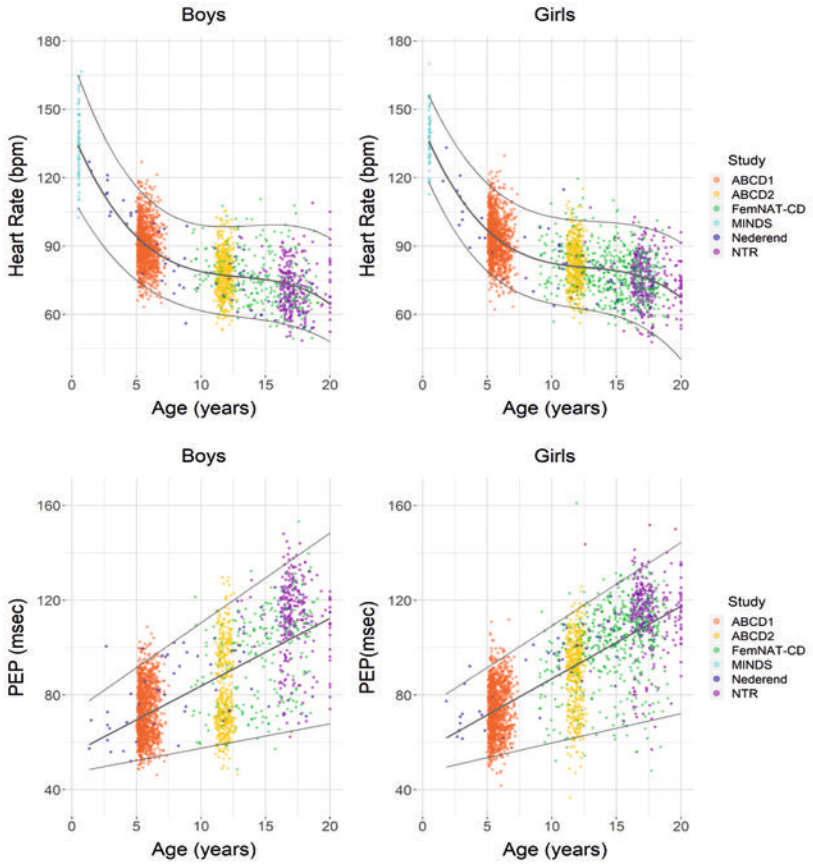

Figure 1. Scatterplots of age-related heart rate and pre-ejection period for boys and girls with cubic linear smoothing of median.

ABCD indicates the Amsterdam Born Children and their Development study; FemNAT-CD, the Neurobiology and Treatment of Adolescent Female Conduct Disorder study; MINDS, the Mother-Infant Neurodevelopment Study; and NTR, Netherlands Twin Register; and PEP, pre-ejection period.

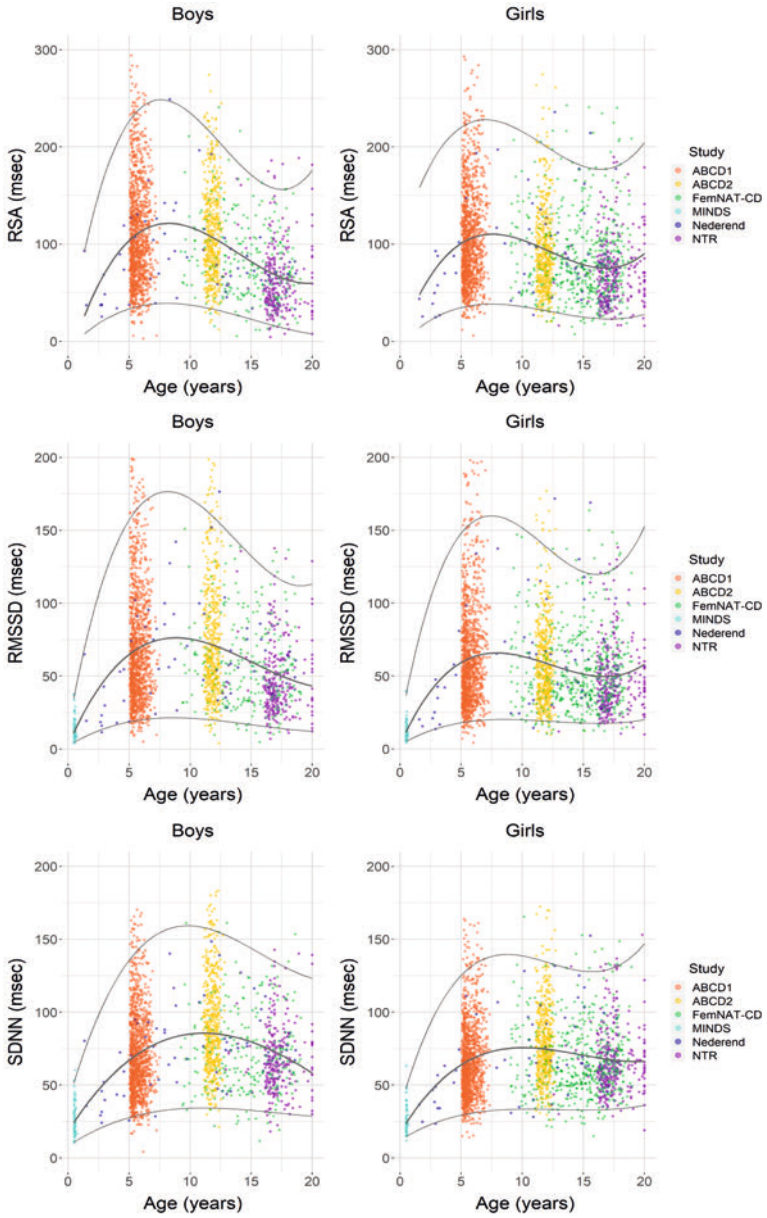

Figure 2. Scatterplots of age-related heart rate variability with Cubic smoothing of median.

ABCD indicates the Amsterdam Born Children and their Development study; FemNAT-CD, the Neurobiology and Treatment of Adolescent Female Conduct Disorder study; MINDS, the Mother-Infant Neurodevelopment Study; NTR, Netherlands Twin Register; RSA, respiratory sinus arrhythmia; RMSSD, root mean square of successive differences between normal sinus beats; and SDNN, SD of the inter-beat interval of normal sinus beats.

The median values and the 2.5th and 97.5th percentile per age group and sex for all variables are shown in Tables 3 and 4. We compared the means of boys and girls with an ANOVA using age and sex as factors. A significant interaction between sex and age was found for HR ($F[11,4787]=2.37$, $P=0.006$), PEP ($F[10,4539]=6.31$, $P<0.001$), RSA ($F[10,4605]=5.42$, $P<0.001$, RMSSD ($F[11,4747]=4.31$, $P<0.001$) and SDNN ($F[11,4777]=3.76$, $P<0.001$). When boys and girls were compared separately for each of the age groups, less than half of the age groups for HR and a quarter of the age groups of PEP, RSA, RMSSD, and SDNN showed a significant difference. Girls had overall higher values of HR at all ages while PEP was only higher in girls at ages 11, 12 and 13 to 15 years (see Table 3) and RSA, RMSSD, and SDNN lower in girls at ages 5, 11, and 12 years (see Table 4). Repeating the analyses using only unrelated children form the NTR study did not change this pattern of results.

Table 3. Age-Related Heart Rate (bpm) and Pre-Ejection Period (ms) per Sex and Age group

	HR (bpm)						PEP (ms)					
	Boys			Girls			Boys			Girls		
		Percentile			Percentile			Percentile			Percentile	
Age, Y	Median	2.5th	97.5th	Median	2.5th	97.5th	Median	2.5th	97.5th	Median	2.5th	97.5th
0.5	134.9	109.6	164.3	137.9	118.0	155.8	NA	NA	NA	NA	NA	NA
1-4	111.4	100.8	125.8	105.1	90.6	129.5	66.6	53.1	95.0	71.2	61.8	90.1
5	90.8	72.6	111.4	94.4*	77.0	113.9	70.3	53.7	93.7	73.1	54.3	94.1
6	88.2	71.2	110.0	90.4	72.4	109.6	74.2	54.6	96.2	75.3	54.8	95.6
7-10	82.8	69.5	99.0	86.4	64.1	108.3	83.9	54.8	108.8	86.2	60.1	109.8
11	77.8	60.0	99.3	83.0*	65.0	102.6	75.8	56.1	115.0	92.0*	58.1	112.6
12	77.5	59.6	99.0	81.5*	61.9	104.3	80.1	57.6	121.1	96.3*	61.7	118.5
13-15	74.2	57.4	97.5	76.7	59.0	98.8	102.4	65.6	125.4	105.4*	66.9	128.5
16	71.0	55.8	92.2	76.8*	59.4	95.5	112.1	71.9	138.4	112.5	74.4	134.0
17	69.5	54.0	91.6	73.3	51.2	94.7	115.8	72.2	138.2	112.8	70.4	133.0
18	68.6	53.3	95.9	75.8*	59.1	97.4	110.3	71.4	136.0	111.1	68.8	130.6
≥19	68.9	55.8	100.1	72.5	55.5	95.5	111.8	77.2	126.3	115.0	88.7	135.9

Note that per variable tests were conducted in 12 age groups and that Bonferroni correction of a nominal alpha of 0.05 would yield a p-value threshold of 0.004. HR indicates heart rate; NA, not applicable; PEP, pre-ejection period.

* *P*<0.001 for difference between Boys and Girls.

Table 4. Age-Related RSA (ms), RMSSD (ms), and SDNN (ms) Per Sex and Age

Age, Y	RSA (ms)						RMSSD (ms)						SDNN (ms)					
	Boys			Girls			Boys			Girls			Boys			Girls		
	Median	Percentile		Median	Percentile		Median	Percentile		Median	Percentile		Median	Percentile		Median	Percentile	
		2.5th	97.5th		2.5th	97.5th		2.5th	97.5th		2.5th	97.5th		2.5th	97.5th		2.5th	97.5th
0.5	NA	NA	NA	NA	NA	NA	11.6	4.9	34.1	11.1	5.2	31.8	24.5	11.5	49.8	23.6	15.0	47.9
1-4	43.0	27.8	112.0	57.5	24.8	99.0	26.4	12.7	62.1	34.8	14.3	74.0	43.0	25.5	78.1	46.7	23.8	71.0
5	99.1	36.0	235.5	92.7*	38.0	223.3	58.2	19.9	172.4	51.6*	19.1	156.4	64.5	29.4	144.4	60.6*	30.1	130.7
6	102.7	36.3	213.2	102.6	37.2	227.6	63.2	19.4	156.5	59.1	19.3	157.9	67.4	28.9	135.8	66.2	31.4	136.0
7-10	92.8	40.9	206.7	102.0	38.3	190.4	61.6	24.3	137.6	63.0	20.4	145.1	67.9	29.9	133.3	69.4	31.5	131.4
11	103.3	39.2	224.7	87.3*	35.3	198.4	64.4	21.5	176.5	54.0*	20.5	137.1	85.3	40.6	162.2	74.9*	38.4	131.4
12	105.2	35.1	210.4	84.0*	25.6	206.5	65.8	22.4	151.2	48.3*	14.2	130.0	84.2	36.5	154.8	72.6*	30.6	137.1
13-15	74.9	18.7	166.8	74.2	21.4	186.7	56.7	13.6	131.3	45.6	18.4	130.0	69.8	26.4	126.8	61.1	27.8	130.3
16	55.3	15.5	151.7	68.3	27.1	150.4	42.1	14.6	112.9	43.0	17.6	109.3	64.7	29.3	125.0	60.4	32.6	122.3
17	58.5	21.2	147.0	70.7	29.8	192.0	42.5	17.8	112.8	47.6	20.2	128.1	65.2	33.2	117.8	65.9	38.4	132.0
18	66.0	17.9	143.3	75.2	20.4	177.8	52.0	15.3	114.2	46.4	14.2	116.1	67.0	34.8	138.5	60.8	36.8	120.1
≥19	51.3	17.4	166.1	70.5	24.1	139.4	38.4	13.5	112.6	45.6	15.3	97.9	68.5	31.4	135.4	65.3	33.9	120.1

Note that tests for respiratory sinus arrhythmia, root mean square of successive differences and SD of inter-beat intervals were conducted in 12 age groups and as all these variables are highly correlated, their results can be regarded as a within-study replication. Therefore, the Bonferroni correction of a nominal alpha of 0.05 would yield a *P* value threshold of 0.001.

NA indicated not applicable; RMSSD, root mean square of successive differences; RSA, respiratory sinus arrhythmia (set at zero); SDNN, SD of inter-beat intervals of normal sinus beats.

* *P*<0.001 for difference between Boys and Girls after LN transformation;

Adjustment for Heart Rate

The HR adjusted ANS parameters, stratified by sex, are shown as scatterplots in Figures S2 and S3. Similar to the unadjusted variables, the cubic model had the highest R square and best fit for the natural log of RSA/HP, RMSSD/HP, and SDNN/HP and the linear model was again chosen for PEP/HP. The trajectories of RSA/HP, RMSSD/HP, and SDNN/HP corresponded to the trajectories of the unadjusted RSA, RMSSD, and SDNN (Figure 2), with an increase from infancy to middle/late childhood followed by a plateau phase with a small decrease during adolescence. Although the trajectory of PEP/HP also corresponded with the trajectory of the uncorrected PEP, the gradual increase of PEP/HP was less steep compared to the unadjusted PEP.

Developmental Changes in the Variance of SNS and PNS Parameters

The variances of the ANS parameters are summarized as SD with 95% CIs per age group and sex in Figure 3. The variance in resting HR was stable across the entire age range, and Levene test confirmed homogeneity of variance in girls ($F[11,2520]= 1.21$, $P=0.272$). For boys, the variances for resting HR were not comparable ($F[11,2267]= 3360$, $P<0.001$), but this was entirely due to age group, 6 months. Removing this measurement rendered the Levene test non-significant ($F[10,2193]=1.15$, $P=0.324$). In contrast to HR, the variances for PEP, RSA, RMSSD, and SDNN were not comparable across all age groups (P-values of Levene tests, all <0.001). The variance of PEP gradually increased until age 12 and plateaued towards adolescence. RSA, RMSSSD and SDNN, showed a steep increase from 0.5 to 5 years, and then plateaued followed by a slight decrease around age 11, stabilizing at the end of adolescence. Homogeneity of variance was largely restored by correcting for skewness in the HRV values by logarithmic transformation (Figure S4) with only RMSSD and SDNN in girls not being equal across age groups (RMSSD $F[11,2502]=1.88$, $P=0.038$ and SDNN $F[11,2517]=2.38$, $P=0.006$).

Temporal Stability from Age 6 to Age 11.5 Years

In the subset of 784 children from the ABCD study measured twice across a 5.5-year period, temporal stability computed as the Pearson correlation coefficient for boys and girls is shown in Table 5. Moderate temporal stability was found for PEP and good stability for HR, RSA, RMSSD and SDNN (Pearson $0.26<r<0.58$; ICC $0.25<r<0.57$). Log transformation of HRV variables barely changed these correlations (Pearson and ICC $0.42<r<0.53$) despite significant longitudinal changes in mean values with age, all of which were compatible with the cross-sectional comparisons of the relevant age groups. Furthermore, adjustment for HP, which adds temporal instability in the HP itself, barely influenced the stability of the ANS parameters (Pearson, $0.28 <r< 0.53$; ICC, $0.27<r<0.54$).

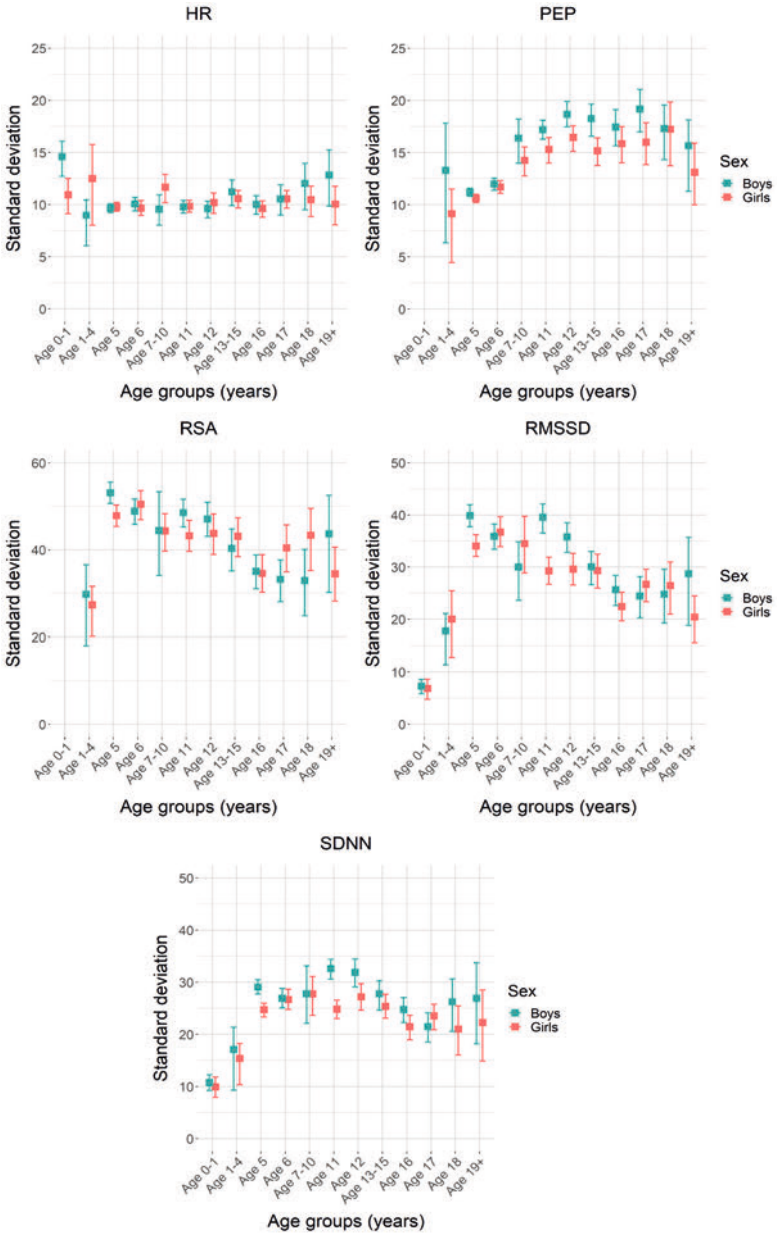

Figure 3. Histograms of SDs per age group for heart rate, pre-ejection period, respiratory sinus arrhythmia, root mean square of successive differences between normal sinus beats and SD of the inter-beat interval of normal sinus beats;

HR indicates heart rate; PEP, pre-ejection period; RMSSD, root mean square of successive differences; RSA, respiratory sinus arrhythmia (set at zero); and SDNN, SD of the inter-beat intervals of normal sinus beats.

Table 5. Correlation of All variables, Untransformed, Natural Log Transformed and Heart Period Adjusted Between 5 to 7 Years and 11 to 12 Years in Children Measured Twice

	Boys (N=398)						Girls (N=386)					
	Mean 5-7, y	±SD	Mean 11-12, y	±SD	Pearson correlation	Intraclass correlation	Mean 5-7, y	±SD	Mean 11-12, y	±SD	Pearson correlation	Intraclass correlation
HR, bpm	90.0	9.9	77.7 †	9.7	0.46 †	0.47 †	93.7	9.8	82.3 †	9.8	0.46 †	0.46 †
PEP, ms	73.3	12.4	81.3 †	17.5	0.33 †	0.31 †	74.3	11.3	89.2 †	15.4	0.26 †	0.25 †
RSA, ms	110.5	48.7	113.4	48.7	0.49 †	0.50 †	105.0	48.3	95.1 †	44.5	0.47 †	0.47 †
RMSSD, ms	69.1	36.2	75.2 †	38.4	0.53 †	0.55 †	60.7	32.3	58.2	29.1	0.46 †	0.47 †
SDNN, ms	71.0	27.0	92.0 †	33.0	0.58 †	0.57 †	66.1	25.7	80.0 †	26.2	0.49 †	0.49 †
LN RSA	4.61	0.46	4.64	0.46	0.49 †	0.50 †	4.56	0.47	4.46 †	0.46	0.43 †	0.43 †
LN RMSSD	4.12	0.54	4.20 *	0.53	0.53 †	0.53 †	3.99	0.52	3.97	0.49	0.42 †	0.42†
LN SDNN	4.21	0.38	4.47 †	0.37	0.53 †	0.53 †	4.14	0.38	4.34 †	0.32	0.45 †	0.45 †
PEP/HP	10.9	2.1	10.4 †	2.6	0.38 †	0.37 †	11.5	2.0	12.1 †	2.6	0.28 †	0.27 †
LN RSA/HP	2.76	0.37	2.65 †	0.37	0.48 †	0.49 †	2.75	0.37	2.54 †	0.37	0.44 †	0.44 †
LN RMSSD/HP	2.30	0.41	2.25 *	0.39	0.53 †	0.54 †	2.22	0.40	2.09 †	0.36	0.43 †	0.43 †
LN SDNN/HP	2.38	0.28	0.28 †	0.27	0.50 †	0.51 †	2.35	0.28	2.42 †	0.24	0.43 †	0.42 †

HP indicates heart period; HR, heart rate; LN, natural log transformation; PEP, pre-ejection period; RMSSD, root mean square of successive differences; RR, respiration rate; RSA, respiratory sinus arrhythmia (set at zero); SDNN = SD inter-beat intervals.

* *P* value <0.05 for difference between the mean at 11 to 12years vs 5to 7years for both boys and girls or Pearson an intraclass correlation.

† *P* value <0.001.

Discussion

To our knowledge, this study is the first and largest multi-cohort study which simultaneously compared HR, PEP and RSA to investigate the maturation of resting cardiac ANS activity in healthy children from infancy to late adolescence. Between the ages of 0.5 to 20 years, resting HR decreased linearly from infancy to the beginning of late childhood (aged 10 years) after which it plateaued with only a minimal further decrease towards the end of adolescence (aged 19 years). PEP linearly increased in both boys and girls throughout all age groups which implies a linear decrease of the cardiac effects of SNS activity with age. Cardiac PNS activity showed the most complex pattern, a clear increase from infancy to middle (girls) or late (boys) childhood, a plateau phase during middle and late childhood, followed by a slight decrease throughout adolescence. Furthermore, whereas the variance in HR was largely stable throughout childhood and adolescence, the variance of the SNS and PNS parameters increased from infancy to early childhood and plateaued or slightly decreased towards adolescence. For PNS, the often-used natural log transformation largely removed this heterogeneity of variance.

A main limitation of this study is the cohort approach in which age groups were compared rather than repeated measurements in the same individuals across the entire 0.5- to 20-age span. For this reason, the 'developmental' trends in Figures 1 and 2 should be interpreted with caution. Furthermore, because of substantial clipping of the thorax impedance PEP and RSA were missing in the age group of 6 months, an important data point for early development. In addition, the RMSSD and SDNN results of this MINDS-cohort should be interpreted with caution acknowledging that body position differed from the other cohorts in this study, lying in supine position instead of sitting. It is known that body position influences cardiac ANS parameters (8). These limitations are balanced by the larger sample size of healthy children available, with the exception of ages 1 to 4 years, compared with previous studies, and the highly comparable and well-validated methodology used to assess PEP and RSA in all age groups. However, substantial caution is needed as PEP is known to be sensitive to preload and afterload effects, which may change across childhood and adolescence in view of the changes in eg, stature and body composition, the size of the heart, HR, arterial stiffness, and mean arterial pressure. Also, as reviewed below, striking resemblance was found between our multi-cohort results and those from longitudinal studies that, although using narrower age ranges, together still cover the entire 0.5- to 20-age span.

A clear and steep increase in HRV (33, 40, 49) and an increase in the amount of myelinated fibres of the vagus nerve (45) in the first months of life suggest that cardiac PNS activity starts to increase after birth. HRV then continues to increase from the first months to early and middle childhood (28, 31, 37, 39-42, 46) and levels off in late childhood/early adolescence (≈age 11) and does not change or decrease during adolescence (24, 29, 34, 36, 40, 42, 46, 50). Such a pattern is highly compatible with the course of maturation seen in our own study and also resembles the pattern reported by the multicohort study of (38), where RSA peaked at age 7 to 8 years, even independently of the definition of the respiratory frequency band used to define the spectral RSA measure. However, another study that compared PNS activity across multiple age cohorts (43) found that the increase in SDNN and RMSSD values from a 10-second electrocardiography strip (uncorrected for HR, their Figure 5 and 6) levelled off around the age of 20 years, which is, much later than in our study (≈11 years). Future research must establish whether this reflects differences in measurement strategy or baseline tasks used to obtain resting values.

For the maturation of cardiac SNS activity, the time course and direction of maturation was still largely unknown. Some longitudinal studies have used the LF:HF ratio as a measure of sympathetic over parasympathetic dominance. These studies showed a decrease of the LF:HF ratio during infancy after which it levels off and may not change or even increase slightly to late adolescence (40, 50). However, the use of the LF:HF ratio to index SNS activity is not unanimously supported (64, 65) and the PEP is considered to be a better index in theory, which has been borne out in empirical validation studies (11, 66, 67).

Most studies investigating developmental trends in PEP showed an increase of mean PEP from infancy to adolescence (27, 30, 32, 35). Matthews et al. (35) showed an increase of mean PEP from 8 to 10 years and higher values of PEP in children aged 15 to 17 years compared with the younger children, but no significant increase of mean PEP from 15 to 17 years, indicating that the increase may level off towards late adolescence. Results from our study suggest a clear maturational effect on mean PEP with a linearly increase from early childhood to late adolescence (Figure 1), meaning that SNS activity decreases over age. However, some caution is needed as PEP is known to be sensitive to preload and afterload effects, which may change across childhood and adolescence in view of the changes in blood pressure and HR. Also, an age-related β-adrenergic desensitization in the elderly has been observed compared with the young (68). If this β-adrenergic desensitization starts already in childhood, the decrease in PEP may partly reflect reduced receptor responsiveness rather than decreases in SNS activity.

All previous studies which reported a significant sex difference in cardiac PNS activity, described a higher PNS-activity in boys compared with girls (47-49), although some studies reported the difference in one specific age group only (41) or in a subset of HRV values (42). Many other studies did not show any sex differences in PNS activity (24, 28, 32, 34, 39, 43, 46, 50, 51). Likewise, there is no consensus in the literature with regards to sex differences in cardiac SNS. A higher mean PEP in boys compared with girls in the ages 8 to 10 and 15 to 17 years was found by some (32, 35) and a lower mean PEP in boys aged 9 to 11 years by others (30).

Our study offers an explanation for these discrepant findings on sex differences in ANS activity. We showed that the peak in PNS activity is reached earlier in girls compared with boys, indicating a difference in maturation; this is likely because of the difference in hormonal changes and their downstream biological effects. An association between HRV and reproductive life stages has been described before (69), and girls are known to enter puberty earlier compared with boys (70). Because of the modulatory effects of sex on the developmental trajectory of the ANS parameters, detection of sex differences may be rather dependent on the exact composition of the age groups studied. In our study, when considering the entire age range, the PEP was lower in boys compared with girls and RSA, RMSSD, and SDNN were higher in boys <13 years. However, not all of these parameters were significantly different across all age groups. Again, the exact age range used to compare boys and girls can determine the outcome of the test for sex differences.

We discovered a significant change in the *variance* of ANS parameters during childhood in both boys and girls which has not been described earlier. However, homogeneity of variance is an assumption of the ANOVA tests often used to compare age groups. The variance of PEP, RSA, and the 2 HRV parameters increased from 6 months to 7 years, peaked in middle childhood, and gradually decreased towards the end of adolescence. The peak in variation during middle childhood may be attributed to differences in maturational speed that will be most manifest in this age range. When applying the often-used natural log transformation on RSA, RMSSD and SDNN, the homogeneity of variance assumption started to hold well across age groups. This is in concordance with previous studies where log-transformed variables with 95% CI curves showed no clear peak in variance (24, 43).

A major aim of the present study was to provide normative absolute values for the sex-specific maturation of cardiac PNS and SNS activity in childhood, which could have substantial clinical use for detecting children at risk for cardiovascular or psychiatric problems. Results dictate substantial modesty about this aim. The

overarching message of Figures 1 and 2 is that there are large interindividual differences in children of the same age and sex. The range is somewhat reduced after natural log transformation and/or adjustment for HP but even then, the variance of same-aged children for both boys and girls remains striking (see Figures S1, S2, and S3). Hence, the normative values or PEP and RSA/HRV, as presented in Tables 3 and 4, will prove useful to detect clearly outlying values that would require further clinical paediatric follow-up, but their clinical use of detecting more subtle deviations in ANS functioning, as is potentially the case in child psychiatric conditions, is hampered by the large individual differences that remain even after stratifying for age and sex.

Causes for the large differences in cardiac SNS and PNS measures can partly be found in between-subject differences in a variety of factors including ethnicity, maturational changes in stature and body composition, the size of the heart, sensitivity of the baroreflex and lung stretch reflexes and respiratory behaviours (frequency and tidal volume), adopted lifestyle patterns (smoking, dietary habits and physical activity), and psychosocial stress exposures. In addition to these between-subject factors, there is a slew of factors in the experimental design that can impact on absolute values of the ANS measures. Time of day, previous physical activity, posture, exact shaping of resting baseline conditions, analytic strategy to quantify SNS and PNS cardiac activity, and ensuing statistical transformations or adjustments are just some of the many factors that impact on reported RSA and PEP levels. We note that this concern mostly pertains to absolute values. It does not disqualify these ANS markers as potential biomarkers of developmental processes when comparing relative ranking of children tested within a fixed experimental design. This is further supported by the good temporal stability of the cardiac ANS activity in the 735 children participating in the ABCD study twice across a 5-year period.

The test-retest correlation of cardiac ANS activity in the 6- to 11-year age range was good for RSA (r=0.47-0.50) and comparable with previous reports of RSA stability in this age range (29, 31, 32, 36). Studies performed in infants showed moderate to good stability of RSA in the first years of life(27, 28, 37), but Dollar et al. (24) show moderate long-term stability in RSA from age 2 to 15 years. In our study, stability was moderate for PEP (r=0.25-0.33) and these estimates were somewhat lower compared with those previously reported for the narrower 5- to 8-year (31), 8- to 11-year (30, 32, 35) and 15- to 17-year periods (35) in which good stability was described. Moreover, a good stability for PEP has even been described in the younger age ranges from 6 months to 5 years (27). In short, the parameters used here to index cardiac ANS activity can be considered to be a stable individual trait

over time, at least with regard to the relative ranking of a child compared with others of the same age and provided that it is measured using the same experimental/ analytical approach.

Conclusion

This study provides maturation curves of resting cardiac PNS and SNS activity in healthy children aged 6 months to 20 years. It shows a differential maturation of the PNS and SNS, with PNS activity increasing rapidly directly after birth, levelling off during middle childhood, and decreasing at the end of adolescence. The SNS, in contrast, shows a monotonic decrease across all ages. Trajectories differ between boys and girls, with the latter showing earlier ANS maturation. Despite the large samples used, the clinical use of the sex-specific centile and percentile values is modest in view of the large individual differences present, even within narrow age bands.

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Supplemental Material

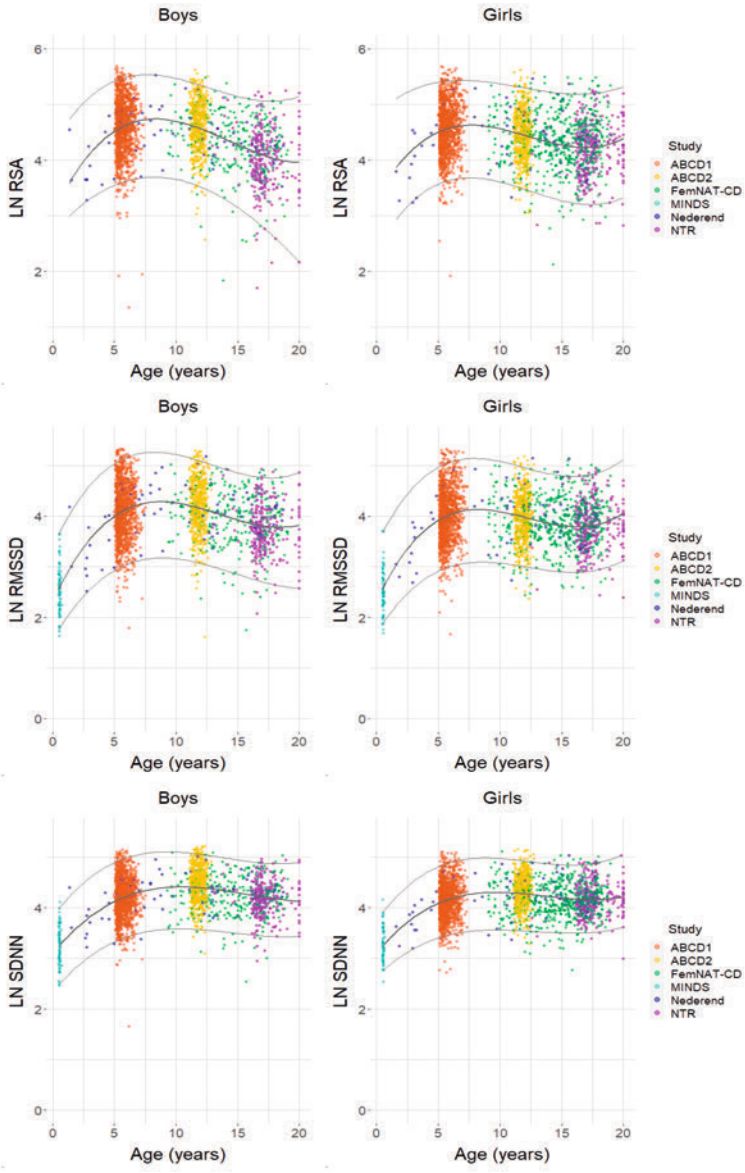

Figure S1. Scatterplot of age-related natural logarithm of RSA, RMSSD and SDNN with Cubic smoothing of median.

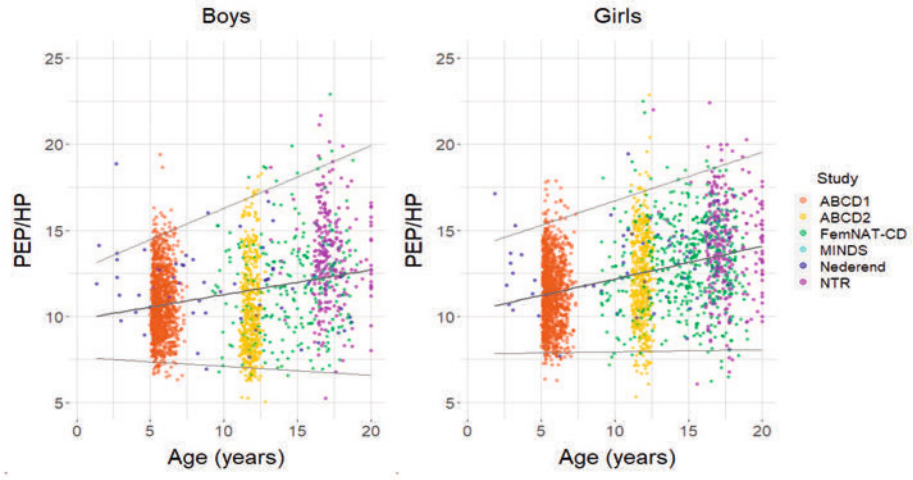

Figure S2. Scatterplot of age related for heart period (HP) corrected PEP with Quadratic smoothing of median.

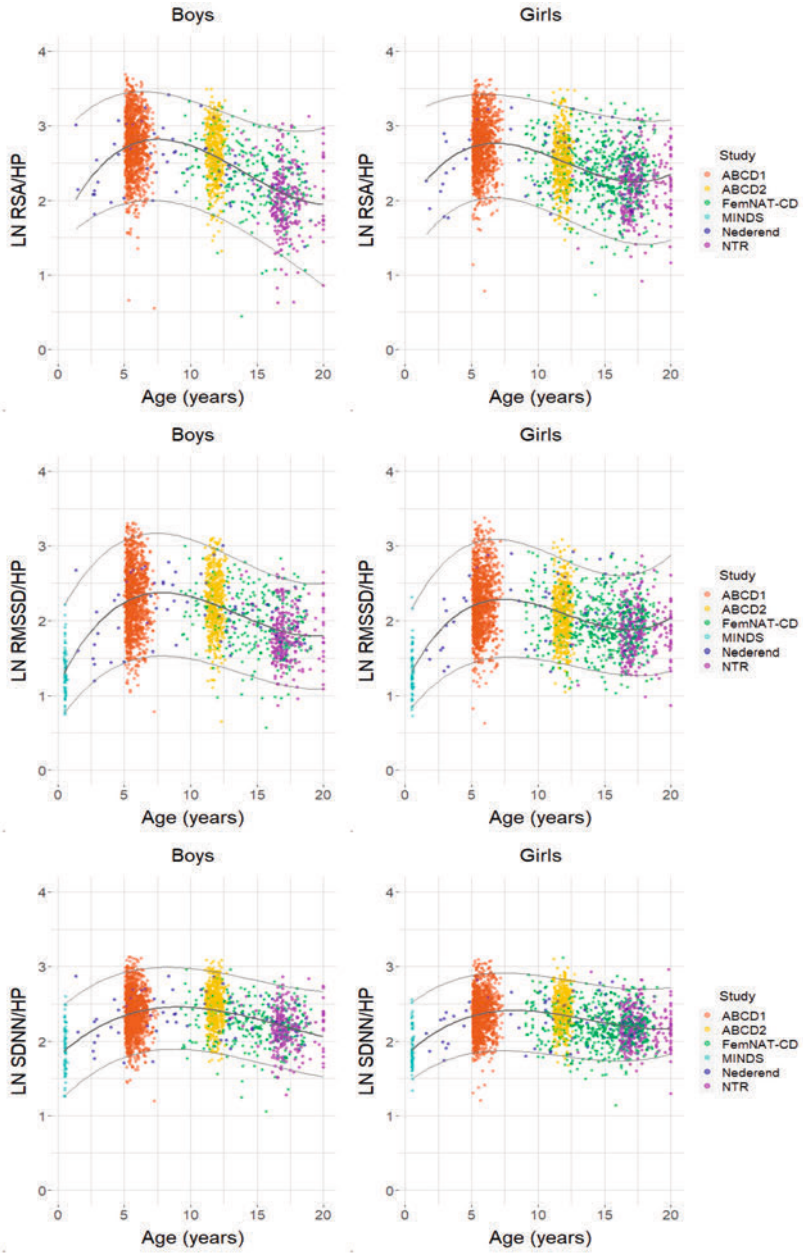

Figure S3. Scatterplot of age related for heart period (HP) corrected natural logarithm of RSA, RMSSD and SDNN with Cubic smoothing of median.

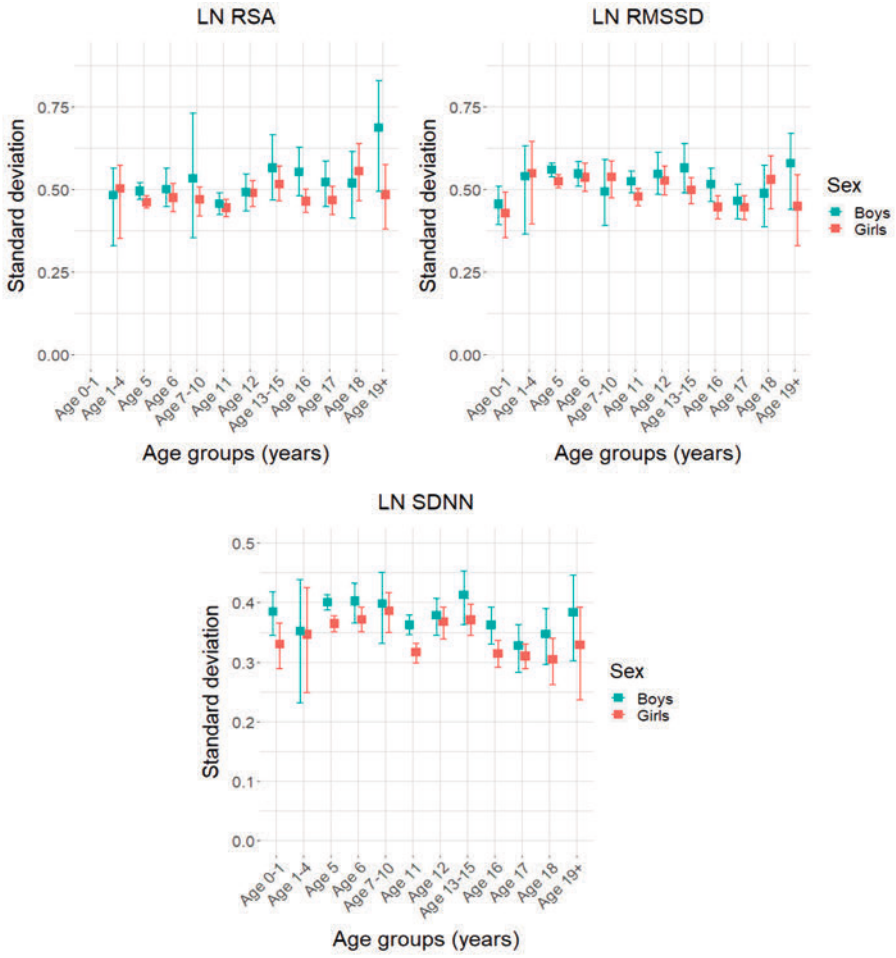

Figure S4. Histograms of standard deviations per age group for natural logarithm of RSA, RMSSD and SDNN.

Chapter 4

Determinants of exercise limitation in contemporary paediatric Fontan patients with an extra cardiac conduit

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Abstract

Background

Although various determinants of exercise limitation in Fontan patients have been studied, most research has been performed in patients who underwent different surgical procedures with differing haemodynamic characteristics. The aim of the current study was to evaluate non-invasively measured cardiovascular parameters and their influence on exercise performance in paediatric Fontan patients with an extracardiac conduit and moderate-good systolic ventricular function.

Methods

Fontan patients, between 8 and 18 years of age, with moderate to good systolic ventricular function and an extracardiac conduit were included. Exercise performance and cardiovascular assessment, comprising echocardiography, aortic stiffness measurement and ambulatory measurement of cardiac autonomous nervous activity were performed on the same day. Healthy subjects served as controls.

Results

Thirty-six Fontan patients (age 14.0 years) and thirty-five healthy subjects (age 12.8 years) were included. Compared to controls, Fontan patients had reduced diastolic ventricular function and increased arterial stiffness. No differences were found in heart rate (HR) and cardiac parasympathetic nervous activity. In Fontan patients, maximal as well as submaximal exercise capacity was impaired, with the percentage of predicted capacity ranging between 54 and 72%. Chronotropic competence, however, was good with a peak HR of 174 (94% of predicted). Lower maximal and submaximal exercise capacity was correlated with a higher HR at rest, higher pulse wave velocity of the aorta and a lower ratio of early and late diastolic flow velocity.

Conclusion

Contemporary paediatric Fontan patients have an impaired exercise capacity with preserved chronotropic competence. Exercise performance correlates with heart rate at rest, diastolic function and aortic stiffness.

Introduction

Surgical Fontan techniques for palliation of a univentricular heart have evolved resulting in improved survival, however, patients still have a decreased life expectancy and significant morbidity, including arrhythmias, thromboembolic complications, heart failure and reduced exercise performance (1, 2). Exercise performance is reduced even in paediatric Fontan patients, and it decreases further with age (1). An inability to increase ventricular preload during exercise prevents an appropriate increase in cardiac output, which is considered an important factor limiting exercise tolerance (3). Other contributing factors included chronotropic incompetence, systolic and diastolic ventricular dysfunction and arterial stiffness (3-8). As there is a direct relation between exercise performance and long-term outcome, a thorough understanding of the mechanisms influencing exercise capacity is important for efforts to improve outcome. Most exercise studies in Fontan patients included patients who underwent different surgical procedures or had different haemodynamic characteristics, which makes a comparison of results difficult. We therefore designed an exercise study in a homogeneous group of paediatric Fontan patients who had an extracardiac conduit and who had moderate to good systolic ventricular function. We evaluated various non-invasively measured cardiovascular function parameters, including echocardiographic parameters, arterial stiffness and cardiac autonomous nervous system activity, and determined their influence on exercise performance. Results were compared to those of healthy controls.

Methods

Study population & design

The study population consisted of Fontan patients aged 8-18 years with an extracardiac conduit, recruited from Leiden University Medical Centre and Amsterdam University Medical Centre. Patients with ventricular dysfunction and those who were unable to exercise were excluded. Healthy controls with a similar age were recruited through advertising in local schools. Written informed consent was obtained from all participants or their parents or guardians. The study was approved by the local ethics committee. Data were collected in a prospective way between July 2017 and October 2019 and all patients had all tests in one day. All participants underwent weight, height and arterial stiffness measurements, echocardiography, and an ambulatory cardiac autonomic nervous system (ANS)

measurement as described below. As adequate reference values of exercise parameters of healthy children exist, cardiopulmonary exercise tests were only performed in Fontan patients and results expressed as percentage of predicted values.

Arterial stiffness measurements

An oscillometric arteriograph device (Tensiomed, Hungary) (9) was used to measure aortic pulse wave velocity (PWVao), aortic augmentation index, systolic blood pressure and pulse pressure. Measurements were performed in supine position with the cuff on the left arm. The software determines measurement accuracy with a standard deviation (SD) of the PWVao (9). However, as children have smaller pulse waves compared to adults, and as movement distorts pulse waves, we analysed each cardiac cycle separately. Measurements were considered valid after a visual check and when a reliable value could be calculated with an SD PWVao <1.0 m/s.

Echocardiography

Transthoracic echocardiograms were performed on a Vivid S6/S60 (GE healthcare, Norway) and images analysed offline using EchoPac (version 203, GE healthcare). If applicable, averages of three consecutive cardiac cycles were used for analysis. Conventional Doppler, Tissue Doppler imaging and speckle-tracking were performed to measure different systolic and diastolic parameters following previously published methods (10). Ratio of peak early conventional and Tissue Doppler diastolic velocity (E/E') was calculated of the lateral wall. Longitudinal global peak strain was obtained if 5/6 segments could be recorded and showed acceptable curves. Furthermore, strain was conducted from both lateral walls when the ventricular septum defect was larger than one segment. In addition, Doppler recordings across the (neo)aortic valve were performed to assess aortic velocity time integral from which, together with heart rate (HR) and measured (neo)aortic annulus diameter, the cardiac index was calculated.

Ambulatory cardiac ANS measurement

We used the VU-ambulatory monitoring system (VU-AMS; VU university, Netherlands, 5 fs version) comprising an impedance measurement, an electrocardiogram, and the VU-Data Analysis and Management Software (VU-DAMS, VU University) to measure several heart rate variability parameters to determine the effect of ANS activity on the heart as described in previously published methods (11). All participants completed a 4-5-min supine baseline measurement. Ectopic beats and artifacts were removed before analysis.

Pre-ejection period (PEP), reflecting cardiac sympathetic activity (12), and respiratory sinus arrhythmia (RSA), mainly reflecting cardiac parasympathetic activity (13), were calculated as described in previously published methods (11). The root mean square of consecutive differences between the beats of normal sinus beats and the interval between the beats of normal sinus beats were calculated as additional cardiac parasympathetic activity parameters. Furthermore, we derived low-frequency (LF;0.04-0.15 Hz) and high-frequency (HF;0.15-0.4 Hz) power using the Fast Fourier Transformation and additionally calculated the LF:HF ratio, a disputed but frequently used measure of sympathovagal balance (14). In addition, respiration rate was derived from the thorax impedance signal as well, as this, along with HR, could affect ANS parameters. To correct for HR remains, however, a subject of debate as adjustment may in fact remove possible important variance of outcomes related to autonomic control (13). We therefore have chosen to report the outcomes of HR and respiration in parallel with the heart rate variability parameters over adjustment procedures.

Cardiopulmonary exercise test

Fontan patients performed an exercise test on an upright bicycle ergometer (Jaeger ER 900; Viasys Healthcare, Germany) with breath-by-breath analysis using a flowmeter (Triple V volume transducer) and a computerized gas analyser (Jaeger Oxycon Champion, Viasys Healthcare or Carefusion Vyntus, Vyaire Medical). Starting wattage and workload increment per minute were determined by patient's age. Patients were encouraged to exercise until exhaustion. From flow and gas analysis and HR measurements per 10 s exercise parameters as defined in previously published parameters could be derived or calculated (15, 16). Maximal exercise parameters were assessed from patients with a peak respiratory exchange ratio ≥ 1.0 and submaximal parameters from all patients. Besides absolute values, the percentage of predicted values adjusted for age and sex were calculated, where applicable, using previous published methods (15-17), except for peak oxygen uptake ($VO_{2\,peak}$) which was adjusted for age, sex, weight and height (18) and oxygen pulse for which we used the data of Ten Harkel et al. (16).

Statistical analysis

Data analysis was performed using SPSS (IBM, version 25). Variables were tested for normality using the Kolmogorov-Smirnov test as well as through visual inspection of histograms and normality plots. LF and HF their ratio were LN transformed before all statistical analyses to remove the significant skewness in the untransformed values so as not to violate the assumption of normal distribution. Continuous data are reported as mean ± SD or as the median with first to third quartile [Q1-Q3] in

case of non-normality, except for the percentage of predicted values which are reported as mean or median with 95% confidence intervals. Categorical data are presented as numbers with percentages. To compare groups, we used the Student's *t*-test or, in case of non-normality, the Mann-Whitney *U* test for continuous data and the Pearson Chi-Square test for categorical data. As aortic arch surgery could affect vascular function, cardiac ANS activity and chronotropic competence, we performed a sub-analysis between patients who did have aortic arch reconstruction and those who did not. Furthermore, a second sub-analyses was performed to show whether there were differences between cardiovascular and exercise parameters in patients with a morphological left of right ventricle. To test whether exercise parameters differed from their reference values, the one sample *t*-test or, in case of non-normality, the one sample Wilcoxon Signed Rank test was used. We tested correlations between exercise and cardiovascular parameters using the Pearson or Spearman correlation coefficients depending on data distribution. For correlation with HR at rest, only patients with a sinus rhythm were used for analysis.

Results

Thirty-six Fontan patients (median age 14.0 years) and 35 healthy controls (median age 12.8 years) were included (Table 1). Age, weight, height, body surface area and sex distribution between Fontan patients and controls were comparable. Twenty-one Fontan patients had a dominant left ventricle (58%), 12 a dominant right ventricle (33%) and 3 an indifferent or undefined ventricle (8%). Fifteen patients (42%) had aortic arch/Norwood surgery. Thirty-three patients (92%) had an initial fenestration at time of surgery and only one patient had a patent fenestration at time of the study (3%), in the remaining 32 patients the fenestration was closed naturally or by device. Furthermore, one other patient had a pacemaker (3%; dual chamber pacing; DDD). The median plasma N-terminal pro brain natriuretic peptide was low (80 ng/L) in the Fontan cohort.

Table 1. Patient Characteristics of Fontan patients and controls

Characteristics	Fontan patients (N = 36)	Controls (N = 35)	*P*-value
Age (years)	14.0 [12.7-16.6]	12.8 [11.1-15.5]	0.202
Males (N,%)	23 (56.1)	18 (43.9)	0.288
Height (cm)	163.0 (14.1)	160.8 (13.5)	0.514
Weight (kg)	51.4 [42.8-60.3]	46.2 [37.0-56.9]	0.133
BSA (m^2)	1.55 (0.3)	1.46 (0.27)	0.189

Table 1. Patient Characteristics of Fontan patients and controls (continued)

Characteristics	Fontan patients (N = 36)	Controls (N = 35)	*P*-value
Diagnosis (N,%)			
Tricuspid atresia	9 (25.0)		
Pulmonary atresia	2 (5.6)		
Double inlet left ventricle	5 (13.9)		
Double outlet right ventricle	1 (2.8)		
Hypoplastic left heart syndrome	8 (22.2)		
Unbalanced atrioventricular septum defect	4 (11.1)		
Other	7 (19.4)		
Main ventricle (N;%)			
Left	21 (58.3)		
Right	12 (33.3)		
Indifferent	3 (8.3)		
Aortic arch/Norwood surgery (N,%)			
Yes	15 (41.7)		
No	21 (58.3)		
Age at Glenn operation (years)	0.52 [0.38-0.79]		
Age at Fontan operation (years)	3.19 (0.65)		
Initial Fenestration (N,%)	33 (91.7)		
Open	1 (3.0)		
Closed (naturally or by device)	32 (97.0)		
Pacemaker (N,%)	1 (2.8)		
NT-pro BNP (ng/L)	80.2 [48.0-131.3]		
AV-valve regurgitation (N,%)			
No	16 (44.4)		
Mild	14 (38.9)		
Moderate	6 (16.7)		
Severe	0 (0.0)		
(Neo)Aortic regurgitation (N,%)			
No	31 (86.1)		
Mild	2 (5.6)		
Moderate	3 (8.3)		
Severe	0 (0.0)		
Cardiac medications (N, %)			
Acetylsalicylic acid	34 (94.4)		
Coumarin derivative	2 (5.9)		
β-blocker	1 (2.8)		
Diuretics	1 (2.8)		

Data expressed as n (%), mean (±SD), and median [Q1-Q3].

BSA = body surface area; NT-pro BNP = N-terminal pro brain natriuretic peptide; SpO_2 = oxygen saturation.

Table 2 shows the results of cardiovascular parameters. Fontan patients had a higher PWVao and augmentation index of the aorta, and a slightly higher systolic blood pressure and pulse pressure compared to healthy controls. Almost all diastolic function parameters showed a worse diastolic function in Fontan patients compared to controls with significant lower Doppler and Tissue Doppler velocities and ratio of peak early and late diastolic velocities (E/A) and higher E/E' ratio. All Fontan patients showed a subjective moderate to good ventricular systolic function on echocardiography. Although Tissue Doppler imaging showed lower systolic velocities in Fontan patients compared to controls, global strain was not significantly different. Cardiac index could be calculated in only 21 patients, as in 15 patients aortic annulus could not be determined. Cardiac index was comparable to healthy controls. Eight patients were excluded from cardiac ANS measurements because of a pacemaker in one patient, and frequent atrial ectopy or a dominant nodal rhythm in seven others. The heart rate variability parameters reflecting cardiac parasympathetic activity were comparable between Fontan patients and controls. PEP was significantly longer in Fontan patients, whereas the LF:HF ratio was higher. HR and respiration rate did not differ between patients and controls.

The results of the exercise test are shown in Table 3. Three patients (8.3%) were not able to achieve a respiratory exchange ratio > 1.0 and were excluded for the maximal exercise results. Furthermore, the patient with the pacemaker was excluded for the chronotropic parameters. There were no adverse events during the exercise tests. Fontan patients showed low exercise capacity, reflected by a low VO_{2peak} (mean 26.8 mL/kg/min), oxygen uptake efficiency slope (OUES) and plateau, and a high slope of respiratory minute volume to CO_2 (VE/VCO_2 slope) with percentage of predicted values ranging from 54 to 72% ($P < 0.001$) and 124% ($P < 0.001$). Oxygen pulse at peak exercise was also decreased with a percentage predicted of 59% ($P < 0.001$) which could reflect a lower stroke volume at peak exercise. Fontan patients showed a good chronotropic competence with a peak HR of 174 (94% of predicted; $P < 0.001$) and percentage HR recovery at 1 min of 35% (116% of predicted; $P < 0.01$).

Table 4 shows the results of the sub-analyses between patients who did have aortic arch reconstruction and who did not. There were no significant differences in any of the analysed parameters, including vascular function, cardiac ANS activity, chronotropic competence and exercise capacity. Furthermore, a second sub-analyses showed that there were no differences in cardiovascular and exercise parameters between patients with a dominant left and right ventricle (Supplementary Tables S1-2).

Table 2. Cardiovascular parameters in Fontan patients and controls at rest

	Fontan patients (N = 36)	Controls (N = 35)	*P*-value
Heart rate (bpm)	71.3 (15.6)	71.0 (10.2)	0.918
SpO^2 (%)	95.1 [94.2-96.3]	99.0 [98.4-99.5]	<0.001
Vascular function			
SBP (mmHg)	118.7 (9.1)	108.9 (8.6)	<0.001
PP (mmHg)	52.9 (8.3)	46.9 (6.8)	0.001
PWVao (m/s)	5.5 (1.1)	4.7 (0.6)	<0.001
AIXao	17.5 (9.9)	11.2 (7.8)	0.006
Ventricular function			
Systolic			
Aortic annulus width (mm)	23.0 (3.1)	20.1 (2.2)	0.001
VTI (cm)	16.1 [14.2-20.9]	21.1 [18.9-23.3]	0.001
CI (L/min/m^2)	3.4 (0.7)	3.3 (0.6)	0.546
TDI septal S' (m/s)	0.04 [0.03-0.05]	0.08 [0.07-0.08]	<0.001
TDI lateral free wall S' (m/s)	0.058 (0.02)	0.106 (0.03)	<0.001
Global strain (%)	-15.3 (3.2)	-16.6 (2.4)	0.062
Diastolic			
E (m/s)	0.66 [0.55-0.82]	0.98 [0.89-1.03]	<0.001
A (m/s)	0.49 (0.20)	0.42 (0.08)	0.069
E/A	1.43 [1.1-2.1]	2.32 [2.0-2.7]	<0.001
TDI septal E' (m/s)	0.072 (0.03)	0.143 (0.03)	<0.001
TDI septal A' (m/s)	0.041 (0.02)	0.053 (0.01)	0.003
TDI lateral free wall E' (m/s)	0.085 (0.04)	0.178 (0.04)	<0.001
TDI lateral free wall A' (m/s)	0.047 [0.03-0.06]	0.055 [0.05-0.07]	0.017
E/E'	8.51 [6.5-12.5]	5.71 [5.13-7.09]	<0.001
Cardiac autonomous nervous activity			
PEP (ms)	122.4 (17.6)	81.3 (15.9)	<0.001
RSA (ms)	68.7 [26.1-110.3]	84.5 [54.7-123.5]	0.104
RMSSD (ms)	60.6 [20.8-107.2]	62.5 [49.4-100.4]	0.611
SDNN (ms)	79.9 [28.0-126.1]	70.7 [55.1-95.9]	0.843
LF (ms^2)	1271 [69-4832]	1243 [575-2173]	0.380
HF (ms^2)	1063 [37-3136]	1264 [559-1264]	0.056
LF:HF ratio	1.48 [0.82-2.64]	0.88 [0.47-1.33]	0.009
Respiration rate (breaths/min)	18.8 (3.0)	18.5 (3.2)	0.686

Data expressed as mean (±SD), and median [Q1-Q3].

A = peak late diastolic velocity; A' = peak late diastolic TDI velocity; AIXao = Augmentation index of the aorta; CI = cardiac index; E = peak early diastolic velocity; E' = peak early diastolic TDI velocity; HF = high frequency power spectral values; LF = low frequency power spectral values; LF:HF = low frequency/high frequency ratio; PEP = pre-ejection period; PP = pulse pressure; PWVao = pulse wave velocity of the aorta; RMSSD = root mean square of successive differences between normal sinus beats; RSA = respiratory sinus arrhythmia zero; S' = peak systolic TDI velocity; SBP = systolic blood pressure; SDNN = standard deviation of the inter-beat interval of normal sinus beats; SpO_2 = oxygen saturation; TDI = tissue doppler imaging; VTI = velocity time integral.

Table 3. Cardiopulmonary exercise test results Fontan patients

	Absolute values	Percentage of predicted
Maximal exercise (N = 33)		
RER_{peak}	1.13 (0.09)	-
WR_{peak} (watt)	118.6 (38.2)	61.3 (56.3-66.3) **
WR_{peak}/kg (watt/kg)	2.2 (0.6)	63.5 (58.0-69.0) **
HR_{peak} (bpm)	173.5 (16.0)	93.5 (90.2-96.7) **
HR reserve (bpm)	94.9 (25.7)	-
HR01 percentage (%)	35.0 [25.2-48.3]	115.8 (102.3-131.0) *
VO_{2peak} (L/min)	1434.5 (337)	-
VO_{2peak} (mL/kg/min)	26.8 (4.7)	54.4 (51.3-57.5) **
Peak O_2 pulse (mL/beat)	8.65 [7.1-9.4]	58.6 (54.6-62.7) **
Submaximal exercise (N = 36)		
VE/VCO2 slope	36.5 [33.5-41.0]	123.8 (118.9-140.5) **
OUES	1402.7 (386.2)	-
OUES/kg	27.2 (5.9)	58.4 (54.3-62.5) **
OUEP	31.6 [29.4-33.8]	72.2 (68.4-74.7) **

Absolute values expressed as mean (±SD), and median [Q1-Q3].
Percentage of predicted expressed as mean or median (95% CI).
HR01 percentage = percentage heart rate recovery at 1 min after exercise; HR_{peak} = heart rate at peak exercise; $HR_{recovery01}$ = heart rate at peak exercise - heart rate at 1 min after exercise; HR reserve = heart rate at peak exercise – heart rate at rest; HR_{rest} = heart rate at rest; OUEP = oxygen uptake efficiency plateau; OUES = oxygen uptake efficiency slope; Peak O_2 pulse = peak oxygen uptake per heart beat; RER_{peak} =respiratory exchange ratio at peak exercise; VE = ventilatory efficiency; VE/VCO2 slope = slope of respiratory minute to CO2 production; VO_{2peak} = oxygen uptake at peak exercise; WR_{peak} = maximal work rate achieved.
* *P*-value <0.01 for difference between exercise values and reference values.
** *P*-value <0.001.

Table 4. Comparison of vascular function, cardiac autonomic nervous activity, chronotropic competence and exercise capacity between patients who had aortic arch reconstruction and who did not

Characteristics	Aortic arch surgery (N = 15)	No aortic arch surgery (N = 21)	*P*-value
Vascular function			
SBP (mmHg)	120.0 [112.0-125.0]	120.0 [115.0-123.0]	1.000
PP (mmHg)	54.1 (9.7)	52.1 (7.3)	0.486
PWVao (m/s)	5.9 [4.8-6.8]	4.9 [4.8-5.7]	0.149
AIXao	13.0 [10.5-18.9]	16.3 [13.3-24.4]	0.215
Cardiac autonomous nervous activity			
PEP (ms)	130.4 [105.4-141.8]	117.6 [112.1-129.5]	0.486
RSA (ms)	64.7 [19.1-103.5]	82.1 [26.1-115.6]	0.664
RMSSD (ms)	50.5 [15.7-126.7]	77.3 [24.2-107.2]	0.568
SDNN (ms)	64.1 [20.4-119.7]	89.6 [39.5-137.7]	0.371
LF	942.4 [37-5531]	1518.7 [385-4832]	0.516
HF	475.5 [17-3920]	1657.9 [74.0-3136]	0.399
LF:HF	1.53 [1.0-2.9]	1.28 [0.7-2.3]	0.516
Respiration rate (breaths/min)	19.9 (2.9)	18.0 (2.9)	0.089
Chronotropic competence			
Patients with maximal exercise (N)	14	19	
HR_{peak} (bpm)	169.0 (15.8)	177.0 (15.7)	0.165
% HR_{peak}	90.9 (9.1)	95.5 (8.6)	0.147
$HR_{01\%}$ (%)	37.7 [30.0-49.3]	32.8 [22.2-45.1]	0.316
% $HR_{01\%}$	125.7 (51.8)	117.0 (40.4)	0.602
Exercise capacity			
Maximal exercise (N)	14	19	
VO_{2peak}(mL/kg/min)	26.8 (3.9)	26.8 (5.3)	0.997
% VO_{2peak}/kg	52.1 (7.3)	56.1 (9.7)	0.198
Submaximal exercise (N)	15	21	
OUES/kg	26.6 [23.7-31.6]	26.9 [22.3-29.8]	0.505
% OUES/Kg	58.2 [56.7-64.4]	58.5 [49.4-63.6]	0.680

Data expressed as mean (±SD), and median [Q1-Q3].
AIXao = Augmentation index of the aorta; HF = high frequency power spectral values; HR_{peak} =maximal heart rate at peak exercise; HR01 percentage = percentage heart rate recovery at 1 min after exercise; LF = low frequency power spectral values; LF:HF = low frequency/high frequency ratio; OUES = oxygen uptake efficiency slope ; PEP = pre-ejection period; PP = pulse pressure; PWVao = pulse wave velocity of the aorta; RMSSD = root mean square of successive differences between normal sinus beats; RSA = respiratory sinus arrhythmia zero; SBP = systolic blood pressure; SDNN = standard deviation of the inter-beat interval of normal sinus beats; VO_{2peak} = oxygen uptake at peak exercise.

Among the cardiovascular parameters, HR at rest (r = -0.540, *P* = 0.001 and r = -0.452, *P* = 0.006), PWVao (r = -0.455, *P* = 0.018 and r = -0.361, *P* = 0.046) and E/A (r = 0.625, *P* < 0.001 and r = 0.421, *P* = 0.010) were found to be correlated with both VO_{2peak} and OUES and are shown Figure 1. An overview of all correlations between VO_{2peak} and OUES and the various cardiovascular parameters is shown in Supplementary Table S3.

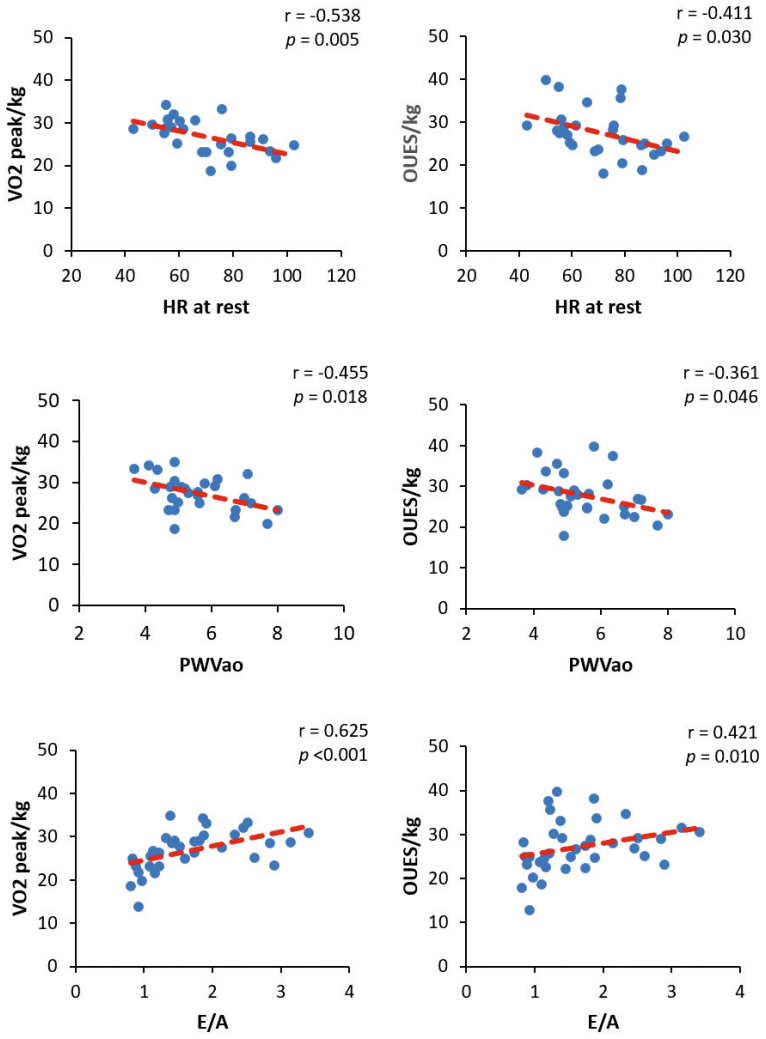

Figure 1. Correlation between VO_2 peak or OUES and HR, PWVao and E/A. E/A = ratio of peak early and late diastolic velocity; HR = heart rate; OUES = oxygen uptake efficiency slope; PWVao = aortic pulse wave velocity; VO_2peak = oxygen uptake peak.

Discussion

Our study demonstrates reduced maximal and submaximal exercise performance in contemporary Fontan patients with moderate to good systolic ventricular function. Stroke volume was reduced at peak exercise, reflected by a decreased oxygen pulse, although chronotropic competence was preserved. A reduced exercise performance was related to impaired diastolic function, increased aortic stiffness and an increased HR at rest.

Exercise requires an adequate increase in cardiac output, which in turn requires an adequate increase in or at least a preserved stroke volume and a sufficient increase in HR. A limited increase in stroke volume during exercise is known to be one of the main limiting factors in a Fontan circulation; the absence of a sub-pulmonary ventricle results in an inability to augment venous return (19). Other factors that may also contribute to a limited increase in stroke volume during exercise in Fontan patients are reduced diastolic and systolic ventricular function and increased arterial stiffness.

Diastolic dysfunction may be caused by an increased stiffness of the systemic ventricle and thereby limit the preload affecting stroke volume. Our study showed that an impaired diastolic function, reflected by an increased E/A ratio, was a determinant of diminished exercise capacity. This finding is in line with a previous echocardiographic study in adult Fontan patients, in which an increased diastolic dysfunction, reflected by an E/E' ratio ≥ 12, was associated with a lower VO_{2peak} (8). In our study, E/E' was not correlated with exercise performance, however, the E/E' was much lower (median 8.5), which could indicate that only a significant increased E/E', indicating significant diastolic dysfunction, might be correlated with reduced exercise capacity. Diastolic dysfunction can already be present shortly after the Fontan operation (20) and is most likely explained by the immediate pre-load reduction post-operatively, resulting in a greater mass to volume ratio altering relaxation and diastolic performance. On the long term, impaired diastolic function tends to persist or even further deteriorate (20, 21). The aetiology of diastolic dysfunction in Fontan patients is not completely understood, and factors such as myocardial fibrosis possibly induced by volume overload and cyanosis pre-operatively, long-term shunts, previous pulmonary artery banding, systemic ventricular outflow tract obstructions, or by renin-angiotensin-aldosterone system activation may play an important role (22).

Systolic ventricular function clearly plays a role in the preservation of stroke volume during exercise and previous Fontan studies also showed that significant systolic ventricular dysfunction was related to limited exercise performance (3). In the present study, which included only patients with moderate to good systolic ventricular function, we found no correlation between systolic function parameters and exercise performance. However, Tissue Doppler imaging values were lower compared to controls, indicating that even in this relatively healthy and young Fontan cohort systolic ventricular function was also decreased and progression of systolic dysfunction in time may contribute to the deterioration of exercise performance on the long term (1).

Increased arterial stiffness leads to a higher afterload which subsequently results in a higher workload of the single ventricle, negatively affecting diastolic and systolic ventricular function and volumes (4, 23). We showed that a higher arterial stiffness, measured as PWVao, was correlated with reduced exercise capacity. AIX was not correlated with exercise performance. However, AIX is an indirect measure of arterial stiffness and is also be influenced by heart rate and stroke volume. The correlation with a higher arterial stiffness corresponds with the majority of studies in paediatric and adult Fontan patients (4, 7, 24). A small study in 17 paediatric and adult Fontan patients, using the same non-invasive methodology, did not show an effect of arterial stiffness on exercise capacity, which may be explained by the small sample size (6).

Furthermore, we showed no difference in arterial stiffness between patients who had aortic arch reconstruction and who did not. Previous studies have shown that not only the non-compliant patch used for aortic reconstruction relates to aortic stiffness, but that also other Fontan patients show arterial stiffness, suggesting other wall abnormalities are present (25, 26). Arterial stiffness could be related to vascular fibrosis, endothelial dysfunction, inflammation and elevated sympathetic activity (25, 27, 28) and has been shown to increase with age (4).

Chronotropic incompetence, defined as the inability to increase heart rate normally with exercise, negatively affects cardiac output, limiting exercise performance (5, 29). In our study, chronotropic competence was preserved and HR at peak was not correlated with exercise performance. However, the degree of chronotropic impairment may vary in Fontan patients. A reduction in maximal HR can be the result of reduction in cardiac filling at exercise (30), sinus node dysfunction or altered ANS control. As markedly impaired cardiac autonomic nerve activity has

been described in adult Fontan patients (31), this may negatively affect chronotropic competence on the long term.

In addition, we showed that there was no difference in HR at peak and ANS control between patients who had aortic arch surgery and who did not. A difference could be expected as at time of arch reconstruction there can be transection of the peri-arterial network of cardiac innervation influencing ANS values and chronotropic competence. Our results suggest that the cardiac innervation in these patients is relatively preserved.

We did find a correlation between a higher resting HR and decreased exercise performance. This finding is intriguing as there is concern about sinus node dysfunction in Fontan patients, which is associated with lower exercise tolerance. However, sinus node dysfunction not only includes bradycardia, but also includes a non-sinus rhythm, lower peak HR at exercise or long pauses (32). Our results are confirmed by a previous study, which showed that sinus bradycardia on its own was not associated with decreased functional status in a group of Fontan patients (33). A lower resting HR is therefore not necessarily unfavourable in Fontan patients.

A possible explanation could be that a lower resting HR provides mechanical advantages by prolonging diastolic filling time to support the preload dependent Fontan circulation (33). Conversely, HR at rest could be increased in patients as a compensation for reduced stroke volume to maintain adequate cardiac output. The correlation with a higher HR might, however, also be caused by sympathovagal disbalance, as can be seen in patients with heart failure (34). Although LF:HF ratio was increased suggesting sympathetic dominance in chronotropic cardiac control, PEP was increased as well suggesting the opposite. Because PEP is also influenced by changes in preload and afterload, it may be a less reliable parameter for sympathetic activity in Fontan patients (35). On the other hand, the LF:HF ratio as sympathovagal balance remains controversial, as LF reflects a mixture of cardiac vagal and cardiac sympathetic activity (14). Therefore, more invasive parameters should be considered in future research to validly measure sympathetic activity and sympathovagal balance in Fontan patients.

Besides limiting factors, there are modifiable factors which are positively associated with exercise capacity, such as the ventilatory and skeletal muscle pump (36). Although not investigated in our study, both factors may augment venous return and are, therefore, important factors in a circulation where a subpulmonary ventricle is lacking. The peripheral muscle pump is the largest contributor to venous return and

lean leg mass has been found to be closely correlated with the increase in cardiac index during exercise (36, 37). Regular physical activity and improving leg muscle strengthening may, therefore, result in better exercise performance (38, 39). While many studies have described potential short-term benefits of physical activity, the benefits of long-term physical activity rehabilitation programs from an early age onwards have not yet been investigated.

Limitations

This study is limited by its cross-sectional design. As in all other Fontan studies, the underlying cardiovascular defect is heterogeneous. However, our group was more homogeneous than in most studies including only paediatric patients with an extracardiac conduit and moderate-good systolic ventricular function. Although we did investigate multiple possible cardiovascular factors of exercise limitation, even more other possible factors have been described previously in Fontan patients, such as skeletal muscle mass/strength, lung function and pulmonary vascular resistance. Measuring all possible factors was not feasible, however could have fully completed the understanding of limited exercise performance in this cohort. In addition, echocardiography has its limitations in assessing diastolic function or ventricular dimensions. Finally, as exercise data in the control group is lacking, we were unable to explore the relationship between different cardiovascular parameters and exercise capacity in the normal population. We were therefore unable to provide a context to what to expect when exploring these relationships in the Fontan population and how to interpret our findings in the Fontan population.

Conclusion

Paediatric Fontan patients with moderate to good systolic ventricular function have an impaired exercise capacity with a preserved chronotropic competence. In this group of young patients impaired diastolic function, increased aortic stiffness and an increased HR at rest seemed to be important determinants of reduced exercise performance. Given that diastolic function further decreases, and aortic stiffness increases over time, both parameters may contribute to the deterioration of exercise capacity in Fontan patients. Further longitudinal studies investigating the effect of changes in these cardiovascular parameters on exercise deterioration are warranted to determine the prognostic value of these markers in Fontan patients.

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Supplementary material

Table S1. Comparison of cardiovascular parameters at rest between patients with a left (LV) or right (RV) ventricle

Characteristics	LV (N=21)	RV (N=12)	*P*-value
Heart rate (bpm)	71.4 (15.6)	68.2 (14.1)	0.577
Vascular function			
SBP (mmHg)	120.0 [112.8-124.5]	120.0 [111.0-123.0]	0.919
PP (mmHg)	52.0 [43-56.5]	54.0 [50.0-57.0]	0.531
PWVao (m/s)	5.0 [4.8-5.9]	5.6 [4.7-6.7]	0.521
AIXao	16.2 [13.4-22.6]	12.0 [9.6-17.8]	0.173
Ventricular function			
Systolic			
CI (L/min/m^2)	3.2 [2.9-3.9]	3.4 [2.8-4.1]	0.965
TDI septal S' (m/s)	0.047 [0.03-0.05]	0.040 [0.03-0.05]	0.200
TDI lateral free wall S' (m/s)	0.061 (0.02)	0.053 (0.01)	0.242
Global strain (%)	-13.9 [-12.4- -17.4]	-15.4 [-13.0- -20.2]	0.570
Diastolic			
E (m/s)	0.69 [0.54-0.82]	0.66 [0.55-0.82]	0.868
A (m/s)	0.45 [0.30-0.64]	0.46 [0.31-0.62]	0.754
E/A	1.45 [1.2-2.0]	1.46 [1.1-2.4]	0.985
TDI septal E' (m/s)	0.070 [0.05-0.10]	0.070 [0.04-0.09]	0.800
TDI septal A' (m/s)	0.047 [0.03-0.06]	0.040 [0.04-0.04]	0.674
TDI lateral free wall E' (m/s)	0.091 (0.04)	0.082 (0.03)	0.492
TDI lateral free wall A' (m/s)	0.050 (0.03)	0.044 (0.01)	0.500
E/E'	8.44 [5.7-12.0]	8.56 [6.7-11.8]	0.897
Cardiac autonomous nervous activity			
PEP (ms)	117.1 [109.7-124.9]	132.3 [112.9-142.7]	0.103
RSA (ms)	53.7 [20.6-109.2]	84.0 [55.0-115.5]	0.310
RMSSD (ms)	55.1 [12.8-101.5]	64.8 [70.8-141.0]	0.551
SDNN (ms)	82.8 [24.7-114.9]	76.7 [54.4-138.3]	0.776
LF (ms^2)	709 [30-4462]	1491 [534-7168]	0.576
HF (ms^2)	1062 [16-2244]	1387 [407-4637]	0.948
LF:HF ratio	1.94 [0.7-2.6]	1.43 [0.8-1.9]	0.723
Respiration rate (breaths/min)	18.6 (3.5)	18.6 (2.1)	0.980

Data expressed as mean (±SD), and median [Q1-Q3].

A= peak late diastolic velocity; A'= peak late diastolic TDI velocity; AIXao= Augmentation index of the aorta; CI= cardiac index; E= peak early diastolic velocity; E'= peak early diastolic TDI velocity; HF = high frequency power spectral values; LF= low frequency power spectral values; LF:HF= low frequency/high frequency ratio; PEP= pre-ejection period; PP= pulse pressure; PWVao= pulse wave velocity of the aorta; RMSSD= root mean square of successive differences between normal sinus beats; RSA = respiratory sinus arrhythmia zero; S'= peak systolic TDI velocity; SBP= systolic blood pressure; SDNN= standard deviation of the inter-beat interval of normal sinus beats; TDI= tissue doppler imaging; VTI= velocity time integral.

Table S2. Comparison of cardiopulmonary exercise test results between patients with a dominant left (LV) and right (RV) ventricle

	LV	RV	P-value
Maximal exercise			
Total patients (N)	18	12	-
RER_{peak}	1.12 [1.04-1.24]	1.14 [1.06-1.16]	0.983
WR_{peak} (watt)	124.7 (40.4)	117.5 (30.3)	0.603
% WR_{peak}	63.6 (13.6)	61.9 (13.8)	0.741
WR_{peak}/kg (watt/kg)	2.22 [1.95-2.60]	2.12 [1.7-2.8]	0.632
% WR_{peak}/kg	66.1 (13.9)	63.8 (14.1)	0.659
HR_{rest} (bpm)	78.1 (19.1)	80.8 (19.3)	0.700
HR_{peak} (bpm)	175.3 (19.8)	170.0 (9.3)	0.335
% HR_{peak}	94.6 (10.9)	91.5 (5.2)	0.309
$HR_{reserve}$ (bpm)	97.3 (28.7)	89.2 (24.3)	0.431
$HR_{01\%}$ (%)	33.9 [25.2-49.3]	39.2 [32.5-49.8]	0.347
% $HR_{01\%}$	116.8 [95-153]	124.0 [107-160]	0.471
VO_{2peak} (L/min)	1511.2 (387)	1396.8 (193)	0.294
VO_{2peak} (mL/kg/min)	28.1 (4.2)	26.0 (3.7)	0.169
% VO_{2peak}/kg	57.7 (8.5)	52.4 (6.0)	0.072
Peak O_2 pulse (mL/beat)	8.9 (2.4)	8.4 (1.1)	0.414
% Peak O_2 pulse	60.4 (13.2)	58.2 (8.8)	0.608
Submaximal exercise			
Total patients	21	12	-
VE/VCO2 slope	37.8 [32.9-40.2]	36.1 [34.0-40.8]	0.956
% VE/VCO2 slope	121.6 (110.9-146.1)	123.8 (115.3-141.2)	0.985
OUES	1416.9 (447.9)	1463.9 (256.0)	0.742
OUES/kg	28.0 [24.6-32.4]	25.0 [23.1-30.2]	0.593
% OUES/Kg	59.7 (51.5-66.7)	56.1 (51.8-61.6)	0.471
OUEP	31.6 [28.3 [34.2]	31.5 [30.7-33.8]	0.699
% OUEP	71.8 (66.6-80.5)	72.8 (71.6-77.2)	0.645

Absolute values expressed as mean (±SD), and median [Q1-Q3]; Percentage of predicted expressed as mean or median (95% CI).

$HR_{01\%}$= percentage heart rate recovery at 1 minute after exercise; % $HR_{01\%}$= percentage of predcited of $HR_{01\%}$; HR_{peak}= heart rate at peak exercise; % HR_{peak}= percentage of predicted of HR_{peak}; HR reserve= heart rate at peak exercise – heart rate at rest; HR_{rest}= heart rate at rest; OUEP= oxygen uptake efficiency plateau; % OUEP= percentage of predicted of OUEP; OUES= oxygen uptake efficiency slope; % OUES/kg= percentage of predicted of OUES/kg; Peak O_2 pulse= peak oxygen uptake per heart beat; % Peak O_2 pulse = percentage predicted of Peak O_2 pulse; RER_{peak}=respiratory exchange ratio at peak exercise; % RER_{peak}= percentage predicted of RER_{peak}; VE/VCO2 slope= slope of respiratory minute to CO2 production; % VE/VCO2 slope= percemtage of predicted of VE/VCO2 slope; VO_{2peak}= oxygen uptake at peak exercise; % VO2peak= percentage of predicted of $VO2_{peak}$; WR_{peak}= maximal work rate achieved; % WR_{peak}= percentage of predicted of WR_{peak}.

Table S3. Correlations of different cardiovascular parameters with exercise performance in Fontan patients.

	VO2peak/kg		OUES/kg	
	r	***P*-value**	**r**	***P*-value**
Heart rate (bpm)	-0.538	**0.005**[a]	-0.411	**0.030**[a]
Vascular function				
SBP (mmHg)	-0.249	0.177[a]	-0.171	0.359[a]
PWVao (m/s)	-0.455	**0.018**[a]	-0.361	**0.046**[a]
AIXao	-0.099	0.615[b]	0.266	0.148[b]
Ventricular function				
Systolic				
CI (L/min/m^2)	0.041	0.868[a]	0.064	0.793[a]
TDI septal S' (m/s)	-0.036	0.850[b]	-0.037	0.840[b]
TDI lateral free wall S' (m/s)	0.042	0.816[a]	0.070	0.686[a]
Global strain (%)	0.022	0.914[a]	-0.041	0.832[a]
Diastolic				
E/A	0.625	**<0.001**[b]	0.421	**0.010**[b]
E/E'	0.091	0.615[b]	0.020	0.909[b]
Cardiac autonomic nervous activity				
PEP (ms)	0.196	0.348[a]	0.175	0.382[a]
RSA (ms)	-0.005	0.980[b]	-0.056	0.778[b]
LF:HF	0.155	0.461[b]	0.265	0.182[b]
Exercise test				
HR_{peak} (bpm)	0.026	0.885[a]	-0.097	0.574[a]
HR reserve (bpm)	0.266	0.141[a]	0.016	0.926[a]

a Pearson correlation; b Spearman correlation
For used abbreviations see Table S1-2

Chapter 5

Fluid responsiveness of ambulant paediatric Fontan patients by passive leg raising

Under review Cardiology in the Young

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Abstract

Background

Passive leg raising (PLR) is used to predict who will benefit from fluid therapy in critically ill patients, including children. Patients with a Fontan circulation may have a different hemodynamic response to a fluid challenge by PLR.

Methods

The hemodynamic response of 35 paediatric Fontan patients from the outpatient clinic (median age 14.0 years) and 35 healthy controls (median age 12.8 years) to PLR was evaluated non-invasively by echocardiography for assessment of e.g., velocity time integral (VTI) across the (neo)aortic valve, blood pressure measurements and respiration. Participants were considered responders when the VTI increased by ≥10%.

Results

Overall, Fontan patients and controls did not differ in the hemodynamic response to PLR. Twelve patients (36%) in the Fontan group and 8 controls (23%) were responders, which was not statistically different (*P*=0.222). Responders in the Fontan and control group also had a similar VTI increase of +18.9% and +15.2% respectively (*P*=0.910). There was no difference in VTI change between Fontan and control non-responders with a decrease of -1.4% and -6.4% respectively (*P*=0.655) and no difference in the amount of patients who were negatively affected by PLR, with a decrease of ≤-10% in VTI in 7 patients (33%) en 9 controls (33%)(*P*=1.00).

Conclusion

The hemodynamic response of ambulant paediatric Fontan patients to PLR is similar to that of healthy controls. Fontan patients who did not respond to PLR were similarly affected as healthy controls. Whether the hemodynamic response is different in critically-ill Fontan patients warrants further investigation.

Introduction

Paediatric and adult critical-care patients often receive fluid therapy to optimize intravascular volume. While fluid administration may improve haemodynamics, excessive administration can result in decreased stroke volume and unwanted side effects (1). A fluid challenge, such as a passive leg raising (PLR) manoeuvre, can be used in critical settings to predict fluid responsiveness. PLR is non-invasive, reversible and has proven to be reliable in predicting volume responsiveness in adult and paediatric populations (2-7).

Studies have shown that approximately 50% of critically-ill paediatric and adult patients with a biventricular circulation increase their cardiac output in response to a fluid challenge (2-5, 8). However, little is known about the responsiveness of Fontan patients with a univentricular circulation. In Fontan patients both caval veins are directly connected to the pulmonary arteries. Venous pressure is required to overcome pulmonary vascular resistance. This means that alterations of fluid balance may have negative consequences for the circulation: while a fluid bolus may result in an increased preload and a beneficial increase in cardiac output, an increase in end-diastolic pressure might also lead to a decrease in transpulmonary gradient and reduced pulmonary blood flow, negatively affecting cardiac output (9). Because Fontan patients are often admitted to the intensive care unit after procedures and may need fluid therapy, it is important to understand how they potentially react to a fluid challenge. Therefore, the aim of this study is to evaluate the hemodynamic response of ambulant paediatric Fontan patients to PLR in comparison with healthy controls.

Methods

Patients between 8 and 18 years of age, who underwent surgery at the Leiden University Medical Centre, were recruited from the outpatient clinic from July 2017 to October 2019. To study a homogenous group, we included patients palliated with an extracardiac conduit and a subjective moderate to good systolic ventricular function. Patients with a pacemaker and an open fenestration were excluded from the study. Healthy children served as controls. The local ethics committee approved the study and written informed consent was obtained from all participants or their parents or legal guardians as appropriate.

To test the reaction to PLR between patients and healthy controls, parameters were measured during supine rest and during PLR. Therefore, in this study, we investigated heart rate, blood pressure, velocity time integral (VTI) across the (neo) aortic valve, stroke volume index (SVI) and cardiac index (CI) for the hemodynamic response, peak hepatic vein flow and inferior vena cava (IVC) collapsibility index for an estimation of change in systemic venous return. Furthermore, since respiration may influence the hemodynamic response, we also evaluated the respiration rate during PLR in this study. Furthermore, we also evaluated if there were differences in baseline characteristics between non-responders and responders in Fontan patients as well as controls, such as e.g., age, sex, BSA, BMI, but also age at operation and diastolic and systolic ventricular function.

For this study, subjects started in supine position for baseline measurements, then the lower extremities were raised 45°, and after three minutes all measurements were performed again. Categorization of responders was defined as ≥10% VTI increase during PLR. Fluid responsiveness is conventionally defined as an increase of at least 10-15% in stroke volume or cardiac output (or one of its surrogates, such as VTI) with good sensitivity and specificity in adults as well as paediatric subjects (3, 4, 6, 10, 11). In this study, VTI was chosen as accurate measurement of the aortic annulus would be difficult in the Fontan population.

To measure VTI, SVI, CI, hepatic venous flow and IVC collapsibility index, transthoracic echocardiography was performed on a Vivid S6/S60 (GE healthcare, Norway). VTI, a measure of blood flow displacement (cm), was measured by pulse wave Doppler recordings across the (neo)aortic valve, from which, together with the (neo)aortic annulus (cm), SVI (ml/m^2) and CI (L/min/m^2) were calculated as follows: $SVI = \frac{\left(\left(\pi * \left(\frac{\text{aortic annulus}}{2}\right)^2\right) * \text{VTI}\right)}{\text{body surface area}}$ and CI=heart rate*SVI.

In addition, Doppler recordings of the hepatic vein were performed to assess peak antegrade flow and the maximum and minimum diameter of the inferior vena cava (IVC) was measured by M-mode during a sniff-test, from which we calculated the proportional change, the IVC collapsibility index. Averages of three consecutive VTI measurements were used for calculations and analysis.

Blood pressure measurements were performed using an oscillometric arteriograph device with the cuff on the left arm (Tensiomed, Hungary). Furthermore, respiration was measured by impedance registration using the VU-ambulatory monitoring system (VU-AMS; VU university, Netherlands, 5fs version).

Analyses were conducted using SPSS statistics (IBM, version 25). To perform reliable inference in the small study group, non-parametric tests were used for all comparisons. Categorical data are reported as numbers with percentages and continuous data are presented as median with first to third quartile [Q1-Q3]. To assess the difference in categorical data, the Chi-square test was used. Differences between patient characteristics, baseline parameters and percentage change to PLR between the different groups were tested by the Mann-Whitney *U*-test. A P-value ≤0.05 was considered significant.

Results

Thirty-five ambulant Fontan patients with a good functional status (median age 14.0 years) and low median plasma NT-Pro BNP of 79.3ng/L and 35 controls (median age 12.8 years) were included in the study (Table 1). Nineteen Fontan patients had a dominant left ventricle (58%), 11 a dominant right ventricle (33%) and 3 an indifferent or undefined ventricle (9%). Although all Fontan patients had a subjective moderate to good systolic ventricular function and a comparable global longitudinal strain compared to healthy controls on echocardiography, Tissue Doppler imaging showed lower systolic velocities in Fontan patients compared to controls. Furthermore, diastolic ventricular function was lower in Fontan patients compared to controls (Table 1).

Table 2 shows the baseline parameters during supine rest and percentage change during PLR between Fontan patients and controls. At baseline, patients had a higher systolic blood pressure and lower VTI, peak hepatic vein flow and IVC collapsibility index compared to controls. Overall, Fontan patients and controls showed similar response to PLR with no difference in percentage change of all parameters.

Table 1. Patient Characteristics

Characteristics	Fontan patients (N=33)	Controls (N=35)	*P*-value
Age (years)	14.0 [12.7-16.5]	12.8 [11.1-15.5]	0.187
Males (N,%)	21 (63.64)	18 (51.4)	0.309
BMI (kg/m^2)	19.2 {17.1-21.0]	17.5 [16.1-19.6]	0.070
BSA (m^2)	1.5 [1.4-1.7]	1.4 [1.2-1.6]	0.103
Main ventricle (N;%)			
Left	19 (57.6)		
Right	11 (33.3)		
Indifferent	3 (9.1)		
Age at Glenn operation (years)	0.5 [0.4-0.7]		
Age at Fontan operation (years)	3.1 [2.7-3.5]		
NT pro-BNP	79.3 [44.4-126.5]		
Systolic ventricular function			
Global longitudinal strain (%)	15.4 [12.8-17.6]	16.6 [14.4-18.2]	0.084
TDI septal S' (cm/s)	4.3 [3.1-5.0]	8.0 [7.0-8.3]	<0.001
TDI lateral free wall S' (cm/s)	5.9 [4.7-7.3]	10.7 [9.3-12.3]	<0.001
Diastolic ventricular function			
E/A	1.5 [1.1-2.2]	2.3 [2.0-2.7]	0.001
E/E'	8.2 [6.2-12.4]	5.7 [5.1-7.1]	<0.001

Data expressed as n (%), mean (±SD), and median [Q1-Q3].
E/A= ratio of peak early and late diastolic velocity; E/E'= ratio of peak early conventional and Tissue Doppler diastolic velocity; BMI=Body mass index; BSA= body surface area; NT-pro BNP= N-terminal pro brain natriuretic peptide; S'= peak systolic TDI velocity.

Table 2. Cardiovascular parameters during supine rest and the percentage change during passive leg raising in Fontan patients vs healthy controls

	Fontan patients		Healthy controls	
	Supine rest	**Percentage change**	**Supine rest**	**Percentage change**
Haemodynamics				
Heart rate (bpm)	64.9 [57.9-89.5]	-5.1 [-12.0-1.4]	69.5 [64.9-76.9]	-4.5 [-12.1-3.1]
VTI (cm)	15.9 [13.8-20.9]	+4.6 [-4.5-14.4]	21.1 [18.9-23.3] *	-0.5 [-10.6-8.3]
SVI (ml/min/ m^2)	50.0 [43.6-54.8]	+6.5 [-1.3-17.6]	47.1 [41.0-52.5]	-1.3 [-10.6-8.1]
CI (L/min/m^2)	3.3 [2.9-4.0]	-0.7 [-9.2-4.9]	3.4 [2.8-3.7]	-3.8 [-13.6-8.5]
Systolic BP (mmHg)	120.0 [115.0-125.5]	0.0 [-3.7-2.5]	108.0 [102.8-115.3] **	-0.4 [-1.9-2.2]
Diastolic BP (mmHg)	65.5 [60.3-71.0]	+0.6 [-3.0-6.5]	61.5 [72.8-83.0]	-0.7 [-6.6-8.0]
Systemic venous return				
Peak hepatic flow (m/s)	0.25 [0.21-0.29]	-5.6 [-16.9-12.2]	0.46 [0.34-0.52] **	-2.2 [-16.2-8.3]
IVC collapsibility index (%)	35.1 [24.5-47.7]	-0.6 [-11.6-13.2]	72.6 [59.7-85.6 **	-3.4 [-13.2-5.6]
Respiration				
Respiration (breaths/min)	19.2 [15.9-20.5]	-0.7 [-6.5-6.9]	18.1 [16.0-20.9]	-0.9 [-5.6-4.7]

Data expressed as mean (±SD), and median [Q1-Q3].

BP= Blood pressure; CI= Cardiac index; IVC= inferior vena cava; SVI= stroke volume index; VTI= velocity time integral.

* *P*-value <0.01 for differences in supine rest and percentage change between Fontan patients and healthy controls.

** *P*- value <0.001.

A total of 12 patients (36%) and 8 controls (23%) responded to PLR with an increase of ≥10%, which was not statistically different (*P*=0.222). In patients, baseline characteristics did not differ between responders and non-responders, including type of main ventricle, age at Glenn and Fontan operation, while in controls, responders had a higher age, body surface area and body mass index, and were predominantly female compared to the non-responders (Table 3).

The parameters of responders and non-responders of Fontan patients and controls during supine rest and percentage change during PLR are depicted in Table 4. Overall, baseline characteristics and reaction to PLR did not differ much. At baseline, IVC collapsibility index of Fontan responders were higher compared to Fontan non-responders and during PLR the IVC collapsibility index decreased in Fontan responders, while in Fontan non-responders it did not change. In contrast, control responders had a lower IVC collapsibility index compared to non-responders, however, there was no difference in percentage change during PLR between both groups. VTI and SVI increased significantly during PLR in Fontan as well as control responders, while CI only increased more in control responders compared to control non-responders. In reaction to PLR, VTI increased similarly in both responder groups (+18.9% in patients versus +15.2% in controls; *P*=0.910). Change of VTI in Fontan and control non-responders did also not differ, with -1.4% and -6.4% respectively (*P*=0.655). Furthermore, there was no difference in the number of patients who were negatively affected by PLR, with a decrease of ≤-10% in VTI in 7 patients (33%) and 9 controls (33%; *P*=1.00).

Table 3. Baseline characteristics of responders and non-responders of Fontan patients and Healthy controls

	Fontan patients		Healthy controls	
	Responders *(N=12)*	**Non-responders** *(N=21)*	**Responders** *(N=8)*	**Non-responders** *(N=27)*
Age (years)	13.6 [11.9-16.0]	14.1 [12.7-17.2]	15.3 [14.4-17.4]	11.7 [10.6-15.3] **
Males (N,%)	6 (50.0)	15 (71.4)	1 (12.5)	17 (63.0) *
BMI (kg/m^2)	18.1 [16.5-20.1]	19.8 [17.5-21.8]	19.0 [17.7-21.0]	17.0 [15.9-18.0] *
BSA (m^2)	1.5 [1.4-1.6]	1.7 [1.4-1.8]	1.6 [1.5-1.7]	1.4 [1.2-1.7] *
Main ventricle (N;%)				
Left	6 (50.0)	13 (61.9)		
Right	5 (41.7)	6 (28.6)		
Indifferent	1 (8.3)	2 (9.5)		
Age at Glenn operation (years)	0.51 [0.37-0.75]	0.50 [0.38-0.73]		
Age at Fontan operation (years)	3.2 [2.6-3.8]	3.0 [2.7-3.4]		
NT pro-BNP	123.3 [42.5-270.6]	78.4 [50.5-105.4]		
Systolic ventricular function				
Global longitudinal strain (%)	13.6 [12.0-16.4]	15.2 [13.0-17.2]	16.0 [13.4-17.5]	17.0 [14.6-18.8]
TDI septal S' (cm/s)	4.0 [3.0-6.0]	4.7 [3.7-5.3]	8.2 [7.8-8.9]	7.8 [7.0-8.3]
TDI lateral free wall S' (cm/s)	6.7 [5.0-7.0]	6.0 [5.7-8.0]	11.5 [9.8-12.8]	10.8 [9.3-12.0]
Diastolic ventricular function				
E/A	1.4 [1.2-2.9]	1.7 [1.0-1.9]	2.6 [1.9-2.7]	2.2 [1.9-2.7]
E/E'	7.9 [4.8-8.4]	6.8 [5.2-9.2]	5.2 [4.3-6.0]	5.9 [5.3-7.1]

Data expressed as mean (±SD), and median [Q1-Q3].

See Table 1 for previously used abbreviations.

* *P*-value <0.05 for differences between responders and non-responders per group.

** *P*-value <0.01.

*** *P*-value <0.001.

Table 4. Cardiovascular parameters during supine rest and percentage change during passive leg raising between responders and non-responders in Fontan patients and healthy controls.

	Responders		Non-responders	
	Supine rest	**Percentage change**	**Supine rest**	**Percentage change**
Fontan patients	*N=12*		*N=21*	
Heart rate (bpm)	77.1 [60.9-88.8]	-10.1 [-17.6-1.6]	63.1 [56.5-92.8]	-4.2 [-10.6-1.4]
VTI (cm)	15.3 [13.1-17.3]	+18.9 [13.3-26.2}	16.9 [14.2-22.1]	-1.4 [-12.9-4.1] ***
SVI (ml/min/ m^2)	46.1 [42.3-52.3]	+18.6 [13.6-23.9]	52.3 [43.4-55.4]	-0.9 [-12.0-5.5] ***
CI (L/min/m^2)	3.3 [3.0-4.2]	+3.4 [-8.2-20.5]	3.0 [2.9-3.8]	-4.7 [-10.9- -0.5]
Systolic BP (mmHg)	120.0 [115.0-122.0]	+1.7 [-1.6-6.3]	120.0 [113.5-125.0]	-0.8 [-4.5-2.2]
Diastolic BP (mmHg)	65.0 [56.0-72.0]	0.0 [-2.7-5.4]	66.0 [60.5-71.0]	+1.3 [-5.7-8.3]
Peak hepatic flow (m/s)	0.25 [0.21-0.26]	0.0 [-13.5-12.2]	0.26 [0.21-0.31]	-6.8 [-21.0-10.3]
IVC collapsibility index (%)	40.5 [35.5-62.3]	-7.8 [24.9-0.7]	30.3 [20.9-40.3] *	+1.9 [-8.7-19.5] *
Respiration (breaths/min)	19.2 [16.3-21.7]	-0.01 [-7.4-6.0]	19.2 [15.7-20.3]	-1.5 [-6.2-7.2]
Controls	*N=8*		*N=27*	
Heart rate (bpm)	67.1 [52.2-82.0]	-9.1 [-13.3-4.1]	70.2 [66.1-76.2]	-2.6 [-11.7-4.9]
VTI (cm)	20.6 [16.0-22.8]	+15.2 [13.5-26.2]	21.1 [18.9-23.6]	-6.4 [-12.4-3.0] ***
SVI (ml/min/ m^2)	42.9 [33.9-55.3]	+15.0 [13.1-28.1]	47.8 [42.3-51.6]	-6.4 [-12.4-3.0] ***
CI (L/min/m^2)	2.7 [2.3-3.7]	+8.2 [-0.5-19.9]	3.4 [3.1-3.7]	-7.6 [-14.5-7.3] **
Systolic BP (mmHg)	116.5 [108.5-119.8]	+0.5 [-3.3-4.3]	106.0 [102.0-111.0] *	-0.9 [-1.9-2.2]
Diastolic BP (mmHg)	65.5 [55.8-71.8]	+3.0 [-6.0-13.5]	61.0 [55.0-67.0]	-2.3 [-6.9-7.0]
Peak hepatic flow (m/s)	0.40 [0.31-0.48]	-2.1 [-16.0-8.3]	0.47 [0.34-0.53]	-2.4 [-17.1-9.2]
IVC collapsibility index (%)	59.4 [37.9-68.3]	+5.1 [-10.1-34.1]	74.3 [62.2-87.1] **	-6.0 [-13.4-1.6]
Respiration (breaths/min)	18.0 [16.1-21.7]	-0.5 [-5.7-4.3]	18.1 [16.0-20.8]	-1.4 [-5.6-4.7]

Data expressed as mean (±SD), and median [Q1-Q3].

BP= Blood pressure; CI= Cardiac index; IVC= inferior vena cava; SVI= stroke volume index; VTI= velocity time integral.

* *P*-value: ≤0.05 for differences in supine rest and percentage change between responders and non-responders in Fontan patients and healthy controls.

** *P*-value <0.01.

*** *P*-value <0.001.

Discussion

Our study demonstrates that ambulant paediatric Fontan patients respond similarly to a fluid challenge by PLR as healthy controls. Furthermore, Fontan patients who did not respond were similarly affected by PLR as healthy controls.

The proportion of responders in both groups, approximately 30%, was lower as compared to previous paediatric studies performed in biventricular patients, where around 50% were responders (3, 4). However, these studies have only been conducted in critical care settings where patients are more likely to be fluid depleted. PLR studies in healthy subjects have so far only been performed in adults and have shown a fluid responsiveness of about 45% (10, 12, 13). The response-rate in our healthy subjects was lower compared to these adult studies and may be due to the fact that adults have a larger blood pool in the lower extremities compared to paediatric subjects (14).

There was no difference in the response of SVI between Fontan patients and controls to PLR. It might be expected that the increased venous pressure in Fontan patients along with venous congestion can result in a reduced response to PLR. On the other hand, Fontan non-responders were not more negatively affected by PLR than controls, which is important to notice as Fontan patients often receive fluid therapy post-operatively in the intensive care unit. In Fontan patients, responders had a higher IVC collapsibility index at baseline and the index decreased during PLR, while in the responders group it did not change. This difference from the control group but can be explained by the fact that the Fontan circulation is a preload dependent circulation requiring venous pressure to overcome pulmonary vascular resistance. While the IVC Collapsibility Index may be useful in Fontan patients to predict fluid response, the effect of fluid loading cannot always be predicted in advance. Although a Fontan circulation is highly dependent on an adequate preload (15), it was shown that in response to a fluid challenge during catheterization most Fontan patients increased their cardiac output, but some showed a substantial decrease in transpulmonary gradient (9). A fluid challenge by PLR prior to fluid administration is thus useful to avoid adverse effects of an unnecessary fluid bolus. Because Fontan patients exhibit a critical fluid balance, the use of a PLR test in the intensive care unit can be very helpful to evaluate hemodynamic status and prevent hypo- or hypervolemia.

This study has some limitations. We included paediatric patients who were not critically-ill, signifying that these results cannot be directly translated to critically-ill patients on the intensive care unit. However, by studying a more homogeneous group we were able to determine the reaction to a fluid challenge in patients with a well-functioning Fontan circulation. Furthermore, previous studies have shown that PLR reflects the effects of fluid administration (2-5), however, the predictability of fluid responsiveness in Fontan patients may be different because of their increased venous pressure and dependence on adequate preload.

Conclusion

Paediatric Fontan patients have a similar hemodynamic response to PLR as healthy controls. Furthermore, patients who did not respond were not more negatively affected by PLR than healthy controls. Whether the hemodynamic response is different in critically-ill Fontan patients warrants further investigation. However, the use of a PLR test in the intensive care unit can be very helpful to evaluate hemodynamic status and to prevent hypo- or hypervolemia, especially in Fontan patients who exhibit a delicate fluid balance.

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Chapter 6

Orthostatic stress response in paediatric Fontan patients and the effect of ACE inhibition

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Abstract

Background

Many cardiocirculatory mechanisms are involved in the adaptation to orthostatic stress. While these mechanisms may be impaired in Fontan patients. However, it is yet unclear how Fontan patients, who exhibit a critical fluid balance, respond to orthostatic stress. Angiotensin converting enzyme inhibitors are often prescribed to Fontan patients, but they may negatively influence orthostatic tolerance. Therefore, we evaluated the response to orthostatic stress in paediatric Fontan patients before and after treatment with enalapril.

Methods

Thirty-five Fontan patients (aged 14 years) with moderate-good systolic ventricular function without pre-existent enalapril treatment were included. Before and after a three-month enalapril treatment period, the hemodynamic response to head-up tilt test was evaluated by various parameters including cardiac index, blood pressure, cerebral blood flow, aortic stiffness and cardiac autonomous nervous activity. Thirty-four healthy subjects (aged 13 years) served as controls.

Results

Fontan patients had a decreased cerebral blood flow and increased aortic stiffness in the supine position compared to controls, while all other factors did not differ. Patients and controls showed a comparable response to head-up tilt test for most parameters. Twenty-seven patients completed the enalapril study with a mean dosage of 0.3±0.1mg/kg/day. Most parameters were unaffected by enalapril, only the percent decrease in cardiac index to tilt was higher after treatment, but the cardiac index during tilt was not lower (3.0L/min/m^2 pre-enalapril versus 2.8L/min/m^2 after treatment; $P = 0.15$).

Conclusion

Paediatric Fontan patients adequately respond to orthostasis with maintenance of blood pressure and cerebral blood flow and sufficient autonomic response. Enalapril treatment did not alter the response.

Introduction

The Fontan procedure is the current surgical palliative method for patients with a univentricular physiology. After Fontan surgery, both caval veins are directly connected to the pulmonary arteries, resulting in a circulation lacking a sub-pulmonary ventricle. Consequently, venous pressure must overcome pulmonary vascular resistance. Any increase in pulmonary vascular resistance will lead to increased central venous pressure resulting in venous congestion and decreased cardiac output (1). As a result, the Fontan circulation has a delicate balance between systemic and pulmonary vascular resistance and a critical fluid balance (2). Many internal or external factors may disrupt this balance, potentially resulting in circulatory impairment.

Investigating determinants of circulatory adaptation can be performed in a non-invasive, ambulatory setting by orthostatic stress testing as during orthostasis a cascade of cardiocirculatory mechanisms must be initiated to maintain an adequate circulation. This cascade starts by unloading of the baroreceptors in reaction to volume unloading, resulting in activation of the sympathetic nervous system, and concurrent vagal withdrawal, resulting in an increase in heart rate, systemic vasoconstriction, and venous return, to be able to maintain blood pressure (BP) and an adequate cerebral blood flow (3,4). In Fontan patients, many of these compensatory mechanisms have been described to be impaired, such as the cardiac autonomic nervous system activity (ANS), with decreased vagal- and increased sympathetic activity, and arterial vascular distensibility (3-7). However, how Fontan patients respond to orthostatic stress, and if it will lead to circulatory impairment, remains incompletely understood. Thus far, the few small previous studies did not investigate cardiac autonomic and hemodynamic response simultaneously and studies that did investigate hemodynamic postural response have even shown differing results (8-11).

In addition, orthostatic stress testing can not only be used to unravel possible mechanisms of circulatory disorders, but can also be used to test the influence of various drugs on the circulatory system, such as angiotensin converting enzyme (ACE) inhibitors. ACE inhibitors are often prescribed in Fontan patients, despite the fact that their efficacy is controversial in these patients (12, 13). In a biventricular circulation the use of ACE inhibitors may lead to orthostatic hypotension (14, 15) and in Fontan patients, with their critical fluid balance, these circulatory effects may be even more deleterious. However, the effect of ACE inhibitors on the orthostatic response in Fontan patients has not yet been investigated.

In the present study, we aimed to unravel the adaptation to orthostatic stress in paediatric Fontan patients by non-invasively examining the cardiovascular and autonomic response to head-up tilt testing (HUTT). In addition, we investigated the effect of enalapril treatment on the response to orthostatic stress in Fontan patients.

Materials and methods

Study population

Fontan patients between 8-18 years old, who were operated at the Leiden University Medical Centre were recruited from July 2017-October 2019. We included patients palliated with an extracardiac conduit and who had moderate-good systolic ventricular function to study a homogenous group. Patients with pre-existent ACE-inhibitors use and those who had difficulties to follow instructions were excluded from the study. Healthy children of similar age were recruited through advertisements at local schools to serve as controls. Written informed consent was obtained from all participants or their parents or guardians. The Medical Ethics Committee of the Leiden University Medical Centre approved the study protocol.

Study design

A combination of a cross-sectional and prospective intervention study was performed.

To test the reaction to orthostatic stress between the different groups, parameters were measured during supine rest and during HUTT. Therefore, in this study we investigated the cardiac ANS activity for the autonomic response, heart rate, BP and cardiac index for the hemodynamic response, peak hepatic vein flow and IVC collapsibility index for an estimation of change in systemic venous return and central venous pressure, and aortic stiffness parameters for the vascular response. Cerebral blood flow was measured as an end result. Furthermore, since respiratory parameters, including respiration rate, end-tidal CO2 (etCO2) and saturation, can influence or be affected by hemodynamic and ANS parameters during HUTT, we also evaluated the response of the respiratory parameters during this study.

At start of the study all participants were placed on a tilt table in supine position to first measure baseline parameters. After baseline measurements and at least 10 minutes of supine rest, a 60^{O} HUTT on a mechanical tilt table with safety belts was performed. Tilt test could prematurely be terminated in case of imminent syncope or at patients request. All measurements performed in supine position were repeated

after 3 minutes in the head-up tilt position as the acute phase has then passed and the parameters of interest have stabilized to the new hemodynamic situation.

After the first study day, Fontan patients started with three-months of oral enalapril treatment at a dosage of 5mg/day. Dosage was further titrated over a period of 2-3 weeks, according to BP measurements, to the target dose of 0.5mg/kg/day or a maximum of 20mg/day. If patients experienced side effects or if the systolic BP fell more than 20%, the dosage was lowered. After three months of treatment the HUTT with all measurements was repeated.

Echocardiographic assessment (for cardiac index and systemic venous parameters), BP and arterial stiffness measurements were performed once in each position. ANS activity, cerebral blood flow, $etCO_2$, saturation and respiration rate were continuously monitored throughout the complete test; final values were obtained by averaging a period of 4 minutes in both positions. In the head-up tilt position the period 3-7 minutes after the start of tilt was taken.

Echocardiography

Transthoracic echocardiography was performed on a Vivid S6/S60 (GE healthcare, Norway). Images were analysed offline using EchoPac (version 203, GE healthcare). Pulse wave Doppler recordings across the (neo)aortic valve were performed to assess aortic velocity time integral from which, by using heart rate and (neo)aortic annulus diameter, cardiac index was calculated as follows:

$$\text{cardiac index} = \frac{\text{heart rate} * \left(\left(\pi * \left(\frac{\text{aortic annulus}}{2}\right)^2\right) * \text{velocity time intergal}\right)}{\text{body surface area}}$$

In addition, Doppler recordings of the hepatic vein were performed to assess peak antegrade flow. Furthermore, the maximum and minimum diameter of the inferior vena cava (IVC) was measured by M-mode during a sniff-test from which we calculated the proportional change, the IVC collapsibility index. Averages of three measurements of each variable were used for analysis, if applicable.

Arterial stiffness and blood pressure

Arterial stiffness, assessed by aortic pulse wave velocity (PWVao) and augmentation index (AIXao), and BP were measured using an oscillometric arteriograph device with the cuff on the left arm (Tensiomed, Hungary) (16). The arteriograph software determines measurement accuracy with a standard deviation of the PWVao, based on at least two consecutive pulse waves. However, as children have smaller pulse waves and tilt leads to even smaller pulse waves, we checked each pulse wave to

decide whether the result of the subsequent analysis could be accepted. If not, we analysed the correct pulse waves separately and used the average of at least two pulse waves for analysis when the standard deviation of the PWVao <1.0m/s.

We initially recorded finger plethysmography (Finometer) data, but preliminary analysis revealed that the Modelflow method was not suitable to analyse the altered circulation in Fontan patients. These data will not be discussed further.

Arterial cerebral blood flow

Mean velocity of both cerebri media arteries was assessed by transcranial Doppler sonography using a non-imaging doppler-Box with DWL Doppler system and QL software version 3.3 (Compumedics Germany, supplied by VCM medical, Netherlands) and two 2MHz probes attached to an adjusted Marc 500 headframe (Spencer Technology, America). For analysis, the artery with the highest mean velocity was used.

Capnography and pulse oximetry

To measure blood oxygen saturation and etCO_2, we used a Nihon Kohden (Japan) finger-clip pulse oximeter and a capONE mainstream CO_2 sensor with a nasal/oral adapter.

Cardiac autonomous nerve system activity and respiration

To measure cardiac ANS activity by heart rate variability parameters and measure respiration, impedance- and electrocardiogram registration was done using the VU-ambulatory monitoring system (VU-AMS; VU university, Netherlands, 5fs version). The VU-Data Analysis and Management Software (VU-DAMS, VU University) were used as described in previously published methods (17). Before analysis, artefacts and ectopic beats were removed. Pre-ejection period (PEP), partly reflecting sympathetic inotropic effect (18), and respiratory sinus arrhythmia (RSA), mainly reflecting parasympathetic chronotropic effects (19), were calculated as described in previously published methods (17). We additionally calculated the standard deviation of the inter-beat interval of normal sinus beats (SDNN) and the root mean square of successive differences between normal sinus beats (RMSSD), both reflecting parasympathetic activity on short-term recordings (20). Furthermore, using the Fast Fourier Transformation we assessed low-frequency (LF;0.04-0.15Hz) and high-frequency (HF;0.15-0.4Hz) power and additionally calculated the LF:HF ratio, a disputed but frequently used measure to reflect sympathicovagal balance (21).

Both HR and respiration may affect cardiac ANS activity. It remains a subject of debate whether it is necessary to correct for these parameters. Some state HR adjustment might remove possible important variance of outcomes related to autonomic control (19). Therefore, we have chosen to report HR and respiration in parallel with the heart rate variability parameters over adjustment procedures (19).

Statistical analysis

Data analysis was performed using SPSS statistics (IBM, version 25). A *P*-value of 0.05 or less was considered as statistically significant. In order to perform reliable inference in the small study group, non-parametric tests were used for all comparisons. Categorical data are reported as numbers with percentages. Continuous data are presented as median with first to third quartile [Q1-Q3]. Comparison of baseline measurements during supine rest and percentage change of parameters during HUTT between Fontan patients and controls were performed with the Mann-Whitney *U* test. Furthermore, we compared the parameters of supine rest and HUTT within each group of subjects, Fontan patients and healthy controls, with the Wilcoxon Signed-Rank Test. The effects of enalapril treatment on baseline supine rest parameters and percentage change during HUTT were evaluated by using the Wilcoxon Signed-Rank Test as well.

Results

From 74 eligible Fontan patients for the study, 36 agreed to participate (49%). Thirty-five healthy controls were recruited. As one patient and one control withdrew before HUTT, 35 Fontan patients (median age 14.0 years) and 34 controls (median age 12.8 years) were analysed (Figure 1). Patients who participated did not differ from those who did not in terms of age (median of 14.0 years [12.6-16.7] for participants vs. 13.0 years [11.7-16.0] for non-participants; *P* = 0.420) and morphology of the main single-ventricle (P = 0.303). Fontan patients did not differ from healthy controls in terms of baseline characteristics (Table 1). Twenty-one Fontan patients had a left (60%), 11 a right (31%) and 3 an indifferent dominant ventricle (9%). Mean age at Fontan operation was 3.2 years. A patent fenestration was present in one patient and one other had a pacemaker (dual chamber pacing; DDD). Fontan patients had a low plasma N-terminal pro brain natriuretic peptide (median 79ng/L). Although all Fontan patients had a subjective moderate to good systolic ventricular function and a comparable global longitudinal strain compared to healthy controls on echocardiography, Tissue Doppler imaging showed lower systolic velocities in Fontan patients compared to controls.

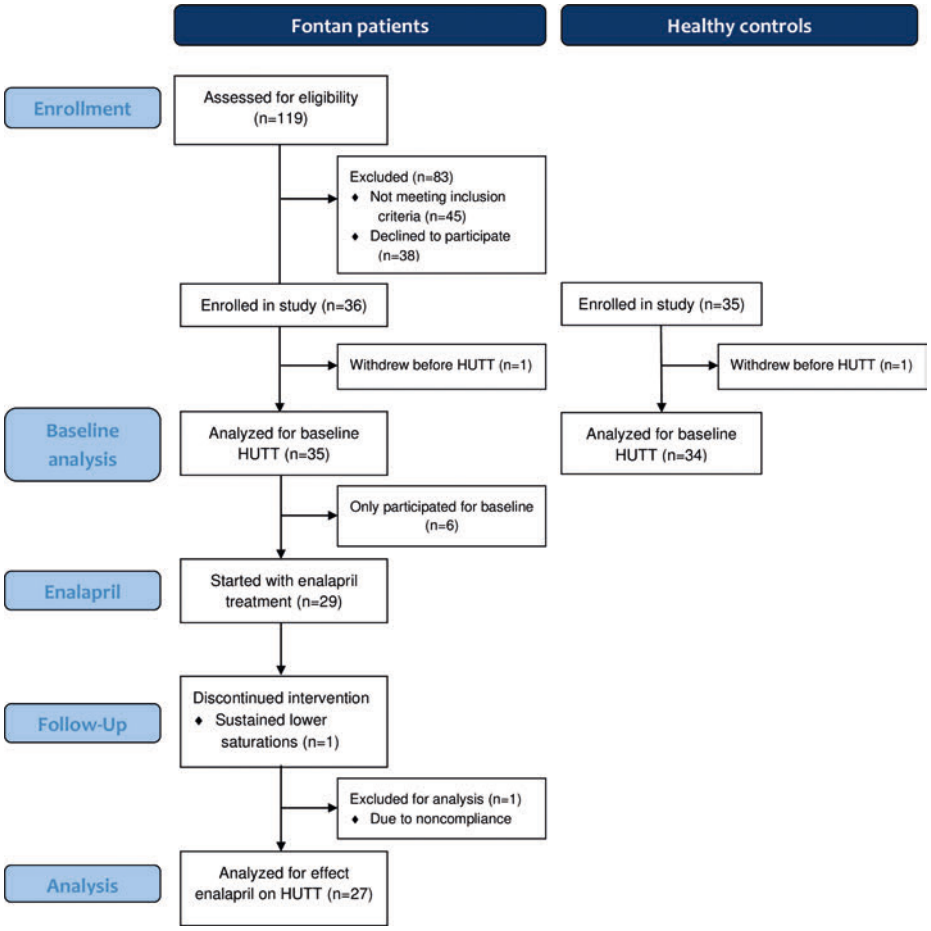

Figure 1. Flow Diagram of the study; HUTT = Head-up tilt testing.

Table 1. Patient Characteristics

Characteristics	Fontan patients (N=35)	Controls (N=34)	*P*-value
Age (years)	14.0 [12.6-16.7]	12.8 [11.0-15.7]	0.193
Males (N,%)	23 (65.7)	17 (50.0)	0.186
Height (cm)	163.9 [153.6-173.0]	160.9 [148.9-170.0]	0.421
Weight (kg)	51.2 [42.5-60.0]	45.4 [36.4-56.2]	0.126
BSA (m^2)	1.5 [1.38-1.72]	1.45 (0.3)	0.121
Diagnosis (N,%)			
Tricuspid atresia	9 (25.7)		
Pulmonary atresia	2 (5.7)		
Double inlet left ventricle	5 (14.3)		
Double outlet right ventricle	0 (0.0)		
Hypoplastic left heart syndrome	8 (22.9)		
Unbalanced atrioventricular septum defect	4 (11.4)		
Other	7 (20.0)		
Main ventricle (N;%)			
Left	21 (60.0)		
Right	11 (31.4)		
Indifferent	3 (8.6)		
Age at Glenn operation (years)	0.50 [0.38-0.76]		
Age at Fontan operation (years)	3.1 [2.8-3.6]		
Patent Fenestration (N,%)	1 (2.9)		
Pacemaker (N,%)	1 (2.9)		
NT-pro BNP (ng/L)	79.3 [45.5-136.1]		
Systolic ventricular function			
Global longitudinal strain (%)	15.2 [12.6-17.5]	16.3 [14.4-18.1]	0.056
TDI septal S' (m/s)	0.043 [0.03-0.05]	0.080 [0.07-0.08]	<0.001
TDI lateral free wall systemic ventricle S' (m/s)	0.060 [0.05-0.07]	0.107 [0.09-0.12]	<0.001
AV-valve regurgitation (N,%)			
No	16 (45.7)		
Mild	13 (37.1)		
Moderate	6 (17.1)		
Severe	0 (0.0)		
(Neo)Aortic regurgitation (N,%)			
No	30 (85.7)		
Mild	2 (5.7)		
Moderate	3 (8.6)		
Severe	0 (0.0)		
Cardiac medications (N, %)			
Acetylsalicylic acid	33 (94.3)		
Coumarin derivative	2 (5.7)		
β-blocker	1 (2.9)		
Diuretics	1 (2.9)		

Data expressed as n (%), mean (±SD), and median [Q1-Q3].

BSA= body surface area; NT-pro BNP= N-terminal pro brain natriuretic peptide; S'= peak systolic TDI velocity, TDI= Tissue Doppler imaging.

During HUTT none of the Fontan patients had imminent syncope while 3 healthy controls (9%) did develop complaints. One control was excluded for analysis as the HUTT had to be stopped at 5 minutes from start HUTT and the other two were not excluded as they developed complaints after all measurements were finished. Cardiac index was calculated for 21 patients, as the aortic annulus could not be assessed in 14 patients. Due to an inadequate echo window, cerebral blood flow could be determined in 19 patients and 27 controls. Furthermore, seven patients were excluded from analysis of cardiac ANS activity due to atrial ectopy, nodal rhythm or pacemaker activity.

Figures 2-5 and Table 2 show the results of the supine and HUTT measurements in Fontan patients and healthy controls. At supine rest, patients had a similar cardiac index, but a lower cerebral blood flow and IVC collapsibility index compared to controls. Furthermore, both PWVao and augmentation index of the aorta were increased and $etCO_2$ was decreased in the Fontan patients at rest. Although, parasympathetic activity was comparable between patients and controls at rest, PEP was significantly longer in Fontan patients, whereas the LF:HF ratio was higher.

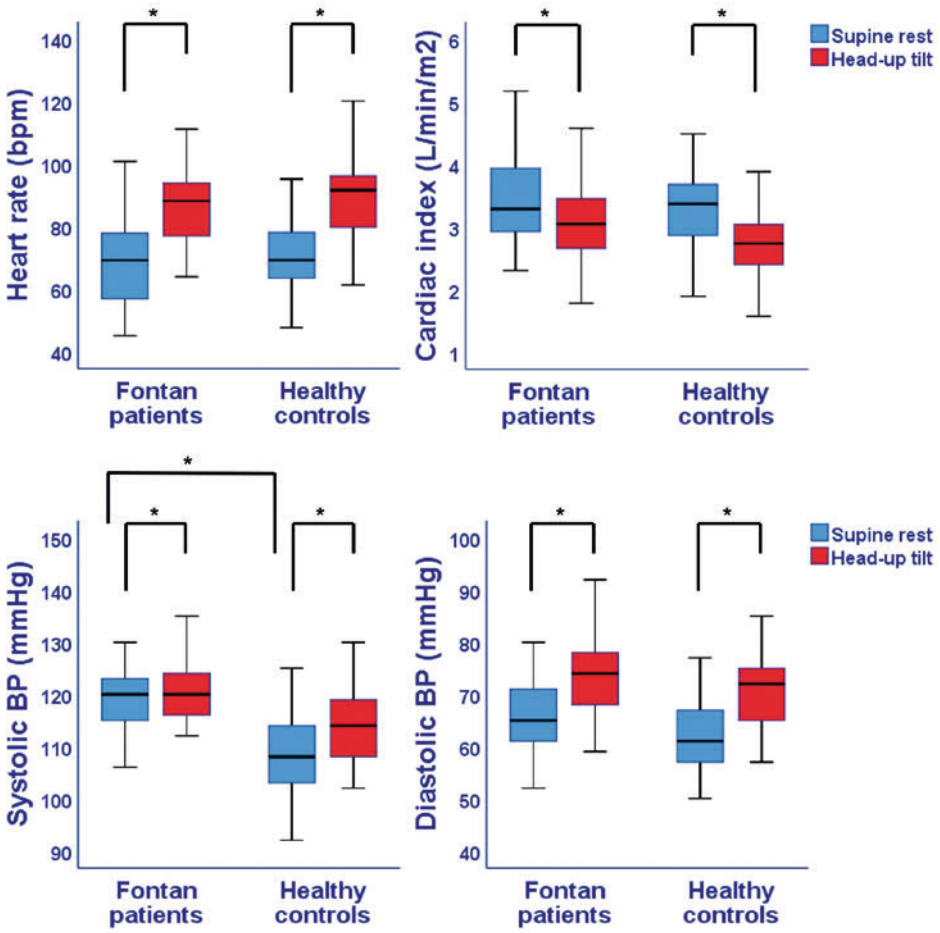

Figure 2. Hemodynamic parameters during supine rest and head-up tilt in Fontan patients and healthy controls.

BP=Blood pressure; CI=Cardiac index; P-value: * <0.05 for difference in supine rest between Fontan patients and controls as well as difference between supine rest and head-up tilt within Fontan patients or healthy controls.

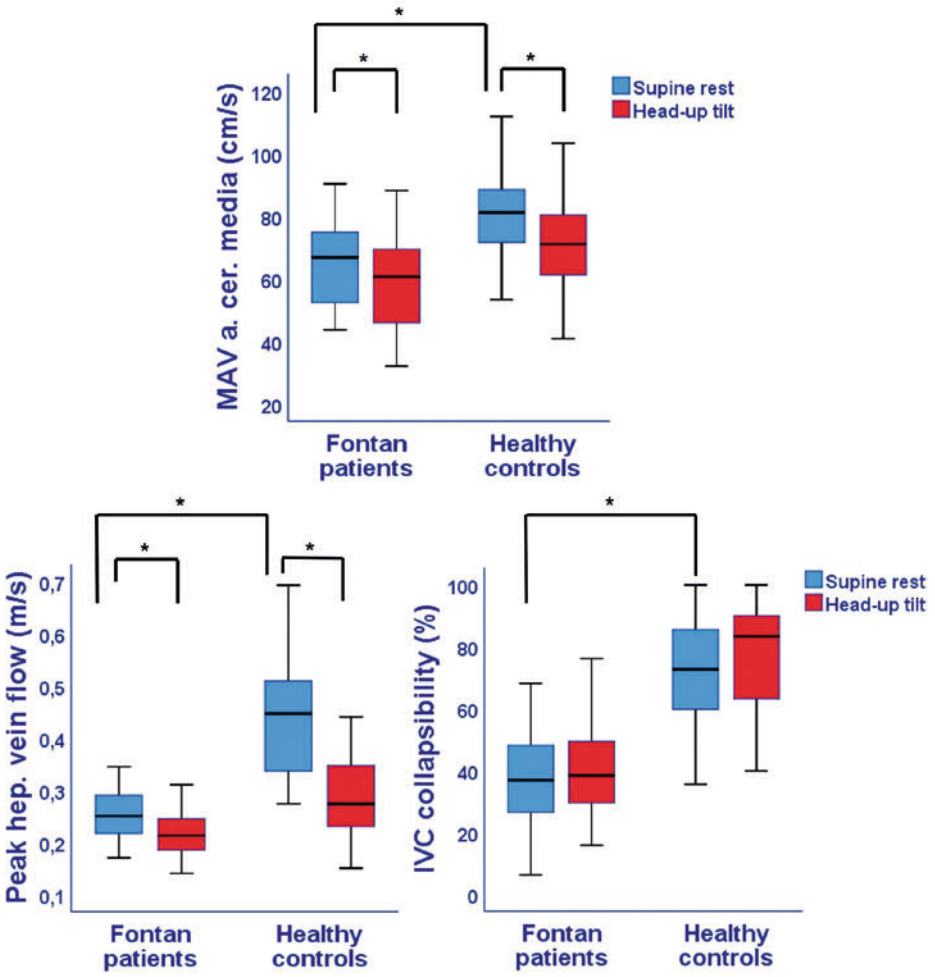

Figure 3. Mean arterial cerebral blood flow, peak hepatic vein flow and collapsibility index of the inferior vena cava in Fontan patients and healthy controls.

IVC= Inferior vena cava; MAV=mean arterial velocity; P-value: * <0.05 for difference in supine rest between Fontan patients and controls as well as difference between supine rest and head-up tilt within Fontan patients or healthy controls.

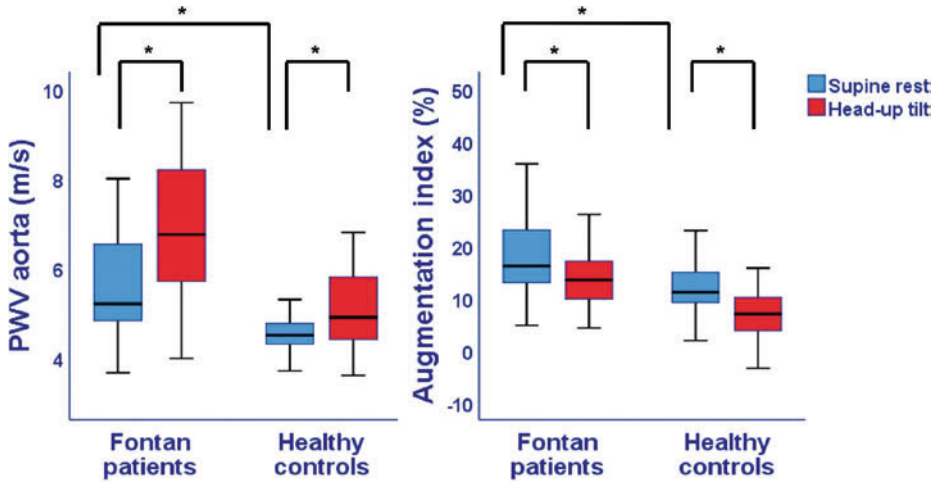

Figure 4. Aortic stiffness parameters during supine rest and head-up tilt in Fontan patients and healthy controls.

PWV=Pulse wave velocity; P-value: * <0.05 for difference in supine rest between Fontan patients and controls as well as difference between supine rest and head-up tilt within Fontan patients or healthy controls.

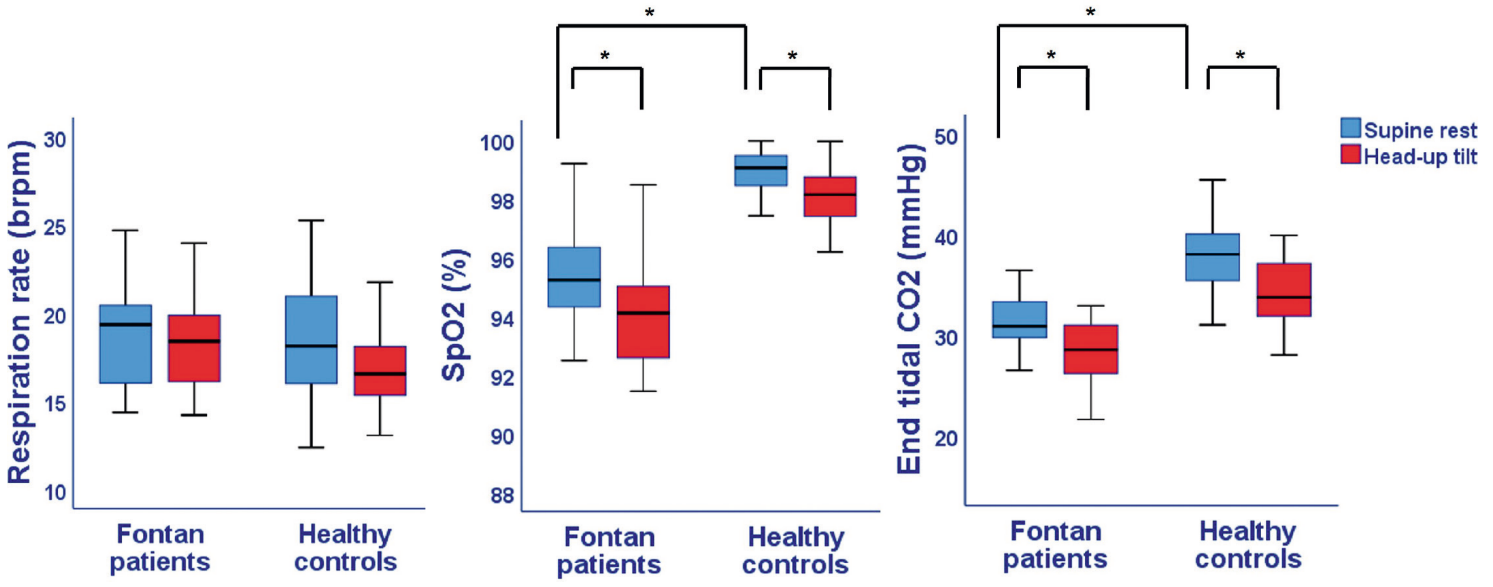

Figure 5. Respiration parameters during supine rest and head-up tilt in Fontan patients and healthy controls.

brpm= breaths per minute; SpO2=saturation; P-value: * <0.05 for difference in supine rest between Fontan patients and controls as well as difference between supine rest and head-up tilt within Fontan patients or healthy controls.

Table 2. Cardiac autonomic nervous system activity parameters during supine and head up tilt in Fontan patients before enalapril treatment and at follow-up

	Fontan patients		Healthy controls	
	Supine rest	**Head-up tilt**	**Supine rest**	**Head-up tilt**
RSA (ms)	70.0 [27.4-112.0]	35.3 [12.1-59.6] ǂǂǂ	84.5 [55.8-119.9]	47.7 [36.6-58.5] ǂǂǂ
RMSSD (ms)	60.6 [20.8-107.2]	25.3 [9.0-35.0] ǂǂǂ	62.5 [49.8-103.1]	30.0 [22.7-36.6] ǂǂǂ
SDNN (ms)	79.9 [28.0-126.1]	40.0 [19.2-54.5] ǂǂǂ	70.7 [55.1-97.6]	56.8 [44.8-70.6] ǂǂ
LF (ms2)	1259.5 [67-4555]	278.3 [67-826] ǂǂ	1229.2 [570-1788]	943.1 [585-1288]
HF (ms2)	944.1 [32-2467]	215.8 [19-462] ǂǂǂ	1234.9 [509-3045]	343.0 [214-546] ǂǂǂ
LF:HF	1.46 [0.8-2.4]	1.84 [1.3-3.3]	0.76 [0.4-1.4] *	2.62 [1.5-4.5] ǂǂǂ
PEP (ms)	124.2 [109.7-134.4]	133.3 [116.2-151.8] ǂǂǂ	82.2 [66.1-93.3]***	108.3 [103.0-119.3] ǂǂǂ

Data expressed as mean (±SD), and median [Q1-Q3].

P-value: * <0.05, *** <0.001 for differences in supine rest between pre-enalapril and follow-up

P-value: ǂǂ <0.01, ǂǂǂ <0.001 for difference between supine rest and head-up tilt parameters in each group of subjects (pre-enalapril and follow-up)

HF = high frequency power spectral values; LF= low frequency power spectral values; LF:HF= low frequency/high frequency ratio; PEP= pre-ejection period; RMSSD= root mean square of successive differences between normal sinus beats; RSA= respiratory sinus arrhythmia; SDNN= standard deviation of the inter-beat interval of normal sinus beats.

During HUTT, most of the parameters increased or decreased with a similar magnitude in both groups (Table 3). However, the percentage increase of systolic BP (P = 0.019) and decrease of hepatic venous flow (P = 0.004) was lower in Fontan patients. The respiration rate decreased slightly more during HUTT in heathy controls (P = 0.013), while saturation decreased more in Fontan patients (P=0.010). Furthermore, the increase of PEP during HUTT in Fontan patients was lower compared to controls (P < 0.001), whereas the LF:HF ratio did not change in the patients (1.5 at rest vs 1.8 at tilt, P = 0.23), while in controls it increased (0.8 at rest versus 2.6 at tilt, P < 0.001).

Twenty-nine Fontan patients started with enalapril treatment (Figure 1). One patient withdrew as a result of sustained lower saturations and one patient was excluded for further analysis due to non-compliance. Ten patients could not reach the targeted dosage due to a decrease in systolic blood pressure (n = 6, 22%) or other adverse events, consisting of syncope (n = 2), dizziness (n = 1) and palpitations (n = 1). Furthermore, one patient reported a hacking cough after completion of the study which disappeared after discontinuation of enalapril. The remaining 27 patients completed the treatment period with a mean dosage of 0.3±0.1mg/kg/day.

The effects of enalapril treatment on all cardiovascular parameters studied in this study during supine rest are depicted in Table 4. Enalapril treatment lowered mean systolic (P=0.006) and median diastolic BP (P=0.03) at supine rest, however, no other parameters at rest were affected by enalapril. Furthermore, after enalapril treatment AV-valve regurgitation changed in only one patient and improved from moderate to mild.

Table 5 shows the results of percentage change during HUTT on Fontan patients before enalapril treatment and after enalapril treatment. Although most changes induced by HUTT were similar before as well as after enalapril treatment, cardiac index decreased significantly more during tilt with enalapril treatment (P = 0.03). However, the mean cardiac index during tilt after treatment was not significantly lower than during tilt before treatment (3.0/min/m^2 before enalapril treatment versus 3.0 L/min/m^2 at follow-up; P = 0.13).

Table 3. Percentage change of parameters during head-up tilt in Fontan patients and Healthy controls

	Fontan patients	Healthy controls
Haemodynamics		
Heart rate (%)	+22.4 [13.9-44.0]	+30.0 [18.3-40.9]
Systolic BP (%)	+2.5 [-0.8-5.7]	+5.9 [+1.4-9.8] *
Diastolic BP (%)	+13.3 [1.5-21.8]	+14.5 [4.6-21.1]
Cardiac index (%)	-9.7 [-18.3- -4.6]	-18.0 [-24.6- -3.1]
Cerebral blood flow		
MAV art. cerebri media (%)	-6.4 [-14.0- -2.7]	-12.1 [-20.5- -4.0]
Systemic venous parameters		
Peak hepatic vein flow (%)	-14.6 [-28.9-0.0]	-31.3 [-47.0- -16.2] **
IVC collapsibility index (%)	+0.2 [-14.0-9.7]	+9.3 [-7.5-25.3]
Arterial stiffness		
PWVao (%)	+16.3 [13.7-38.6]	+16.1 [8.5-23.7]
AIXao (%)	-4.3 [-10.5- -1.2]	-6.3 [-9.1- -0.9]
Respiration		
Respiration rate (%)	-1.7 [-5.0-3.7]	-7.1 [-11.2- -3.4] *
SpO_2 (%)	-1.8 [-2.5- -0.9]	-0.8 [-1.4- -0.1] *
$EtCO_2$ (%)	-8.5 [-11.3- -5.8]	-7.8 [-12.2- -4.2]
Cardiac ANS activity		
RSA (%)	-44.0 [-62.7- -25.7]	-47.0 [-54.3- -31.8]
RMSSD (%)	-55.5 [-69.4- -27.1]	-52.7 [-68.4- -44.3]
SDNN (%)	-44.5 [-61.1 -22.0]	-18.4 [-35.4- -3.2] **
LF (%)	-44.2 [-82.2- -15.6]	-5.6 [-46.9-53.0] *
HF (%)	-70.1 [-85.1- -24.6]	-71.3 [-81.2- -54.2]
LF:HF (%)	+26.1 [-34.5-158.5]	+228.7 [108.0-433.4] **
PEP (%)	+6.9 [0.3-16.9]	+31.6 [16.0-61.1] ***

Data expressed as median [Q1-Q3].

P-value: * <0.05, ** <0.01, *** <0.001 for difference in percentage change

AIXao= Augmentation index of the aorta; BP= Blood pressure; bpm= beats per minute; $EtCO_2$= End-tidal carbon dioxide; HF = high frequency power spectral values; IVC= Inferior vena cava; LF= low frequency power spectral values; LF:HF= low frequency/high frequency ratio; MAV art. cerebri media= Mean arterial velocity arteria cerebri media; PEP= pre-ejection period; PWVao= Pulse wave velocity of the aorta; RMSSD= root mean square of successive differences between normal sinus beats; RSA= respiratory sinus arrhythmia; SDNN= standard deviation of the inter-beat interval of normal sinus beats; SpO_2= oxygen saturation.

Table 4. Comparison of baseline measurements during supine rest between Fontan patients before enalapril treatment and at follow-up

	Pre-enalapril	Follow-up
Haemodynamics		
Heart rate (bpm)	63.1 [54.1-76.6]	63.3 [53.7-82.1]
Systolic BP (mmHg)	120.0 [111.5-124.0]	112.0 [109.0-121.5] **
Diastolic BP (mmHg)	65.0 [60.5-70.5]	61.0 [59.0-65.5] *
Cardiac index (L/min/m^2)	3.2 [2.9-3.5]	3.3 [2.9-4.1]
Cerebral blood flow		
MAV art. cerebri media (cm/s)	59.7 [50.8-69.0]	56.9 [48.8-67.6]
Systemic venous parameters		
Peak hepatic vein flow (m/s)	0.26 [0.21-0.30]	0.28 [0.23-0.34]
IVC collapsibility index (%)	38.4 [29.6-56.2]	38.1 [26.4-46.5]
Arterial stiffness		
PWVao (m/s)	5.2 [4.9-6.2]	5.3 [4.3-5.8]
AIXao (%)	17.2 [13.4-23.9]	18.7 [13.8-21.6]
Respiration		
Respiration rate (brpm)	19.4 [16.7-21.5]	19.3 [16.6-19.9]
SpO_2 (%)	95.2 [94.4-96.4]	95.1 [94.2-96.0]
$EtCO_2$ (mmHg)	30.6 [29.5-32.9]	30.4 [28.2-32.3]
Cardiac ANS activity		
RSA (ms)	81.5 [42.2-111.1]	66.7 [35.7-88.2]
RMSSD (ms)	78.0 [40.8-110.6]	68.8 [30.0-94.8]
SDNN (ms)	86.9 [46.4-132.4]	79.2 [43.0-100.7]
LF (ms^2)	1464.0 [339-4648]	1495.3 [336-2835]
HF (ms^2)	1062.5 [209-2690]	1084.0 [221-2150]
LF:HF	1.6 [0.8-2.5]	1.4 [0.9-2.3]
PEP (ms)	124.9 [110.9-137.8]	131.3 [113.5-139.4]

Data expressed as median [Q1-Q3].
P-value: * <0.05, ** <0.01 for difference in percentage change
For previously used abbreviations see Table 3; brpm= breaths per minute

Table 5. Percentage change of parameters during head-up tilt in Fontan patients before enalapril treatment and at follow-up

	Pre-enalapril	Follow-up
Haemodynamics		
Heart rate (%)	+25.7 [18.9-44.2]	+27.0 [17.9-38.9]
Systolic BP (%)	+3.2 [0.0-7.3]	+3.3 [-3.1-10.5]
Diastolic BP (%)	+17.2 [4.0-24.0]	+11.1 [8.9-21.5]
Cardiac index (%)	-6.7 [18.2-2.4]	-20.5 [-27.6- -6.8] *
Cerebral flow		
MAV art. cerebri media (%)	-5.1 [21.8- 1.2]	-2.4 [-18.0-3.8]
Systemic venous parameters		
Peak hepatic vein flow (%)	-21.0 [-29.3- -3.6]	-13.6 [-34.9-1.1]
IVC collapsibility index (%)	+0.2 [-14.6-24.2]	+5.8 [-6.2-24.1]
Arterial stiffness		
PWVao (%)	+21.2 [13.8-38.9]	+24.4 [15.5-36.8]
AIXao (%)	-4.3 [-10.5- -1.2]	-2.9 [-10.0- -0.5]
Respiration		
Respiration rate (%)	-3.0 [-6.4-2.0]	-3.2 [-12.0-3.4]
SpO_2 (%)	-2.0 [-2.5- -0.9]	-1.5 [-2.9- -0.7]
$EtCO_2$ (%)	-7.3 [-10.8- -5.6]	-10.1 [-13.4- -5.8]
Cardiac ANS activity		
RSA (%)	-44.0 [-65.5- -12.3]	-34.3 [-61.9- -8.6]
RMSSD (%)	-57.6 [-76.0- -28.8]	-52.2 [-71.1- -27.4]
SDNN (%)	-47.6 [-64.5- -25.5]	-27.1 [-61.6- -5.8]
LF (%)	-49.5 [-89.6- -18.0]	-62.7 [-88.0- -25.0]
HF (%)	-70.2 [-89.6- -22.2]	-73.4 [-86.6- -51.6]
LF:HF (%)	+13.6 [-37.5-130.7]	+60.9 [-18.9-181.7]
PEP (%)	+6.9 [-0.2-19.5]	+5.6 [-1.3-20.0]

Data expressed as median [Q1-Q3].
P-value: * <0.05 for difference in percentage change
For used abbreviations see Table 3.

Discussion

Our study demonstrated that Fontan patients responded adequately to orthostatic stress, with normal response to HUTT for most parameters, including BP, cardiac index, aortic stiffness and vagal activity. Furthermore, enalapril treatment resulted in a lower BP, but did not hamper the response to orthostatic stress.

HUTT is generally used to test orthostatic tolerance of syncope patients (22). During HUTT, redistribution of blood volume to the lower body occurs which results in unloading of the baroreceptors (23). This activates the sympathetic nervous system and causes concurrent vagal withdrawal, to increase heart rate, systemic vasoconstriction and venous return and maintain BP and cerebral blood flow (23, 24). When these compensating mechanisms are impaired, arterial pressure will decrease, resulting in a decrease of BP and, when cerebral circulatory mechanism also fail, insufficient cerebral blood flow to maintain consciousness (24).

The cardiocirculatory resting condition from Fontan patients differed from that of healthy controls, so a different response to HUTT might be expected. At supine rest, patients had a lower IVC collapsibility index, which most likely is the result of venous pooling, necessary to increase central venous pressure to an extent that it may function as the driving force to push blood through the pulmonary circulation. An increased arterial stiffness at rest has previously been described in Fontan patients and may reflect vascular dysfunction and increased sympathetic activity (10). The lower cerebral blood flow in Fontan patients at rest probably is the result of increased aortic stiffness and central venous pressure combined with cerebral vasoconstriction induced by the lower $etCO_2$ (25, 26). The origin of decreased $etCO_2$ is as yet unclear, although thoracic impedance is described to be reduced after thoracotomy, influencing pulmonary efficiency (27).

An adequate response to HUTT necessitates integrated responses by the autonomic nervous system, vascular function, regulation of cerebral blood flow. Previous studies have shown a reduced baroreflex sensitivity and markedly impaired cardiac ANS activity in Fontan patients, reflected in an increased sympathetic and decreased parasympathetic activity, however, the majority of these studies were performed in adult patients (3, 5, 10, 11). In contrast to these studies, we showed that parasympathetic activity was not impaired during resting conditions and showed a normal response upon HUTT. The LF:HF ratio did not increase further upon HUTT but was already increased in supine position which could indicate that sympathetic activity was already at the upper end in resting condition (28). However, the LF:HF

ratio as sympathicovagal balance is controversial as LF reflects both para- and sympathetic activity. Furthermore, PEP, another parameter of sympathetic activity was increased as well in Fontan patients, suggesting a decreased sympathetic activity. However, PEP is also influenced by changes in pre- and afterload and is therefore a less reliable parameter of sympathetic activity in Fontan patients and also when used during postural changes. During HUTT, PEP increased in both groups while a decrease was expected due to the known increase in sympathetic activity. However, this response of PEP to postural change is consistent with previous studies and is due to a decrease in preload and an increase in afterload (29, 30). The decreased preload leads to a longer PEP via a decrease of end-diastolic volume which results in a decrease of strength of cardiac contraction mediated by the Frank-Starling mechanism. The increased afterload prolongs the PEP, which ends at aortic valve opening, because it takes longer for the ventricular pressure to build up to the level of the aortic pressure.

Although sympathetic activity could not be reliably measured, our Fontan patients showed an adequate HR response to orthostasis with a similar increase in heart rate as in healthy controls indicating an intact cardiac autonomic regulation. An adequate vagal response to orthostatic stress in Fontan patients conflicts with one previous study investigating parasympathetic activity during standing in 8 adult patients showing a blunted parasympathetic response (11). Age may have influenced this difference in results, as impaired autonomic nervous system activity has been demonstrated in mainly adult Fontan patients.

Aortic stiffness increased normally during tilt in our patients, despite it was already increased at rest, as illustrated by an increase in PWVao, a direct measure of stiffness unaffected by heart rate and stroke volume as is known from augmentation index (31). The increase in stiffness during orthostatic stress confirms the result of two previous small studies, with respectively 8 and 18 adult Fontan patients, where an increase in systemic vascular resistance and plasma norepinephrine were found (8, 10).

Cerebral blood flow decreased in both groups in reaction to tilt, but sufficient flow was maintained in all Fontan patients since none of the patients developed syncope, even despite a lower cerebral blood flow in Fontan patients at rest. Cerebral blood flow during tilt is not only directly affected by a lowering of the cardiac index, but a decrease in cardiac index also causes a decrease in $etCO_2$, resulting in cerebral vasoconstriction (32, 33).

IVC collapsibility index in Fontan patients remained low during tilt. Patients seem to adapt to the Fontan circulation to prevent venous pooling while standing and avoid a deleterious drop in central venous pressure and cardiac index. As observed by a study of Krishnan et al. (9), venous pooling might be prevented by an increase in venous tone and peripheral arterial resistance, especially in the legs, in Fontan patients which results in decreased venous capacitance and compliance. The integrity of the regulating mechanisms is further underscored by a similar change in cardiac index in Fontan patients as compared to controls. In older Fontan patients a larger decrease in cardiac output upon tilt has been demonstrated, suggesting that the adaptive mechanisms may be falling short during long-term follow-up (10).

A further challenge for adaptive mechanisms to tilting occur after enalapril treatment. Enalapril treatment resulted in a lower BP at rest but did not lead to orthostatic hypotension. Although orthostatic hypotension has been described as a side effect of ACE inhibitors, it usually occurs only as a first-dose phenomenon or during treatment in conjunction of dehydration or other hypotensive drugs (14, 15). Furthermore, the percentage decrease of cardiac index was higher after treatment with enalapril, however the cardiac index during tilt was not significantly lower as compared with values before treatment. Previous studies performed in normal male subjects and hypertensive patients also showed that ACE inhibition did not result in a lower cardiac index during HUTT (34, 35). The vasodilator effect of ACE inhibitors is apparently minimal, otherwise vasodilation would have resulted in increased venous pooling and respectively a decreased central venous pressure and cardiac index, which could be particularly detrimental in Fontan patients.

In clinical perspective, this study demonstrated that well-functioning asymptomatic patients at a relatively early stage after the completion of the complex Fontan surgery have a normal response to orthostatic stress comparable to healthy controls. However, as it is known that the Fontan circulation will deteriorate on the long term, ultimately resulting in Fontan failure, Fontan patients may respond abnormally at a later stage. The response to orthostatic stress testing may be useful to distinguish underlying mechanisms of Fontan failure during follow-up. In addition, it may help to evaluate the effects of heart failure medication in patients with an impaired Fontan circulation.

This study had some limitations. We included paediatric patients with an extracardiac conduit and moderate-good systolic ventricular function and therefore created a selection bias. However, our aim was to investigate a more homogenous group of asymptomatic patients to comprehend the adaptive mechanisms of a

well-functioning Fontan circulation which may form a basis for further research to elucidate the development of Fontan failure. Our results are limited to the investigated population and could not be generalized. Furthermore, the non-invasive study set-up has its limitations as, for example, venous pressure could not be determined and might have completed the understanding of the adaptive mechanisms. Finally, sympathetic activity could not be directly determined from our measurements and more invasive measurements of sympathetic activity are necessary in future research.

Conclusion

Paediatric patients with a Fontan circulation can respond adequately to orthostatic stress and maintain adequate BP, cardiac output and cerebral blood flow. We found no evidence of autonomic circulatory impairment. Enalapril treatment resulted in a lower BP at rest but did not alter the response to orthostatic stress and did not lead to orthostatic hypotension or a lower cardiac index during HUTT.

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Supporting information

S1 Checklist/CONSORT Flow Diagram (PDF):
https://doi.org/10.1371/journal.pone.0273940.s001

S1 Dataset (XLSX): https://doi.org/10.1371/journal.pone.0273940.s002

S1 Protocol sAFE trial (PDF)l: https://doi.org/10.1371/journal.pone.0273940.s003

Chapter 7

3-month Enalapril Treatment in Paediatric Fontan Patients with Moderate to Good Systolic Ventricular Function

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Abstract

Many Fontan patients with and without systolic ventricular dysfunction are being treated with angiotensin-converting enzyme (ACE) inhibitors, despite its effectiveness remaining unclear. In the present study, we evaluated the short-term effect of enalapril on exercise capacity, vascular and ventricular function in paediatric Fontan patients with moderate-good systolic ventricular function. Fontan patients between 8 and 18 years with moderate-good systolic ventricular function and without previous ACE inhibitor treatment were included and were treated with enalapril for 3 months. During the first 2 weeks, the dosage was titrated according to systolic blood pressure (SBP). Exercise tests, ventricular function assessed by echocardiography, arterial stiffness measurements, and plasma levels of N-terminal pro-B–type natriuretic peptide assessed before and after a 3-month enalapril treatment period was compared. A total of 28 Fontan patients (median age 13.9 years, 6 to 15 years after Fontan operation) completed the study with a mean dosage of 0.3 ± 0.1 mg/kg/d. A total of 6 patients (21%) experienced a significant drop in SBP and 6 others (21%) experienced other adverse events. Enalapril treatment lowered the SBP (from 110 to 104 mm Hg, $p = 0.003$) and levels of N-terminal pro-B–type natriuretic peptide (from 80 to 72 ng/L, $p = 0.036$). However, enalapril treatment did not improve exercise capacity, ventricular function, or arterial stiffness. In conclusion, short-term ACE inhibition has no beneficial effect in Fontan patients with moderate-good systolic ventricular function.

Introduction

Although survival of Fontan patients has improved, life expectancy is still less than normal, and many patients suffer from morbidities. Exercise performance, diastolic and systolic ventricular function are already reduced at a young age (1, 2). Because severe diastolic and systolic dysfunction may not be present yet, both functions deteriorate over time in these patients, which may eventually result in heart failure (1, 2). In patients with a biventricular heart circulation, angiotensin-converting enzyme (ACE) inhibitors have become the cornerstone of systolic heart failure treatment as they have been shown to improve exercise performance, diastolic and systolic ventricular function and decrease systemic vascular resistance in adult and paediatric patients with mild to severe systolic heart failure (3-10). Because its effectiveness in biventricular heart patients, many Fontan patients are currently treated with ACE inhibitors, including those without overt systolic ventricular dysfunction, despite the lack of evidence of its efficacy in this patient population (11, 12). Therefore, in this study we evaluated the effect of ACE inhibition in paediatric Fontan patients with moderate-good systolic ventricular function and hypothesized that it may improve exercise performance and ventricular and vascular function. Additionally, adverse events and tolerability were evaluated.

Methods

Fontan patients from 8 to 18 years old who were operated at the Leiden University Medical Centre were recruited from July 2017 to October 2019. Patients with pre-existent ACE inhibitor use and those unable to exercise were excluded. Written informed consent was obtained from all participants or their parents or guardians. The study was approved by the Medical Ethical Committee Leiden-Den Haag-Delft.

For this study patients were treated with enalapril, an ACE inhibitor, for 3 months. This period was chosen as previous studies have shown beneficial effects of ACE inhibitors within 12 weeks of treatment, especially on exercise capacity, the primary end point of this study (3, 5, 13, 14). Initial enalapril dosage was 5 mg/day and was titrated, as tolerated, to the target dose of 0.5 mg/kg/day or a maximum of 20 mg/day. Enalapril dosage was titrated by blood pressure measured weekly for at least 2 weeks after initiation of treatment. If systolic blood pressure (SBP) fell >20%, or if patients experienced side effects, the dosage was lowered. Renal function (urea and creatinine blood levels) was assessed at baseline, after 2 weeks of treatment with the maximal tolerated dosage, and at the end of the study. At baseline patients were asked if they were familiar with syncope, dizziness, low blood pressure, or if they

experienced other complaints, such as palpitations. During the titration period and after 3 months of treatment, patients were asked about it again. At baseline, and after 3 months of treatment, a cardiopulmonary exercise test, echocardiography, arterial stiffness measurement, and blood sample were performed, as described later.

Exercise testing was performed on an upright bicycle ergometer (Jaeger ER 900; Viasys Healthcare, Höchberg, Germany) with breath-by-breath analysis using a flowmeter (Triple V volume transducer) and computerized gas analyser (Jaeger Oxycon Champion, Viasys Healthcare or Carefusion Vyntus, Vyaire Medical). Starting wattage and workload increment per minute were determined by the age of the patient. Patients were encouraged to exercise until exhaustion. Tests were considered maximally performed when the peak respiratory exchange ratio was ≥1.0. Maximal exercise parameters were only assessed in patients with a maximal test. Peak work rate, heart rate at peak, peak oxygen uptake ($VO_{2\ peak}$), $VO_{2\ peak}$ per heartbeat, the respiratory minute to CO_2 production slope (VE/VCO_2), and the oxygen uptake efficiency slope were derived from the exercise test following previously published methods (15).

Transthoracic echocardiography was performed on a Vivid S6/S60 ultrasound machine (General Electric Healthcare, Norway). Images were stored and analysed offline using EchoPac (version 203, GE Healthcare, Little Chalfont, United Kingdom). Measurements of 3 consecutive cardiac cycles were averaged for analysis. Pulse wave Doppler recordings were performed across the atrioventricular valve to assess early and late diastolic velocities and calculate the ratio of those velocities (E/A). Through Tissue Doppler imaging, myocardial velocity curves from the basal part of the single ventricles' lateral wall and ventricular septum were obtained to assess peak systolic and peak early and late diastolic velocities. Furthermore, the ratio between the pulse wave and tissue Doppler peak early diastolic velocities (E/E') of the lateral wall was calculated. Longitudinal global peak strain, evaluating systolic performance, was obtained from the dominant ventricle using speckle-tracking strain analysis from the 4-chamber apical view as previously described (16). To assess global longitudinal strain, at least 5 of 6 segments had to show acceptable curves. Furthermore, if the ventricular septum defect was larger than 1 segment, the strain was conducted from both lateral walls.

The oscillometric arteriograph device (Tensiomed, Budapest, Hungary) was used to measure pulse wave velocity of the aorta, augmentation index of the aorta, and central SBP. Measurements were performed in a supine position with the cuff on

the left arm. The arteriograph software calculates average values and determines the accuracy of the measurement with an SD. Since automatic calculation was not always possible because of small pulse waves or movement, we analysed each cardiac cycle individually using the software. Measurements were considered valid after a visual check and when a reliable value could be calculated with an SD of the pulse wave velocity of the aorta <1.0 m/s.

From a venous puncture plasma creatinine, urea, and N-terminal pro-B–type natriuretic peptide (NT-pro BNP) levels were assessed.

Data analysis was performed using SPSS Statistics software (Version 25, IBM, New York, United States). Variables were tested for normality with histograms and QQ-plots. Continuous data are reported as mean ± SD or as median with first to third quartile (Q1-Q3) in case of non-normality. Categorical data are presented as a number with percentages. A paired sample t-test or a Wilcoxon signed-rank test for non-normal distributed values were used for comparison between pre-enalapril and follow-up measurements. A p <0.05 was considered significant. The primary end point for this study was exercise performance, and specifically the $VO_{2\ peak}$. Using Cohen's D of 0.49 as effect size, calculated with mean and SD from previously published data (17), an alpha of 0.05% and 80% power, we calculated that 35 subjects would be sufficient to detect a 10% increase in $VO_{2\ peak.}$

Results

A total of 74 Fontan patients were eligible for inclusion of which 36 agreed to participate (49%). Patients who participated did not differ from those who did not in terms of age (median of 14.0 years [12.7 to 16.6] for participants vs. 13.0 years [11.7 to 16.0] for nonparticipants; *P*=0.364) and morphology of the main single-ventricle (p = 0.374). As only 6 patients participated in the baseline measurements, a total of 30 patients were enrolled in this study and started with enalapril treatment. During the study 1 patient withdrew at the request of parents and 1 patient was excluded for further analysis because of medication noncompliance. The remaining 28 patients completed the study of whom baseline characteristics are summarized in Table 1. Initial diagnosis and ventricular morphology differed in the group.

An overview of blood pressure measurements and plasma urea and creatinine levels is shown in Table 2. SBP was significantly lower during the study as compared with baseline. Plasma levels of urea and creatinine did not change significantly. Eleven patients could not reach the targeted dosage because of a decrease in

SBP (n = 6, 21%) or other adverse events, consisting of syncope (n = 2), dizziness (n = 2), and palpitations (n = 1). Furthermore, 1 patient reported a hacking cough after completion of the study which disappeared after discontinuation of enalapril. This means that 21% of the patients experienced adverse events other than the predetermined drop in SBP, which was higher when compared with baseline where 2 patients (7.1%) reported that they were familiar with syncope (n = 2) and palpitations (n = 1). All patients completed the study with a mean dosage of 0.29 ± 0.1 mg/kg/day, dosages ranged from 5 to 20 mg/day.

Table 1. Patient Characteristics of the study population

Characteristics	**n = 28**
Age (years)	13.9 [13.0-16.7]
Males	18 (64%)
Height (cm)	164.1 (12.5)
Weight (kg)	53.3 (12.6)
BSA (m^2)	1.57 (0.2)
Oxygen saturation (%)	95.6 (2.1)
Diagnosis	
Tricuspid atresia	6 (21%)
Pulmonary atresia	1 (4%)
Double inlet left ventricle	4 (14%)
Double outlet right ventricle	1 (4%)
Hypoplastic left heart syndrome	8 (29%)
Unbalanced atrioventricular septal defect	4 (14%)
Other	4 (14%)
Main ventricle	
Left	13 (46%)
Right	12 (43%)
Undifferentiated	3 (11%)
Age at Glenn operation (years)	0.5 [0.37-0.75]
Age at Fontan operation (years)	3.1 (0.6)
Type Fontan tunnel	
TCPC-EC	28 (100%)
Initial Fenestration	26 (92%)
Open	1 (4%)
Closed (naturally or by device)	25 (96%)

Data expressed as n (%), mean (±SD), and median [Q1-Q3].
BSA = body surface area, TCPC-EC = Total cavopulmonary connection with an extracardiac conduit.

Table 2. Systolic blood pressure and plasma urea and creatinine levels during trial

	Week 1	Week 2	Week 3	Week 5-6	3 Months
	Baseline	Control BP 1	Control BP 2	Control plasma urea and creatinine	Follow-up
Systolic BP (mmHg)	120.4 (9.2)	109.9* (12.5)	111.0** [99-119]		117.7** (10.2)
ΔSBP from start (%)		-8.6 (10.2)	-7.3 (9.2)		
Creatinine (umol/L)	62.5 [56-68]			64.5 [501-73]	63.0 [58-72]
Urea (mmol/L)	5.3 (±1.1)			5.7 (±1.5)	5.2 [4.5-6.38]

*P-value <0.05 for difference between control and follow-up measurement vs pre-enalapril values; **P-value <0.01
Data is presented as mean (± standard deviation) or median [Q1-Q3].
BP = Blood pressure; SBP = Systolic blood pressure.

Table 3. Cardiopulmonary exercise test results

	Pre-enalapril	Enalapril	P-value
Maximal exercise (n=25)			
RER_{peak}	1.13 (0.08)	1.12 (0.07)	0.383
WR_{peak} (watt)	120.4 (36.3)	123.0 (38.8)	0.261
HR_{peak} (bpm)	172.8 [164-184]	173.6 [164-185]	0.637
VO_{2peak} (ml/kg/min)	26.2 (4.7)	26.7 (6.5)	0.691
Peak O_2 pulse (ml/beat)	8.53 (1.6)	8.56 (2.1)	0.900
Submaximal exercise (n=28)			
VE/VCO_2 slope	36.1 [32.6-40.8]	38.1 [34.1-42.5]	0.133
OUES/kg	27.2 (6.0)	26.9 (6.5)	0.706

Data expressed as mean (SD) or median [IQR].
HR_{peak} = maximal heart rate at peak exercise, RER_{peak} = respiratory exchange ratio at peak exercise, OUES = oxygen uptake efficiency slope, Peak O_2 pulse = maximal oxygen uptake per heartbeat, VE/VCO_2 slope = slope of respiratory minute to CO_2 production, VO_{2peak} = peak oxygen uptake, WR_{peak} = maximum work rate achieved.

Results of the exercise tests are shown in Table 3. A total of 3 patients (11%) were not able to achieve a respiratory exchange ratio ≥1.0 and were excluded for comparison of the maximal exercise parameters. The Fontan patients showed on average a low exercise capacity at baseline, reflected by a low VO_{2peak} and high VE/VCO_2 slope. A 3-month treatment with enalapril did not improve any of the exercise parameters.

Table 4 depicts the results of the cardiac and vascular function. On echocardiography, all patients showed a subjective moderate to good systolic ventricular function. At

baseline, Tissue Doppler peak systolic velocities were decreased and global peak longitudinal strain was similar compared with normal controls as was already reported in a previous study.(18) Diastolic ventricular function was decreased at baseline as well, with a low E/A ratio and high E/E' ratio, compared with normal controls from our previous published data. Furthermore, atrioventricular valve regurgitation was moderate in 6 and mild in 11 patients. (Neo)Aortic valve regurgitation was moderate in 3 and mild in 2 patients. All echocardiographic parameters, systolic and diastolic, did not change with enalapril treatment. The level of NT-pro BNP, although already low at baseline, decreased significantly after treatment with enalapril. Central SBP significantly decreased. Although the values of the augmentation index of the aorta tended to decrease, this did not reach the threshold for statistical significance. Pulse wave velocity of the aorta showed no difference either.

Table 4. Comparison of cardiac and vascular function (n =28)

	Baseline	Follow-up	P-value
Heart rate (bpm)	63.8 [60.0-82.3]	75.2 [55.0-85.7]	0.387
Cardiac function			
NT-Pro BNP (ng/L)	80.2 [48.4-146.8]	71.5 [42.1-136.4]	0.036
Systolic			
TDI septal S' (m/s)	0.043 [0.03-0.05]	0.043 [0.04-0.05]	0.857
TDI lateral free wall S' (m/s)	0.056 (0.02)	0.055 (0.01)	0.780
Global longitudinal strain (%)	15.3 (3.5)	14.6 (3.1)	0.139
Diastolic			
E (m/s)	0.58 [0.5-0.8]	0.64 [0.6-0.7]	0.819
A (m/s)	0.48 [0.3-0.6]	0.43 [0.4-0.5]	0.106
E/A	1.43 [1.1-1.9]	1.51 [1.2-1.9]	0.361
TDI septal E' (m/s)	0.070 [0.05-0.09]	0.073 [0.06-0.09]	0.454
TDI septal A' (m/s)	0.041 (0.02)	0.043 (0.02)	0.430
TDI lateral free wall E' (m/s)	0.083 (0.03)	0.078 (0.03)	0.379
TDI lateral free wall A' (m/s)	0.037 [0.03-0.06]	0.042 [0.03-0.05]	0.931
E/E'	8.8 [6.7-11.9]	7.7 [7.3-11.7]	0.864
Vascular function			
Central SBP (mmHg)	109.9 (9.6)	104.3 (9.2)	0.003
PWVao (m/s)	5.31 (0.9)	5.17 (1.2)	0.502
AIXao (%)	19.4 (9.7)	17.0 (9.8)	0.062

Data expressed as mean (SD) or median [IQR].

A = peak late diastolic velocity, A' = peak late diastolic TDI velocity, AIXao = Augmentation index of the aorta, E = peak early diastolic velocity, E' = peak early diastolic TDI velocity, Global longitudinal strain = Global longitudinal strain from the four-chamber apical view, NT-pro BNP = N-terminal pro brain natriuretic peptide, PWVao = pulse wave velocity of the aorta, S' = peak systolic TDI velocity, SBP = systolic blood pressure, TDI = Tissue Doppler imaging.

Discussion

Our study evaluating the effect of a 3-month enalapril treatment in paediatric Fontan patients, demonstrated no improvement of exercise capacity nor systolic and diastolic ventricular function, and arterial stiffness. SBP and NT-pro-BNP levels, however, did reduce slightly. Nonetheless, a large proportion of patients had significant side effects.

ACE inhibition has been the cornerstone of the treatment of congestive heart failure in adult patients. Studies have shown beneficial effects of enalapril in patients with reduced ejection fraction with dosages ranging from 2.5 to 40 mg/day (3, 7-10). Interestingly, some of these studies found no relation between the dose of enalapril and clinical outcome (9, 10). However, the role of ACE inhibition in patients with diastolic heart failure with preserved ejection fraction is unknown as a recent meta-analysis showed that randomized trials have not demonstrated a survival benefit of ACE inhibitors in these patients (19). In paediatric patients with acquired and congenital heart disease associated with congestive heart failure and patients with a volume-overloaded biventricular circulation, enalapril dosages between 0.15 to 0.5 mg/kg/day have been effective (4, 6, 14, 20). In the present study, we aimed for a maximal enalapril dose of 0.5 mg/kg/day with a maximum of 20 mg. The dosage reached after the introduction phase ranged from 5 to 20 mg/day with a mean of 0.29 ± 0.1 mg/kg/day, which correlates well with paediatric and adult dosages presented in the literature.

ACE inhibitors are frequently used in Fontan patients. In 2 previous observational studies, 36% and 57% of the Fontan patients were treated with ACE inhibitors (11, 12). In the study by Anderson et al. (11) the use of ACE inhibitors in paediatric Fontan patients correlated with severe atrioventricular valve regurgitation and right ventricular morphology but not with the presence of ventricular dysfunction. In fact, in the study by Wilson et al. (12) 2/3 of the patients receiving enalapril did not have systolic ventricular dysfunction indicating that in most Fontan patients ACE inhibitors are prescribed as a preventive therapy, although proof of effectiveness is lacking. In this study, we showed that a 3-month enalapril treatment had no demonstrable impact on exercise capacity, nor on systolic or diastolic function in paediatric Fontan patients with moderate-good systolic ventricular function.

We did find a reduction in blood pressure and levels of NT-pro-BNP after a 3-month enalapril treatment. The effect on blood pressure might implicate a reduction in vascular resistance. However, both aortic stiffness parameters, including

augmentation index and pulse wave velocity, did not change significantly. As both parameters are affected by blood pressure, a small change can be expected based on blood pressure lowering, which does not always indicate that there is a reduction in aortic stiffness. Other retrospective studies were also unable to demonstrate an improvement in endothelial function in paediatric Fontan patients during treatment with ACE inhibitors (21, 22). We did find a slight decrease in NT-pro-BNP levels which suggests improved filling pressures. Although E/E' did not change, there may have been an effect on filling pressures, which were not measured in this study. A small catheterization study reported a reduction of elevated filling pressures after enalapril treatment in patients with univentricular hearts, either before or after Fontan completion, suggesting an improvement of diastolic function (23). Another positive effect of enalapril might be the preservation of renal function in Fontan patients as previously shown in a retrospective study (24).

Two previous studies have prospectively evaluated the effect of enalapril in 10 and 18 paediatric Fontan patients respectively (25, 26). Both showed that no parameter, including vascular resistance, cardiac index at rest, diastolic and systolic ventricular function, exercise capacity, and cardiac autonomous nervous activity improved after a 3 to 6-month enalapril treatment period; these results are comparable with ours. Although no effect on functional outcome was observed after several months of treatment, long-term enalapril treatment could have a beneficial effect. A previous study with enalapril in biventricular patients with congestive heart failure showed that survival curves did not separate until 18 months of treatment (8). Long-term beneficial effects on ventricular failure may be due to inhibition of the cardiac renin-angiotensin-aldosterone system (RAAS), thereby limiting cardiac remodelling and cardiomyocyte fibrosis (27). High levels of renin and aldosterone can be found in adult patients with congenital heart disease, including patients with single ventricle physiology (28). Furthermore, levels of renin and aldosterone have been shown to correlate with symptom severity and ventricular dysfunction in these patients (28) and therefore RAAS activation may play a role in the deterioration of the Fontan circulation. However, if long-term treatment with ACE inhibition or other RAAS inhibition may have beneficial effects in Fontan patients warrants further investigation.

Our study showed that a large proportion of patients experienced a significant adverse event which may raise concerns regarding how well ACE inhibition is tolerated by this patient cohort. Lowering blood pressure in a chronic volume unloaded circulation with limited preload reserve, because of the lack of a subpulmonary ventricle, is likely to cause negative effects. Because of the limited or

even absent preload reserve, afterload reduction will not lead to increased cardiac output as typically seen in biventricular circulations, but in a Fontan circulation may even cause a decrease in cardiac output which could be detrimental (29) One previous small study in paediatric Fontan patients has shown a lower increase in cardiac index during exercise after treatment with enalapril (25).

Because many Fontan patients are treated with ACE inhibitors, the ventricular function must be severely impaired to limit cardiac output in a Fontan circulation (29). Preload has been recognized as one of the main limiting factors of cardiac output increase in Fontan patients, of which pulmonary vascular resistance is one of the main contributors. Because low pulmonary vascular resistance is a prerequisite for a functioning Fontan circulation, pulmonary vasodilators have emerged as a promising medical therapy in the prevention of Fontan failure. The effect of pulmonary vasodilators in Fontan patients has already been investigated in a few large randomized studies and showed an increase in VO_{2peak} of 3% to 5% after treatment (30).

This study has some limitations. Our study includes a small sample size, with the predefined recruitment target not being reached despite many eligible participants. Nonetheless, it is the largest prospective study of enalapril in Fontan patients yet, and the power to conclude that enalapril has no beneficial effect was enhanced by lower-than-expected variance. Although the underlying cardiovascular defects were heterogeneous as in other Fontan studies, our sample was relatively homogenous as only paediatric patients with extracardiac conduit and moderate-good systolic ventricular function were included. We did not study the effect of ACE inhibition in Fontan patients with significant ventricular dysfunction. The treatment period chosen could have been too short, however, the treatment period was chosen based on previous studies which showed a beneficial effect on exercise parameters within 4 to 12 weeks after initiating treatment with ACE inhibitors, which was the primary end point of interest (3, 5, 13, 14). Lastly, echocardiography has its limitations in assessing diastolic function or ventricular dimensions. However, both cardiac catheterization and MRI would have increased the burden in the present study because repeated measurements were necessary.

In conclusion, our study demonstrated that a 3-month enalapril treatment period in paediatric Fontan patients with moderate-good systolic ventricular function does not enhance exercise capacity or improve vascular and echocardiographic ventricular function. Enalapril, however, did reduce SBP and NT-pro BNP levels. Nonetheless, enalapril was not well tolerated as a large proportion of patients

experienced adverse events. Based on these results, we conclude that short-term ACE inhibition has no beneficial effect in Fontan patients with moderate-good systolic ventricular function.

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Chapter 8

Treatment and outcome of plastic bronchitis in single ventricle patients: a systematic review

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Abstract

Plastic bronchitis (PB) is a life-threatening complication in single ventricle (SV) patients of which the exact pathophysiology, outcome and optimal treatment are still unclear. This study aims to systematically review the literature to give insight into the characteristics, outcome and management options of SV patients with PB. A systematic review was conducted, using the electronic database PubMed to find records published up to August 2018, describing SV patients and PB in which characteristics, treatment and/or outcome were adequately described per case. A total of 577 records were screened of which 73 had sufficient data describing 133 SV cases with PB. Most cases had completed a Fontan palliation (*n* = 126) with a median interval between Fontan completion and diagnosis of PB of 18.4 months (Q1-Q3 5.0-36.3). Overall mortality was 15.2% and was associated with diagnosis of PB within 12 months after Fontan palliation (5-year survival of 56.1% ≤12 months vs 94.8% >12 months, *P* = 0.002) and a higher age at Fontan completion (47.4 months for non-survivors vs 36.0 months for survivors, *P* = 0.015). Most patients received a combination therapy from 3 different treatment strategies, i.e. therapy for relief of airway obstruction, anti-inflammatory treatment and treatment to improve haemodynamics of the Fontan physiology (55.1%). In conclusion, SV patients who are diagnosed with PB within 12 months after Fontan palliation have a higher risk of mortality. Moreover, most cases received a combination therapy consisting of all 3 treatment strategies.

Introduction

Plastic bronchitis (PB) is a rare and life-threatening condition characterized by the formation of mucofibrinous casts within the tracheobronchial tree leading to airway obstruction and potentially asphyxia. PB has been described as a complication of respiratory disorders, lymphatic abnormalities and infections but particularly occurs in patients with a single ventricle (SV) after Fontan palliation (1) in which the prevalence has been estimated to be 2-8% (2-4). However, literature describing PB in Fontan patients usually consists of case reports and case series. As a result, knowledge of patient characteristics, survival and possible factors associated with disease outcome is limited. PB has a multifactorial pathogenesis, in which genetic and environmental factors, inflammation and the Fontan physiology itself, with elevated systemic venous pressure, seem to play an important role (5). There is a wide range of therapeutic strategies of which no specific regimen has proven to be consistently effective. A better understanding of SV patients with PB and all therapeutic options is important to recognize patients at risk and improve treatment strategies.

This study aims to systematically review the literature to give insight into the characteristics and outcome of patients with PB after SV palliation, as well as the therapeutic options to treat PB. Furthermore, a case report is presented to illustrate treatment options.

Case report

A boy born with tricuspid atresia and ventricular septal defect received an atrioseptectomy, banding of the main pulmonary artery and bidirectional Glenn anastomosis at the age of 3 months without complication. At the age of 4 years, there were low pulmonary artery pressures post-Glenn (11-12 mmHg) and good left ventricular function. Therefore, the patient received an extracardiac total cavopulmonary connection with a fenestration and division of the main pulmonary artery. The age at which the Fontan operation was performed is in concordance with our institutional standards (6). At the age of 6, 9 months after fenestration closure with an Amplatzer septal device, the patient developed PB with episodes of respiratory distress and daily coughing up large casts.

Diagnostic evaluation showed normal cardiac function, an unobstructed cavopulmonary circuit with a mean pressure of 15-17 mmHg, absence of aortopulmonary collaterals and a mild asymptomatic sinus node dysfunction with

a nodal escape rhythm of 75 beats per minute. There appeared to be a relation between the onset of PB and the fenestration closure. Because of recurrent life-threatening episodes, despite therapy with salbutamol and hypertonic saline, there was opted for an aggressive approach by lowering the pulmonary artery pressure by a Fontan takedown in combination with atrial pacing. The Fontan takedown consisted of removal of the entire extracardiac conduit, reconnection of the inferior caval vein to the right atrium and placement of a central aortopulmonary shunt to improve saturation. Furthermore, for atrial pacing a pacemaker system (A*trial-pacing Atrial-sensing Inhibited-response Rate-adaptive (AAIR)*) was implanted.

Postoperative treatment consisted of azithromycin, a medium-chain triglyceride diet and nebulization with mucolytics, bronchodilators and beclomethasone. Postoperatively, bronchoscopy was necessary to remove a large cast (Figure. 1) and sildenafil, bosentan and nebulization with alteplase were added. Eventually, symptoms of PB ceased and all medications were successfully weaned without recurrence. Due to severe cyanosis and reduced exercise intolerance, it was decided to redo a total cavopulmonary connection with fenestration 4.5 years after Fontan takedown. Haemodynamic assessment before the operation showed low pulmonary artery pressures (6-7 mmHg). Postoperatively, the patient's physical condition improved substantially and currently, >1.5 years post-redo-Fontan completion, he had no recurrence and only receives phenprocoumon and azithromycin.

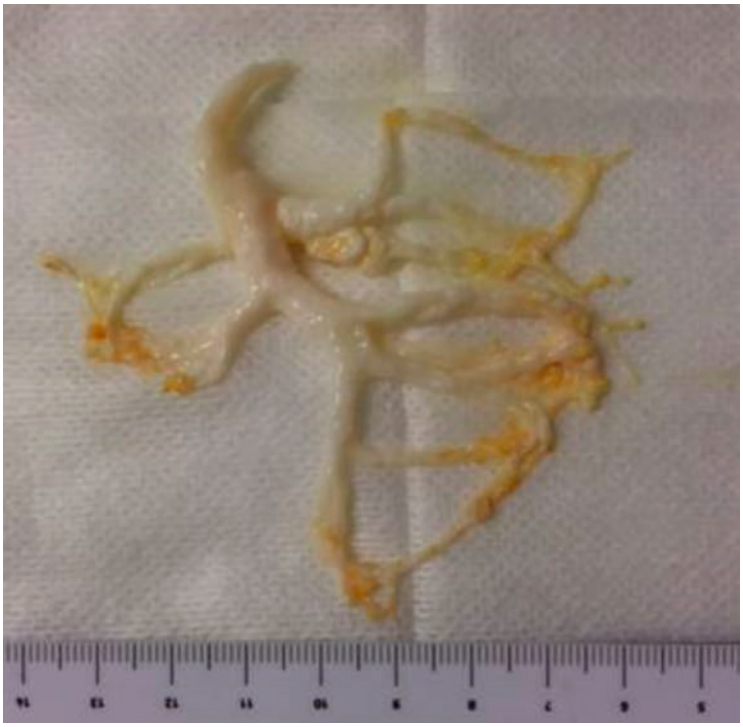

Figure 1. Expectorated bronchial cast

Materials and Methods

A systematic review of published studies describing SV patients with PB was performed using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement protocol (7). The PubMed database was used to search relevant records published up to August 2018, date of search 13 August 2018 (Figure 2). The following search terms were used: plastic bronchitis OR bronchial cast OR bronchial casts. The search was performed by the primary author (L.M.H.).

Titles and abstracts were screened and selected based on the following predefined criteria: the entire or partial study population consisting of SV patients who were diagnosed or treated for PB and characteristics, treatment and/or outcome being well described per case. All full-text records were studied, and non-English records were excluded. The case presented by our own centre was also included.

The following data were extracted: publication year, number of SV cases with PB and per case gender, type SV, type palliation, age at PB diagnosis, age at Glenn or Fontan

operation, follow-up time after diagnosis, history of chylothorax or protein-losing enteropathy (PLE), treatment received and outcome after treatment. Therapeutic options were divided into relief of airway obstruction, anti-inflammatory treatment and treatment improving haemodynamics of the Fontan physiology. Furthermore, characteristics between patients who survived or died were compared as well as survival between patients diagnosed within or after 12 months after Fontan completion.

Statistical analyses

Categorical data were extracted per case based on the presence or absence of a certain variable. Regarding the continuous data, all available data per individual case were directly extrapolated from the article. Although most articles provided data per individual case, a few articles only provided data on a group level. For those articles we had to make assumptions and therefore we used the available median of the entire group that was described in the article. This was necessary for 9 cases and only for the follow-up time since diagnosis of PB. Moreover, for 1 article we had to use 2 medians' to extract the necessary data for 2 cases (8). Data are presented as numbers with percentages for categorical data and median with first to third quartiles (Q1-Q3) for continuous data. To determine risk factors for negative outcome, continuous data were analysed using the Mann-Whitney *U*-test and categorical data using the Fisher's exact test. Kaplan-Meier curves were conducted to present overall survival and to compare the difference in survival between patients who were diagnosed with PB within or after 12 months after Fontan completion using the log-rank test.

SPSS (version 23) software was used. A *P*-value of 0.05 or less was considered statistically significant.

Results

Of the 577 records found in PubMed, 88 contained titles and abstracts that met the inclusion criteria. After studying the records in full text, 73 describing 133 PB cases were included in the review (Figure. 2). Fifty-three records consisted of a single case report, 18 records consisted of small case series and 2 records consisted of larger case series (see Supplementary Material, *Table S1*).

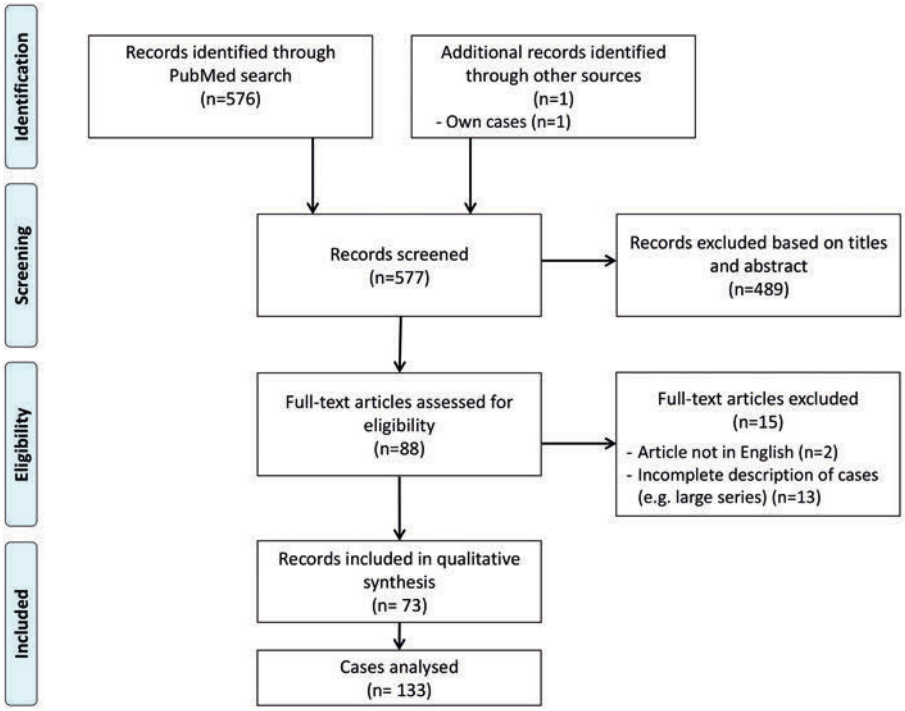

Figure 2. Preferred Reporting Items for Systematic Reviews and Meta-Analysis flow diagram of search results.

Patients characteristics are described in Table 1. Most cases were diagnosed with PB after Fontan palliation (*n* = 126) with a median age at the diagnosis of 60.0 months and the median time from Fontan to diagnosis of 18.4 months. Seven patients had a bidirectional Glenn anastomosis at the time of diagnosis. The median follow-up time after diagnoses was 18.0 months. After diagnosis, 10 cases needed extracorporeal membrane oxygenation support of which 2 eventually died. Overall mortality was 15.2% with a 5-year survival of 77.4% (Figure. 3). Furthermore, mortality seemed to have decreased over time as the mortality rate in articles published before 2012 was found to be 24.4% compared to 10.5% in articles published between 2012 and 2018. The median time to death after diagnosis was 3.5 months. Resolution of PB occurred in 51.5%, with 26.8% still experiencing symptoms at the end of follow-up.

Table 1. Characteristics and outcome of cases

Variables	
Sex (*n* = 104)	
Male	66 (63.5)
Female	38 (36.5)
Type single ventricle (*n* = 104)	
Single left ventricle	51 (49.0)
Single right ventricle	53 (51.0)
Type palliation (*n* = 133)	
Fontan	126 (94.7)
Glenn	6 (4.5)
Hemi-Fontan	1 (0.8)
Age at diagnosis of PB (*n* = 112, months)	60.0 (41-83.8)
Age at Glenn operation (*n* = 5, months)	6.0 (5.1-72.5)
Interval between Glenn operation and PB (*n* = 5, months)	12.0 (9.5-64.1)
Age at Fontan operation (*n* = 98, months)	36.6 (25.8-51.5)
Interval between Fontan operation and PB (*n* = 110, months)	18.4 (5-36.3)
Follow-up since diagnosis of PB (*n* = 126, months)	18.0 (6.8-37.1)
History of chylothorax	35 (26.3)
History of or occurrence of PLE alongside PB	6 (4.5)
Patients who received ECMO	10 (7.5)
Outcome at follow-up (*n* = 132)	
Death	20 (15.2)
Alive	112 (84.8)
Symptom free of PB at follow-up	68 (51.5)
Still symptoms of PB at follow-up	30 (22.7)
Unknown	14 (10.6)

All values are expressed as *n* (%) or median (Q1-Q3).
ECMO: Extracorporeal membrane oxygenation; PB: Plastic Bronchitis; PLE: Protein losing enteropathy.

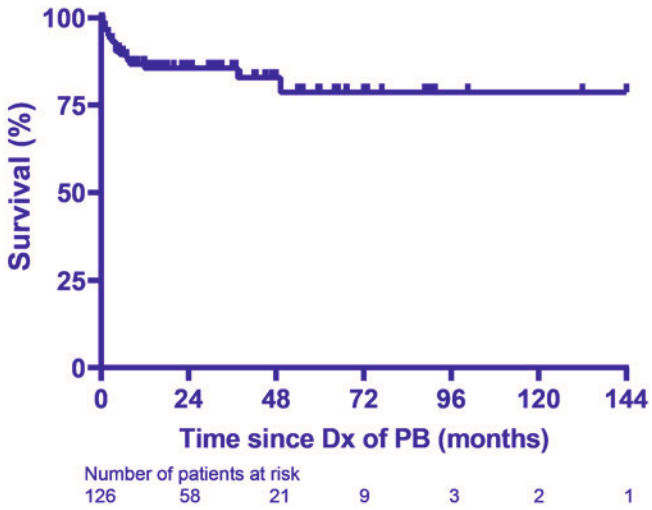

Figure 3. Kaplan–Meier curve of overall survival of Fontan patients after Dx with plastic bronchitis. Dx: diagnosis; PB: plastic bronchitis.

An older age at the time of Fontan operation (Table 2; 47.4 months for non-survivors vs 36.0 months for survivors) and the diagnosis of PB within 12 months after Fontan completion (Figure 4; 5-year survival of 56.1% with diagnosis ≤12 months vs 94.8% >12 months) were associated with an increased risk of mortality.

Table 2. Comparison of the characteristics between patients who survived or died after diagnosis of PB

Characteristics	Alive (n = 113)	Death (n = 20)	P-value
Gender (*n* =104)			1.000
Female	32 (36.4)	6 (37.4)	
Male	56 (63.6)	10 (62.5)	
Type single ventricle (*n* = 104)			1.000
Single left ventricle	45 (49.5)	6 (50.0)	
Single right ventricle	46 (50.5)	6 (50.0)	
Age at diagnosis of PB (*n* = 111, months)	60.0 (39.6-84.0)	55.0 (49.7-90.8)	0.854
Age at Fontan operation (*n* = 97, months)	36.0 (25.0-48.0)	47.4 (42.9-75.0)	0.015
Interval between Fontan operation and PB (*n* = 109, months)	23.9 (6.0-43.2)	6.0 (3.0-12.8)	0.052
History of chylothorax	25 (22.1	9 (45.0)	0.387
History of/or occurrence of PLE alongside PB	5 (4.4)	1 (5.0)	1.000
Patients who received ECMO	7 (6.2)	2 (10.0)	0.279

All values are expressed as n (%) or median (Q1-Q3).

ECMO: Extracorporeal membrane oxygenation; PB: Plastic Bronchitis; PLE: Protein-losing enteropathy.

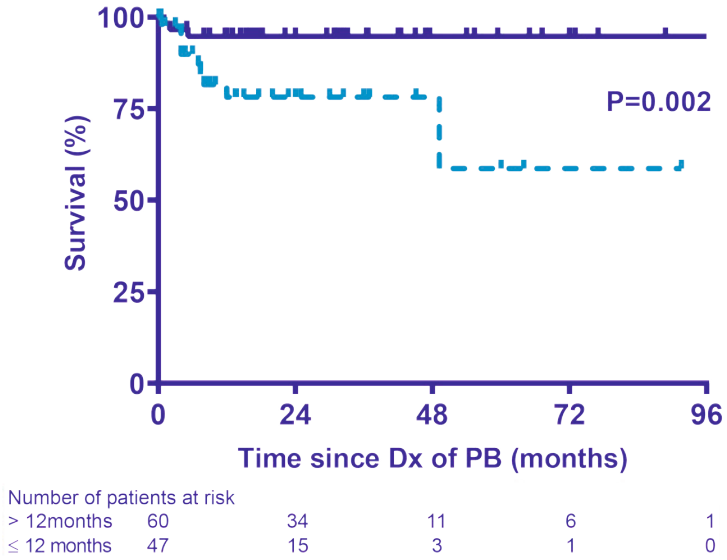

Figure 4. Kaplan–Meier curve of survival of Fontan patients after Dx with plastic bronchitis based on period of Dx after Fontan palliation, Dx within 12 months (light blue dotted line) and after 12 months (dark blue solid line) after Fontan palliation. The P-value of the log-rank test between groups is indicated in the graph. Dx: diagnosis; PB: plastic bronchitis.

Treatments were divided into 3 different treatment strategies (Table 3): (i) therapy for relief of airway obstruction, (ii) anti-inflammatory treatment and (iii) treatment improving the haemodynamics of the Fontan physiology. Most patients received a combination therapy from all 3 different treatment strategies (55.1%). In 6 patients treatment was not defined. Relief of airway obstruction was the most frequently reported strategy (91.3%), especially during the acute phase of PB. More than 50% of the patients received a combination of different aerosolized drugs for cast relief. Anti-inflammatory therapy was the least frequently reported (65.4%). Therapy improving haemodynamics was variable and consisted of optimizing the Fontan circulation or cardiac function (61.4%), antiarrhythmic therapy (8.7%), inhibition of lymph leakage/production (33.1%), decompression of the Fontan circulation (23.6%) and heart transplantation (9.4%).

Table 3. Description of therapeutic options applied for treatment of PB

	Total (n =127)
Relief of airway obstruction	116 (91.3)
Bronchoscopic cast extraction	62 (48.8)
Chest physiotherapy	31 (24.4)
Mechanical ventilation therapy	6 (4.7)
Fibrinolytics (total)	75 (59.1)
Tissue plasminogen activator/ Urokinase	70 (55.1)
Nebulized heparin	10 (7.9)
Mucolytics (total)	73 (57.5)
Bronchodilators	76 (59.8)
Anti-inflammatory	83 (65.4)
Corticosteroids	74 (58.3)
Antibiotics	37 (29.1)
Haemodynamics (CO/CVP/Lymph)	102 (80.3)
Optimizing Fontan circulation or cardiac function	78 (61.4)
Fontan conversion	1 (0.8)
Fontan revision	2 (1.6)
Surgical or interventional relief of Fontan circuit obstruction	25 (19.7)
Valve replacement	2 (1.6)
Ligation/embolization of aortopulmonary collaterals	17 (13.4)
Pulmonary vasodilators (total)	57 (44.9)
Bosentan	18 (14.2)
Sildenafil	54 (42.5)
Systemic prostacyclin	2 (1.6)
Heart failure therapy/medication	22 (17.3)
Anti-arrhythmic therapy	11 (8.7)
Pacemaker therapy	9 (7.1)
Ablation	1 (0.8)
Antiarrhythmic agents	1 (0.8)
Inhibition of lymph leakage/production	42 (33.1)
Low fat diet	13 (10.2)
Octreotide	1 (0.8)
Ligation/embolization of thoracic duct or lymphatic vessels	33 (26.0)
Decompression of Fontan circulation	30 (23.6)
Creation, dilation or stenting of fenestration	24 (18.9)
Takedown of Fontan circulation	6 (4.7)
Heart transplantation	12 (9.4)

All n (%) values represent number of cases in which the value was described. CO: cardiac output; CVP: central venous pressure; PB: Plastic bronchitis.

Discussion

PB is a rare complication in SV patients. Through collecting all available SV cases, we were able to describe characteristics, survival and therapy of 133 cases. Major findings include an overall mortality of 15.2%, diagnosis within 12 months after Fontan palliation and a higher age at Fontan operation, both of which are associated with increased risk of mortality. Moreover, the majority of cases received a combination therapy consisting of all 3 treatment strategies.

Mortality in this review was considerably lower compared to previous reports. A case-control study reported an overall mortality of 32.0% in Fontan patients diagnosed before 2012 (9). If we compare mortality within our review, mortality is higher in cases published before 2012 which may imply improving survival. Moreover, recent studies of PLE in Fontan patients also reported improved survival (10). Nevertheless, these higher survivals could be caused by improvement in the survival of all Fontan patients. Furthermore, mortality of PLE is higher (26.0-40.0%) (6, 10) and resolution after treatment much lower (31%)(6) when compared to PB (51.5%). Although both diseases share a similar pathological basis, PB is an acute disease that typically appears shortly after Fontan operation while PLE typically appears in a more chronic fashion several years after operation (6, 10).

One study reported that an earlier onset of PB after the Fontan palliation was associated with a higher risk of mortality (9), a risk factor we also found. However, early onset of PB may be a manifestation of a poor Fontan circulation and may therefore be correlated with an increased risk of death. Furthermore, we found an older age at Fontan operation as an additional risk factor. Whether this finding has been influenced by other factors could not be determined. For example, patients may have been operated late because they were suboptimal Fontan candidates. Nevertheless, it seems to be in line with most series that older age at Fontan operation is an independent risk factor for poor outcome (11-13). Other variables of interest for mortality, such as elevated Fontan pressures and ventricular function, or possible risk factors for the development of PB, could not be analysed in our study. In our case, the onset of PB seemed to be related to an elevated Fontan pressure after fenestration closure.

Treatment

We divided treatments into 3 therapeutic strategies including relief of airway obstruction, anti-inflammatory treatment and treatment improving haemodynamics of the Fontan physiology. Most patients described in the literature, including our

case, received a combination of all treatment strategies, which eventually led to the successful management of PB in the majority of cases. Therefore, based on all cases included in this systematic review, we developed a treatment algorithm in which treatment after the acute phase consists of a combination of all 3 different treatment strategies (Figure 5).

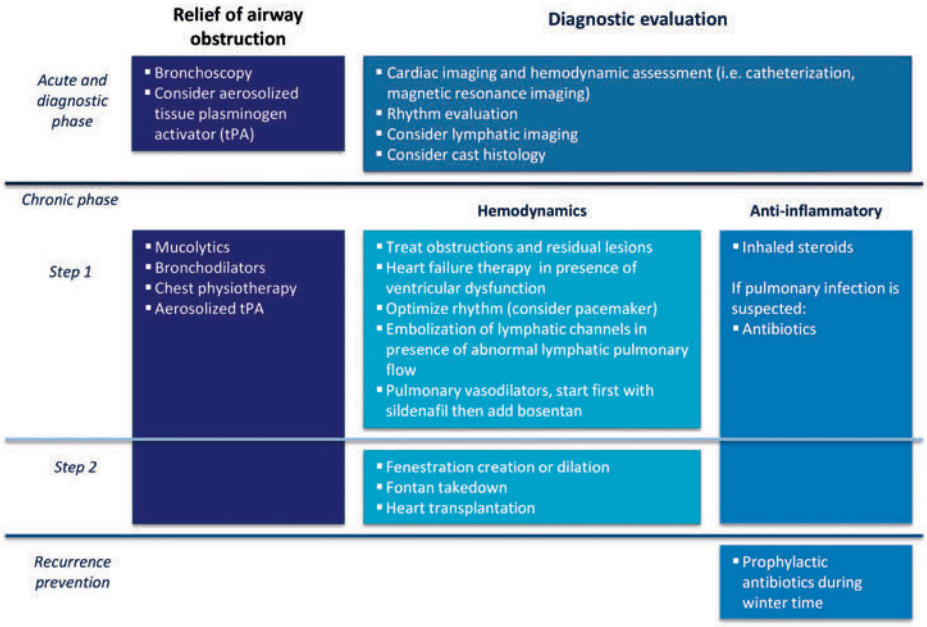

Figure 5. Treatment algorithm for Fontan patients diagnosed with plastic bronchitis. tPA: tissue plasminogen activator.

Relief of airway obstruction

In emergency cases, immediate bronchoscopy should be considered. However, complete lavage is not always possible as casts are often fragile. Alternatively, mechanical ventilation with positive–negative pressure (14-17) or high frequency jet ventilation (18) can be used for cast relief. However, a need for high airway pressure and repeated bronchoscopies should be avoided as both will decrease passive blood flow through the pulmonary system, negatively affecting the cardiac output of Fontan patients (19). With the absence of acute respiratory failure, treatment with chest physiotherapy, bronchodilators, mucolytics and fibrinolytics can be initiated to enhance cast expectoration or to induce the lysis of casts. Inhalation of plasminogen activator, a fibrinolytic, has proven to be an effective and safe treatment for cast

reduction (20-23), acute exacerbations (24) and chronic therapy in preventing cast formation (21, 25). Although all these therapies may be effective for cast relief, they are not able to prevent recurrence.

Anti-inflammatory treatment

Because some casts consist of inflammatory cells (21, 26) and since there is a relation between PB and acute respiratory infections (2), anti-inflammatory treatment has been frequently used. This most often consists of inhaled corticosteroids and antibiotic treatment. Yet, evidence of effectiveness of steroids is limited. However, a case with acellular casts resolution appeared after the introduction of corticosteroids (27) and another case remained cast free while chronically on steroid treatment (26). Inhaled steroids may be effective as it is believed that altered Fontan haemodynamics cause mucosal injury inducing mild airway inflammation. Furthermore, as cast recurrence has been associated with respiratory infections (21), prophylactic antibiotic treatment may be a useful adjunct therapy for recurrence prevention, as shown in our case and another case report (28).

Therapy improving the Fontan circulation

The Fontan physiology itself also contributes to the development of PB. A lower cardiac output, elevated systemic venous pressure and abnormal lymphatic flow may contribute (5). Therefore, a thorough diagnostic evaluation of the Fontan circulation is essential to determine a therapeutic strategy. Nevertheless, the onset of PB can occur in Fontan patients with relative normal Fontan pressures with preserved cardiac function without residual lesions (21, 26).

Relief or repair of residual lesions and obstructions have to be considered. Resolution of PB has been reported after successful interventions for ventricular, arterial and venous obstruction atrioventricular valve regurgitation and aorta-pulmonary collaterals (21). Although these results are promising, not every intervention in each individual will lead to successful treatment (5, 29, 30). In addition, medical therapies may also improve haemodynamics and optimize the circulation. Pulmonary vasodilators, sildenafil and bosentan in particular have shown to be an effective and safe treatment that can be administered chronically (21, 26, 31-33).

Improvement in cardiac output can be obtained by antiarrhythmic therapy. Atrial pacing may restore chronotropic incompetence and atrioventricular synchrony but has only led to the resolution of PB in some cases (1, 34, 35), while others report less favourable results (5, 23, 25, 36, 37).

Inhibition of lymph production or leakage could be an effective therapy, especially in those who previously had a chylothorax or currently have chylous casts. A low-fat or medium-chain triglyceride diet has proven to be successful (37, 38). However, recurrence can occur when the diet is stopped (37). Furthermore, interruption of lymphatic flow through embolization or ligation has shown to improve symptoms and even temporarily resolution (26). However, not all patients have abnormal lymphatic flow (26). Therefore, lymphatic imaging should ideally be part of the diagnostic evaluation.

If the Fontan circulation cannot be further optimized, then decompression should be considered. A Fontan takedown to a Glenn physiology as well as fenestration of the Fontan pathway can result in reduced venous pressures and improved cardiac output. However, both therapies have shown various effects, with results ranging from no effect (39-41) to the improvement of symptoms (21, 26, 31, 39, 42) or even complete resolution (21, 36, 43-46). Although catheter-created fenestration is the least invasive method, the chance of spontaneous closure is high (36). Furthermore, when surgery is considered, a takedown will have the largest effect on lowering venous pressures and improving cardiac output but also will result in significant cyanosis, which can be a complicating factor if PB persists. In our case, the severe PB, which developed after fenestration closure, resolved after temporary Fontan takedown and appeared to be a successful therapy by drastically decreasing the pulmonary circulation. After the resolution of casts and a long recovery period, re-Fontan procedure with a relatively large fenestration was feasible without PB recurrence.

Besides improving the hemodynamic of the Fontan circulation itself, a heart transplantation could be considered in some cases. Although Fontan patients with PB have a high mortality peri-transplantation (47, 48), long-term mortality is comparable to the general paediatric heart transplant population (47). As residual casts may be present, airway clearance during and early after transplantation is necessary to reduce postoperative morbidity (49, 50). Cardiac transplantation could be very successful for treating PB, as in a large group of Fontan patients with PLE resolution occurred in almost all cases after heart transplantation (51).

Limitations

This review includes only patients already diagnosed with PB, and therefore we could not identify risk factors for PB itself. Furthermore, missing data and reporting bias were present due to the inclusion of case reports. For example, we were not

able to report the type of Fontan operation performed. Potential risk factors such as haemodynamic measurements of cardiac catheterization could not be studied, as they were only infrequently reported in the cases. Due to the possible presence of publication bias, the study population may also not be representative of the total population of Fontan patients with PB. However, missing data and reporting bias may have biased results in both directions. Finally, due to the many therapy types, it was only possible to provide an overview instead of an analysis of different therapeutic strategies.

Conclusion

Most SV patients with PB are diagnosed early after the Fontan operation with approximately one-sixth dying shortly after diagnosis. Furthermore, diagnosis of PB within 12 months after Fontan palliation and a higher age at Fontan operation are associated with an increased risk of mortality. Whether these findings are independent predictors for mortality could not be investigated. Although treatments varied enormously and none has proven to be consistently effective, this study together with our own experience suggests that therapy of PB consisting of relief of airway obstruction, anti-inflammatory treatment and treatment improving haemodynamics of the Fontan physiology can be an effective strategy to cure PB. In addition, a temporary Fontan takedown could be a life-saving therapy. During this period, PB can resolve, pulmonary circulation can recover and the Fontan circulation could be restored successfully at a later time. Nevertheless, large multicentre cohort studies are warranted to unravel the exact pathophysiology to determine which patients are at risk for developing PB and which therapeutic strategy is most effective.

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Supplementary materials

Table S1. Overview of included records

Study	N cases	Age at diagnosis of PB	Interval Fontan operation until PB	Follow-up since diagnosis of PB	Outcome	Therapy										
						Symptomatic cast relief		Anti-inflammatory-treatment			Hemodynamics (CO/CVP/Lymph)					
		median (min-max) months	median (min-max) months	median (min-max) months	Died (N cases)	Y/N	N cases	Y/N	N cases	Y/N	N cases Tot	Opt	Rhyt	Lymp	Dec	HTx
Okada et al. 2018 [16]	1	192	171	0,5		Yes	1	Yes	1	No						
DePopas et al. 2017 [41]	3	60 (72-108)	24 (24-48)	45 (42.5-73)		Yes	3	No		Yes	3	1	1	2	1	
Parent et al. 2017 [50]	3	-	-	54 (48-90)		Yes	3	Yes	3	Yes	3			1		3
Perez Ruiz et al. 2017 [52]	1	17	-	1	1	Yes	1	No		No						
Unseld et al. 2017 [8]	4	50.4 (39.6-61.2)	15.6 (4.8-27.6)	58.2 (12-88.8)	2	ND		ND		ND						
Charlagorla et al. 2016 [43]	1	60	30	24		Yes	1	Yes	1	Yes	1				1	
Dori et al. 2016 [26]	16	65.6 (20.8-136.6)	20.6 (0-51.6)	32.2 (13.7-100.6)	1	Yes	15	Yes	13	Yes	16	16	1	15	3	1
Guevara et al. 2016 [53]	1	-	-	-		Yes	1	No		Yes	1			1		
Ugaki et al. 2016 [29]	1	51	3	8	1	Yes	1	No		Yes	1	1		1	1	
Wesnerowicz et al. 2016 [45]	2	79.8 (21-138.5)	52.8 (9-96.5)	26.8 (24-29.5)		Yes	2	Yes	1	Yes	2	2			1	
Chung et al. 2015 [54]	1	60	51	65		Yes	1	No		Yes	1			1		
Hallbergson et al. 2015 [39]	2	84 (38.4-129.6)	2.9 (0.8-5)	14 (4-24)	1	No		No		Yes	2				2	

Table S1. Overview of included records (continued)

Study	N cases	Age at diagnosis of PB median (min-max) months	Interval Fontan operation until PB median (min-max) months	Follow-up since diagnosis of PB median (min-max) months	Outcome Died (N cases)	Therapy										
						Symptomatic cast relief		Anti-inflammatory-treatment			Hemodynamics (CO/CVP/Lymph) N cases					
						Y/N	N cases	Y/N	N cases	Y/N	Tot	Opt	Rhyt	Lymp	Dec	HTx
Jasinovic et al. 2015 [55]	2	66.5 (56-77)	-	20.5 (3-38)	1	Yes	2	No		No						
Singhi et al. 2015 [56]	1	62	2	9		No		No		Yes	1	1				
Tanase et al. 2015 [38]	4	29 (21.5-45)	10 (0.5-24)	10.8 (5-25)		Yes	4	No		Yes	4	4		4		
Avitable et al. 2014 [21]	14	-	18.0 (0.3-184.8) (1 Missing)	32.4 (3-54)		Yes	14	Yes	14	Yes	14	14		1	7	3
Colaneri et al. 2014 [24]	1	156	84	12		Yes	1	Yes	1	Yes	1	1				
Dori et al. 2014 [57]	1	68	32	11,7		Yes	1	Yes	1	Yes	1	1		1		
Eason et al. 2014 [58]	1	60	24	12		Yes	1	Yes	1	Yes	1	1				
Lis et al.; 2014 [59]	1	18	-	1,1		Yes	1	Yes	1	Yes	1	1				
Parent et al. 2014 [49]	1	-	-	-		Yes	1	No		Yes	1			1		1
Brooks et al. 2013 [15]	2	54.0 (41-66.9)	36.5 (24-48.9)	132 (1 Missing)		Yes	2	Yes	1	No						
Ezmigna et al. 2013 [60]	1	72	24	15.4		Yes	1	Yes	1	Yes	1			1		
Grutter et al. 2013 [61]	1	144	60	18		Yes	1	Yes	1	Yes	1	1				
Kobayashi et al. 2013 [40]	1	42	6	8		Yes	1	Yes	1	Yes	1	1			1	
Kunder et al. 2013 [46]	4	33 (24-36)	1 (3 Missing)	7.5 (3-144)		Yes	4	Yes	4	Yes	3	2			2	
Lubcke et al. 2013 [23]	1	144	120	9		Yes	1	Yes	1	Yes	1		1			
Singhal et al. 2013 [62]	2	101.5 (83-120)	65.5 (35-96)	7.5 (2-13)		Yes	2	Yes	1	Yes	2	2		1	1	
Do et al. 2012 [42]	1	132	72	9		Yes	1	Yes	1	Yes	1	1			1	

Table S1. Overview of included records (continued)

Study	N cases	Age at diagnosis of PB median (min-max) months	Interval Fontan operation until PB median (min-max) months	Follow-up since diagnosis of PB median (min-max) months	Outcome Died (N cases)	Therapy											
						Symptomatic cast relief		Anti-inflammatory-treatment		Hemodynamics (CO/CVP/Lymph)							
											N cases						
						Y/N	N cases	Y/N	N cases	Y/N	Tot	Opt	Rhyt	Lymp	Dec	HTx
Elahi et al. 2012 [63]	1	48	6	0,33		Yes	1	No		No						
Grutter et al. 2012 [64]	4	43 (25-54)	4 (0-10)	7 (0-10)	2	Yes	4	Yes	4	Yes	4	4				
Kovesi et al. 2012 [65]	1	13	-	67,4		Yes	1	Yes	1	Yes	1	1				
Larue et al. 2012 [5]	2	121 (38-204)	79 (2-156)	36 (30-42)		Yes	2	Yes	2	Yes	2	2	1		1	1
Nawa et al. 2012 [66]	1	96	60	1		Yes	1	No		Yes	1	1				
Parikh et al. 2012 [37]	1	132	110	72		Yes	1	Yes	1	Yes	1	1	1	1		
Wirbelauer et al. 2012 [67]	1	60	36	24		Yes	1	No		Yes	1	1		1		
Elmallah et al. 2011 [14]	1	72	-	42		Yes	1	Yes	1	Yes	1					1
Laubisch et al. 2011 [68]	1	48	24	32		Yes	1	Yes	1	Yes	1	1				1
Silva et al. 2011 [69]	1	60	-	2,4	1	Yes	1	Yes	1	Yes	1				1	
Guimaraes et al. 2010 [70]	1	54	14	3		Yes	1	No		Yes	1	1				
Preciado et al. 2010 [19]	3	36 (8-36)	6 (2 Missing)	2 (0-4.7)	1	Yes	3	Yes	2	No						
Reinhardt et al. 2010 [31]	4	117.6 (81.6-148.8) (1 missing)	78 (42-96) (1 missing)	21.4 (12-30.8) (2 missing)		No		No		Yes	4	4			3	1
Do et al. 2009 [22]	1	68	2	1		Yes	1	No		Yes	1	1		1		
Zahorec et al. 2009 [18]	2	127.4 (74-180.8)	1.4 (0.8-2)	32 (4-60)	1	Yes	2	Yes	1	No						
Cajaiba et al. 2008 [71]	1	192	144	0,16	1	Yes	1	No		No						

Table S1. Overview of included records (continued)

Study	N cases	Age at diagnosis of PB median (min-max) months	Interval Fontan operation until PB median (min-max) months	Follow-up since diagnosis of PB median (min-max) months	Outcome	Therapy										
						Symptomatic cast relief		Anti-inflammatory-treatment			Hemodynamics (CO/CVP/Lymph) N cases					
					Died (N cases)	Y/N	N cases	Y/N	N cases	Y/N	Tot	Opt	Rhyt	Lymp	Dec	HTx
Verghese et al. 2008 [72]	1	32	14			Yes	1	No		No						
Zaccagni et al. 2008 [73]	1	180	108	17		Yes	1	No		Yes	1	1				
Nayar et al. 2007 [74]	1	102	15	2	1	Yes	1	Yes	1	Yes	1			1		
Haseyama et al. 2006 [32]	1	38	2	9		No		Yes	1	Yes	1	1				
Apostolopoulou et al. 2005 [33]	1	92	8	91,6		Yes	1	Yes	1	Yes	1	1			1	
Shah et al. 2006 [17]	2	48 (1 Missing)	36 (1 Missing)	14.5 (5-24)		Yes	1	Yes	1	Yes	2			2		
Peleg et al. 2005 [75]	1	75	3	4	1	Yes	1	Yes	1	No						
Tzifa et al. 2005 [76]	1	41	5	8,5		Yes	1	Yes	1	Yes	1	1				
Wakeham et al. 2005 [25]	1	50	35	31		Yes	1	Yes	1	Yes	1	1	1	1		
Wilson et al. 2005 [36]	1	61	1	15		Yes	1	Yes	1	Yes	1		1		1	
Yalcin et al. 2005 [77]	1	90	60	17		Yes	1	No		Yes	1	1				
Barber et al. 2004 [34]	1	54	6	15		Yes	1	No		Yes	1	1	1			
Chaudhari et al.; 2004 [44]	1	42	1	36		Yes	1	Yes	1	Yes	1				1	
Dicindio et al. 2004 [78]	1	28	10	0,46	2	Yes	1	Yes	1	No						
Ishman et al. 2003 [79]	1	60	7	12		Yes	1	No		No						
Onoue et al. 2003 [27]	1	96	60	36,7		Yes	1	Yes	1	No						

Table S1. Overview of included records (continued)

Study	N cases	Age at diagnosis of PB median (min-max) months	Interval Fontan operation until PB median (min-max) months	Follow-up since diagnosis of PB median (min-max) months	Outcome Died (N cases)	Therapy										
						Symptomatic cast relief		Anti-inflammatory-treatment			Hemodynamics (CO/CVP/Lymph) N cases					
						Y/N	N cases	Y/N	N cases	Y/N	Tot	Opt	Rhyt	Lymp	Dec	HTx
Brogan et al. 2002 [1]	1	65	5	20		Yes	1	Yes	1	Yes	1		1	1		
Costello et al. 2002 [80]	1	53	27	5,2		Yes	1	Yes	1	No						
Stiller et al. 2002 [81]	2	57 (42-72)	26.5(17-36)	18 (12-24)		Yes	2	Yes	2	Yes	2	1		2		
Hug et al. 2001 [82]	1	47,4	1,4	0		Yes	1	No		No						
McMahon et al. 2001 [30]	1	30	6	7,4		Yes	1	Yes	1	Yes	1	1				
Setzer et al. 2001 [83]	1	72,2	0,16	0,16	1	Yes	1	No		No						
Quasney et al. 2000 [35]	1	60	24	36,5		Yes	1	Yes	1	Yes	1		1			
Collorodi et al. 1990 [84]	1	84	-	-	1	Yes	1	No		No						
Languepin et al. 1999 [85]	1	24	9	64		Yes	1	Yes	1	Yes	1	1		1		
Seear et al. 1997 [86]	3	96 (60-144)	12 (12-12) 1Missing	0.69 (0.46-1.2)		Yes, 2ND	1	Yes, 2ND	1	No, 2ND						
Bowen et al. 1985 [87]	1	101	5	37	1	No		Yes	1	No						
Own case	1	70	19	77		Yes	1	Yes	1	Yes		1	1	1	1	

CO= Cardiac output; CVP= Central Venous Pressure; Dec= Therapy for decompression of Fontan circulation; HTx= Heart transplantation; Lymp= Therapy for inhibition of lymph leakage/production; N cases = Number of cases; ND = Not defined; Opt= Therapy for optimizing Fontan circulation; Rhyt= Anti-arrhythmic therapy; Y/N= Yes/No.
* Not all variables that were investigated in this systematic review are included in this table.

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Chapter 9

General discussion and future perspectives

Despite improvement in survival, Fontan patients still have an increased risk of morbidity and mortality (1, 2). However, a clear therapeutic strategy to improve Fontan circulation and prevent Fontan failure is still lacking. While many centres have focused their therapy on treating or preventing ventricular dysfunction using angiotensin converting enzyme (ACE) inhibitors, evidence of their efficacy in these patients is still lacking (3, 4). Accordingly, in this thesis, we studied the effects of ACE inhibition on various cardiovascular and hemodynamic properties of the Fontan circulation of paediatric patients. In addition, to further elucidate the effectiveness of ACE inhibition in Fontan patients and understand the pathophysiology of Fontan failure, we also studied the haemodynamics of the Fontan circulation of paediatric patients. Different pathophysiological mechanisms may influence the Fontan circulation negatively, such as systolic and diastolic ventricular dysfunction, reduced vascular compliance and autonomic failure. Since little is known about the normal development of autonomic function in healthy children, we studied both normal development of autonomic function as well as the autonomic function of paediatric Fontan patients. At last, in this thesis, the literature of plastic bronchitis, a severe complication of the Fontan circulation, was systematically reviewed to evaluate several aspects of plastic bronchitis in Fontan patients.

All different parts of this thesis with associated findings will be discussed below. First the results of cardiac autonomic function in healthy children and paediatric Fontan patients will be discussed. Thereafter, the results of evaluation of the Fontan circulation and effects of ACE inhibition are evaluated. At last, results of the systematic review of plastic bronchitis will be explicated.

Cardiac autonomic nervous activity

The autonomic nervous system (ANS), consisting of the parasympathetic- (PNS) and sympathetic nervous system (SNS), influences the circulation by regulating, e.g., blood pressure and heart rate. The interest in cardiac ANS activity has increased in recent years because of its relevance for cardiovascular disease and mortality (5). However, the normal development of cardiac ANS activity is still not fully understood. Therefore, in this thesis in **chapter 3**, we assessed the maturation of the cardiac PNS and SNS activity by measuring different non-invasive PNS activity parameters, i.e., respiratory sinus arrhythmia and two heart rate variability parameters, and a SNS activity parameter, i.e., pre-ejection period. For this study, cardiac PNS and SNS activity parameters from 5 different studies were combined, resulting in a multi-cohort study of 4820 healthy subjects aged 0.5 to 20 years (6-10). Our study showed that PNS activity increased rapidly after birth, levelled off during middle childhood

(≈11 years), and then decreased into adolescence, while SNS activity showed a linear decrease from early childhood to late adolescence. Such patterns were highly compatible with previously performed studies (11-28). Furthermore, there was a difference in maturation between sexes with PNS activity peaking later in boys than in girls. This later peak in boys is likely due to differences in hormonal changes since girls are known to enter puberty earlier than boys. Although the maturational trends are clear there is a large interindividual variability in absolute ANS parameters. This interindividual variability can be caused by various factors including ethnicity, differences in development of stature, body composition and entering puberty, differences in loading conditions, heart size, sensitivity of the baroreflex and lung stretch reflexes and respiratory behaviours (frequency and tidal volume), adopted lifestyle patterns (smoking, dietary habits, and physical activity), and psychosocial stress exposures. Moreover, even experimental factors can impact absolute values, such as time of day, posture, previous physical activity, and even analytic strategy to quantify SNS and PNS cardiac activity. This may limit the usefulness of individual absolute parameters, although group comparisons can still be valuable.

In **chapter 4**, we compared absolute values of cardiac ANS activity of Fontan patients with healthy controls to get an interpretation of baseline values. While PNS activity was comparable between both groups, the pre-ejection period was increased in Fontan patients suggesting lower SNS activity. This is in contrast with previous studies in adult Fontan patients who showed an impaired autonomic function with decreased PNS- and increased SNS activity (29-31). However, caution is needed with interpretation of pre-ejection period in Fontan patients as it is known to be sensitive to preload and afterload effects, which are both known to be different in Fontan patients. Our findings suggest a preserved ANS function in young Fontan patients being treated in the present era. Future research should consider more invasive parameters to measure SNS activity, as no other good non-invasive alternative exists. Since autonomic dysfunction has been described in adult Fontan patients and autonomic function may play a role in the deterioration of the Fontan circulation, it is interesting for future research to investigate the maturation of cardiac ANS activity in Fontan patients.

Evaluation of the Fontan circulation

Multiple pathophysiological mechanisms may impact the Fontan circulation. A combination of exercise stress testing and the use of acute fluid challenges, either fluid loading or fluid depletion may give more insight in these mechanisms.

Exercise stress testing

During exercise stress testing an adequate increase in cardiac output is mandatory, being influenced by heart rate, preload, contractility, and afterload. In Fontan patients the exercise capacity is known to be impaired. As shown in **chapter 4,** the percentage of predicted exercise capacity can range from 54-72% in one cohort, differing on the parameter used, which is comparable with previous literature (32-37).

The main limiting factor of Fontan circulation is the inability to augment venous return due to the lack of a ventricle for the pulmonary circulation. In our patient population, we observed an adequate increase in heart rate, i.e., a good chronotropic competence, while stroke volume was reduced at peak exercise. We found that decreased diastolic function, influencing preload, and increased arterial stiffness, influencing afterload, were correlated with decreased exercise performance. It is known that diastolic dysfunction and increased arterial stiffness are present in Fontan patients at an early age and that both may worsen over time and therefore may contribute to deterioration of exercise performance (38-42). In contrast to other studies systolic function, i.e., contractility, was not correlated with exercise performance in our study. This may be explained by the selection criteria of our study cohort, excluding patients with significant systolic dysfunction, (1). Although we investigated the relationship between exercise capacity and various cardiovascular parameters we did not study other parameters such as the impact of residual obstructions in the Fontan tunnel or pulmonary arteries or increased pulmonary vascular resistance that will lead to energy loss and reduced pulmonary blood flow (37, 43, 44).

Acute fluid challenges

While acute fluid challenges were previously performed more frequently in invasive (laboratory) studies, in recent decades it has also been performed non-invasively by means of passive leg raising, mimicking fluid loading, and head-up tilt table testing, reflecting acute fluid unloading.

Passive leg raising as a test to evaluate the hemodynamic response to an increase in preload has not been used in Fontan patients. Passive leg raising is a non-invasive, reversible, and reliable test to predict volume responsiveness. In this thesis, in **chapter 5**, hemodynamic reaction to passive leg raising was studied in Fontan patients and healthy controls. Overall, the hemodynamic response of Fontan patients was similar to the response in healthy controls. Moreover, Fontan patients who did not respond with an increase in stroke volume were not more

negatively affected by passive leg raising than controls. However, in a previous catheterization study (45) an increase in preload was related to an increase in end-diastolic pressure, decreasing the transpulmonary gradient and cardiac output in part of the patients. Since the response to an increase in preload may be affected by differences in loading conditions, this can explain the different results from our study as compared to literature.

Head-up tilt testing can be used to test the acute orthostatic stress response. A good reaction to orthostatic stress requires activation of the sympathetic nervous system and concurrent vagal withdrawal to increase heart rate, systemic vasoconstriction, and venous return to be able to maintain adequate blood pressure and cerebral blood flow (46, 47). Previous studies, mostly performed in adult Fontan patients, have shown differing results with overall an impaired cardiac ANS activity and decreased baroreflex sensitivity, resulting in a blunted autonomic response to orthostatic stress (30, 31, 45, 48). In contrast to these studies, in **chapter 6,** we showed that paediatric patients had a sufficient autonomic response during head-up tilt testing while maintaining adequate blood pressure and cardiac output. Sufficient autonomic response was demonstrated by an increase in heart rate and aortic stiffness, both indicative of intact autonomic regulation, as well as by the same degree of decrease in cardiac PNS activity between patients and controls. As previous studies were performed in adult patients, age may have affected the difference in results. Furthermore, cardiac output was maintained which may imply that patients are likely to be able to adapt to the Fontan circulation and to prevent venous pooling while standing and avoid a deleterious drop in central venous pressure. Although paediatric patients seem to adapt, studies in adult Fontan patients show a large decrease in cardiac output in response to tilt, suggesting that the adaptive mechanisms may be falling short on the long-term (48). It is interesting to further investigate both patients who respond well and those who do not, to clarify the difference and to possibly identify risk factors for deterioration of the circulation over time.

ACE inhibition

Since many Fontan patients, even without systolic ventricular dysfunction, are currently treated with ACE inhibition without evidence of efficacy, in this thesis, in **chapters 6 and 7**, we investigated the effect of ACE inhibition on the Fontan circulation. The previously mentioned methods to evaluate the circulation were also used to evaluate the effects of a 3-month treatment with enalapril on the circulation.

We showed that a 3-month enalapril treatment had no effect on the exercise performance of the Fontan patients and did not improve arterial stiffness, diastolic nor systolic ventricular function, which was comparable with the previous two smaller studies performed in Fontan patients (49, 50). Enalapril did, however, lower the systolic blood pressure and NT pro-BNP levels. Lower levels of NT pro-BNP might suggest lower filling pressures, although not directly measured in this study, this effect of enalapril has previously been shown in a small catheterization study in univentricular heart patients (51). Furthermore, although enalapril lowered the blood pressure at rest, it did not lead to orthostatic hypotension or a decrease in cardiac output during head-up tilt testing. During the titration period a large proportion of patients experienced side effects (42%), such as a significant drop in systolic blood pressure, dizziness, and syncope. After the titration period patients were given a stable dosage, which was better tolerated. In other words, ACE inhibition has a vasodilatory effect in Fontan patients leading to lowering the blood pressure, but after the titration period most side effects have disappeared. This habituation is further supported by the good orthostatic tolerance under enalapril treatment.

Although we did not show beneficial effects of enalapril on the short-term, on the long-term cardiac renin-angiotensin-aldosterone system (RAAS) inhibition could limit cardiac remodelling and cardiomyocyte fibrosis (52). However, future studies are warranted to investigate if ACE inhibition or other RAAS inhibition may have beneficial effects in Fontan patients on the long-term.

Plastic Bronchitis

When the Fontan circulation fails in single ventricle patients, plastic bronchitis can manifest as a serious complication. Plastic bronchitis is characterized by the formation of mucofibrinous casts in the tracheobronchial tree, leading to airway obstruction and possible suffocation. Systematically reviewing the literature in **chapter 8** showed that the overall mortality rate in single ventricle patients was 15.2%, with a lower overall mortality rate of 10.5% in cases published after 2012. Mortality was associated with diagnosis of plastic bronchitis within 12 months of the Fontan palliation, which could indicate that early onset of plastic bronchitis may be a manifestation of a poor Fontan circulation. Furthermore, a higher age at Fontan operation was found as an additional risk factor, however, whether this finding has been influenced by other factors could not be determined as many other possible risk factors were often missing from records.

Concerning treatment for plastic bronchitis, we found that most cases received a combination therapy consisting of relief of airway obstruction, inflammatory treatment, and treatment to improve the haemodynamics of the Fontan circulation, which eventually led to the successful management of plastic bronchitis in most cases. Therefore, based on the data from our systematic review, we developed a treatment algorithm in which the acute phase may exist of bronchoscopy for direct cast relief and aerosolized tissue plasminogen activator can be considered, next to diagnostic evaluation. After the acute phase, treatment may best be consisting of a combination of all three treatment strategies. Improving the haemodynamics can consist in this phase of optimizing the Fontan circulation by medical therapy or surgery, anti-arrhythmic therapy and/or inhibition of lymph leakage. When the plastic bronchitis is still not improving, the haemodynamics could be improved by more invasive strategies, i.e., decompression of the circulation by creation of a fenestration or Fontan takedown, and even heart transplantation could be considered. In our case report, presented in this review, a temporary Fontan takedown was lifesaving, and during this period, the plastic bronchitis could resolve, the pulmonary circulation recovered, and the Fontan circulation restored successfully later. Finally, when the plastic bronchitis has resolved, we recommend that patients start prophylactic antibiotics as preventive therapy, as recurrence has been associated with respiratory infections (53).

Although a good overview of alle cases in the literature was given, much data from many cases were lacking to be able to determine more exactly which patients may be at risk for developing plastic bronchitis and which treatment option/strategy will work best. Large multicentre cohort studies are warranted to unravel the exact pathophysiology to determine which patients are at risk for developing plastic bronchitis and which therapeutic strategy is most effective.

Conclusions and future perspectives

In this thesis several aspects of the Fontan circulation were studied in a relatively homogeneous group of paediatric patients, with an extracardiac conduit and moderate to good systolic ventricular function, to evaluate not only the effectiveness and side effects of ACE inhibition, but also study the limitations of exercise capacity and haemodynamic reaction to acute fluid challenges.

We demonstrated that ACE inhibition on the short term does not show positive effects on exercise capacity, cardiovascular and ventricular function, but besides the side effects during the titration period, also had no negative effects on the

circulation itself. When evaluating the circulation itself further, we showed that even relatively healthy young Fontan patients have an impaired exercise capacity which is correlated with diastolic dysfunction and aortic stiffness. During exercise, patients showed a good chronotropic competence, implicating a preserved cardiac autonomic function. Preserved autonomic function could also be conducted from the sufficient response to acute fluid depletion, i.e., orthostatic stress. In contrast to limited exercise capacity, paediatric Fontan patients are still able to respond well to acute fluid changes and seem able to adapt well to the circulation.

As shown in this thesis, future therapeutic strategies should also be aimed at treating and preventing diastolic dysfunction and aortic stiffness in Fontan patients to optimize and hopefully prevent deterioration of the circulation. However, both diastolic dysfunction and increased aortic stiffness are difficult to treat and no single treatment option is yet available. Diastolic dysfunction may be caused by impaired ventriculo-arterial coupling and myocardial fibrosis (54, 55). The optimal treatment would keep ventricular filling pressure low and enhance ventricular pull, however, such an effective lusotropic drug is still lacking. In addition, anti-hypertensive drugs can be prescribed to lower the systemic vascular resistance, such as ACE inhibitors, and they may lower the blood pressure by a vasodilator effect but not effectively improve the aortic compliance and reduce aortic stiffness, as shown in this thesis. Possible treatment for improving aortic stiffness with anti-inflammatory drugs or endothelin-A receptor antagonist have shown some promising but also conflicting results in patients with a biventricular heart circulation (56). However, each treatment may only impact a part of the cause of the aortic stiffness. Future research is needed to first determine the exact pathophysiology and then develop medical therapies that can improve both diastolic function and arterial stiffness.

Furthermore, although we recognized diastolic dysfunction and arterial stiffness as limiting factors, we did not study all possible potential limiting factors and only studied a relatively specific group of patients. For example, the pulmonary circulation, which has been recognized as one of the major limiting factors of Fontan circulation, has not been investigated. Nevertheless, improving the pulmonary circulation by pulmonary vasodilators has been the key focus of some recent large randomized studies and showed a slight increase in exercise capacity after treatment, which is promising (57). Whether this will prevent circulatory deterioration in the long term, however, needs further investigation. In addition, although systolic ventricular function was not a limiting factor in our population, systolic function is known to deteriorate, leading to heart failure on the long term. Therefore, preventing systolic ventricular function to deteriorate and treating systolic heart failure will still be one

of the therapeutic targets. However, from this thesis, we can conclude that there is no beneficial effect of short-term ACE inhibition in Fontan patients with moderate to good systolic ventricular function. If ACE inhibition might play a role in treating patients with significant systolic dysfunction has still to be determined.

As the Fontan population consists of a heterogeneous group of patients, the cause of deterioration of the circulation and eventually Fontan failure can vary between patients. Future studies are needed not only to develop medical therapies for the various possible limiting factors, but also to be able to recognize the limiting factors and determine which patients benefit most from which therapy.

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Chapter 10

Nederlandse samenvatting

De Fontan procedure bestaat uit een reeks operaties, die wordt verricht bij patiënten met een univentriculair hart om zo de overleving en de kwaliteit van leven van deze patiënten te kunnen verbeteren. Bij een univentriculaire hartafwijking is er maar één functionele ventrikel, ook wel hartkamer genoemd, aanwezig. Dit kan zowel de linker- als de rechterventrikel zijn. In een univentriculaire circulatie wordt het bloed van zowel de long- als de systeemcirculatie rondgepompt door de enige aanwezige ventrikel. Deze situatie leidt zonder ingrijpen tot vroegtijdige complicaties en overlijden. Bij de Fontan procedure worden de pulmonale en systemische circulatie van elkaar gescheiden door de systemische veneuze retour rechtstreeks naar de longslagaders te leiden. Stapsgewijs zal hierbij eerst de vena cava superior en later de vena cava inferior direct op de pulmonaal arteriën worden aangesloten. De laatste stap, het aansluiten van de vena cava inferior op de pulmonaal arteriën kan door middel van een intra-atriale tunnel of een extra cardiale tunnel. Na de Fontan palliatie ontbreekt er een ventrikel die bloed door de pulmonale circulatie pompt. Hierdoor is na palliatie veneuze druk noodzakelijk om de pulmonale vaatweerstand te overwinnen om zo het bloed passief door de pulmonale circulatie te laten stromen.

De laatste decennia is, door vooruitgang in chirurgische technieken, de overleving verbeterd en is het aantal patiënten met een Fontan circulatie dat volwassen wordt toegenomen. Ondanks verbetering in overleving hebben veel Fontan patiënten complicaties, zoals onder andere ritmestoornissen en hartfalen, en is er nog steeds sprake van een verminderde levensverwachting. Een duidelijke therapeutische strategie om de Fontan circulatie te verbeteren en falen van de Fontan circulatie te voorkomen ontbreekt echter nog. Hoewel veel centra hun therapie hebben gericht op het behandelen of voorkomen van systolische ventriculaire disfunctie met behulp van angiotensine-converterende enzym (ACE) remmers, ontbreekt nog steeds bewijs voor de werkzaamheid daarvan bij deze patiënten. In dit proefschrift bestudeerden we daarom de effecten van ACE-remming op verschillende cardiovasculaire en hemodynamische eigenschappen van de Fontan circulatie van pediatrische patiënten. Om de effectiviteit van ACE-remming bij Fontan patiënten verder op te helderen en de pathofysiologie van Fontan falen te begrijpen, bestudeerden we bovendien de hemodynamiek van de Fontan circulatie van pediatrische patiënten. Om een homogene groep patiënten te kunnen onderzoeken werden voor de studie alleen patiënten met een goede of licht verminderde systolische ventrikel functie en met een extra-cardiale Fontan tunnel geïncludeerd. Verschillende pathofysiologische mechanismen kunnen de Fontan circulatie negatief beïnvloeden, zoals systolische en diastolische ventrikeldisfunctie, verminderde vasculaire compliantie en falen van het autonome zenuwstelsel. Aangezien er weinig bekend is over de normale

ontwikkeling van de autonome functie bij gezonde kinderen, bestudeerden wij zowel de normale ontwikkeling van de autonome functie als de autonome functie van pediatrische Fontan patiënten. Ten slotte is in dit proefschrift de literatuur over plastische bronchitis, een ernstige complicatie van de Fontan-circulatie, systematisch bestudeerd om verschillende aspecten van plastische bronchitis bij Fontan patiënten te evalueren.

Alle verschillende onderdelen van dit proefschrift met bijbehorende bevindingen worden hieronder besproken. Eerst zullen de resultaten van de cardiale autonome functie bij gezonde kinderen en pediatrische Fontan patiënten worden besproken. Daarna worden de resultaten van de evaluatie van de Fontan circulatie en de effecten van ACE-remming geëvalueerd. Tenslotte zullen de resultaten van een systematisch literatuuronderzoek van plastische bronchitis worden toegelicht.

Cardiale autonome zenuwactiviteit

Het autonome zenuwstelsel, bestaande uit het parasympathische en sympathische zenuwstelsel, beïnvloedt de bloedsomloop door onder meer de bloeddruk en de hartslag te regelen. De belangstelling voor cardiale autonome zenuwactiviteit is de laatste jaren toegenomen vanwege de relevantie ervan voor bij hart- en vaatziekten. De normale ontwikkeling van cardiale autonome zenuwactiviteit wordt echter nog steeds niet volledig begrepen. Daarom hebben we in dit proefschrift in **hoofdstuk 3** de rijping van de cardiale parasympathische en sympathische activiteit beoordeeld door het meten van de parasympathische activiteit, middels het berekenen van respiratoire sinus aritmie en de hartslag variabiliteit, en de sympathische activiteit, middels het berekenen van de pre-ejectie periode. Voor deze studie werden cardiale parasympathische en sympathische waarden uit 5 verschillende studies gecombineerd, wat resulteerde in een multi-cohortstudie van 4820 gezonde proefpersonen in de leeftijd van 0.5 tot 20 jaar (6-10). Uit onze studie bleek dat de parasympathische activiteit snel toenam na de geboorte, afvlakte tijdens de kindertijd (≈11 jaar), en vervolgens afnam tot in de adolescentie, terwijl de sympathische activiteit een lineaire afname vertoonde van de vroege kindertijd tot in de late adolescentie. Dergelijke patronen kwamen goed overeen met eerdere studies (11-28). Daarnaast was er een verschil in rijping tussen jongens en meisjes, waarbij de parasympathische activiteit bij jongens een latere piek liet zijn in vergelijking met meisjes. Deze latere piek bij jongens is waarschijnlijk het gevolg van verschillen in hormonale veranderingen, aangezien meisjes eerder in de puberteit komen dan jongens. Hoewel de rijpingstrends duidelijk zijn, is er een grote interindividuele variabiliteit in absolute autonome zenuwactiviteit. Deze interindividuele variabiliteit

kan worden veroorzaakt door verschillende factoren, waaronder etniciteit, verschillen in ontwikkeling van lichaamsgrootte, lichaamssamenstelling en start van de puberteit, verschillen in vullingsstatus, hartgrootte, gevoeligheid van de baroreflex, longstrekreflexen en ademhalingsgedrag (frequentie en teugvolume), aangenomen leefstijlpatronen (roken, voedingsgewoonten en lichaamsbeweging), en psychosociale stressblootstellingen. Bovendien kunnen ook de testomstandigheden de absolute waarden beïnvloeden, zoals het tijdstip van de dag, de lichaamshouding, eerdere lichamelijke activiteit en zelfs de analytische strategie om de hartactiviteit van de sympathicus en parasympathicus te kwantificeren. Dit kan het gebruik van individuele absolute parameters beperken, hoewel groepsvergelijkingen toch waardevol kunnen zijn.

In **hoofdstuk 4** vergeleken we de absolute waarden van de cardiale autonome zenuwactiviteit van Fontan patiënten met die van gezonde controles. Terwijl de parasympathische activiteit tussen beide groepen vergelijkbaar was, was de pre-ejectieperiode verhoogd bij Fontan patiënten, wat wijst op een lagere sympathische activiteit. Dit staat in contrast met eerdere studies bij volwassen Fontan patiënten die een veranderde autonome functie lieten zien met verminderde parasympathische- en verhoogde sympathische activiteit. Voorzichtigheid is echter geboden bij de interpretatie van de pre-ejectieperiode bij Fontan patiënten, omdat deze gevoelig is voor de effecten van pre- en afterload van het hart, die beide anders zijn bij Fontan patiënten in vergelijking met gezonde controles. Onze bevindingen wijzen op een behouden autonome functie bij jonge Fontan patiënten die in het huidige tijdperk worden behandeld. Met toekomstig onderzoek met meer invasieve metingen kan wellicht beter inzicht worden verkregen in de sympathische activiteit aangezien niet-invasieve metingen beperkte waarde hebben. Aangezien autonome disfunctie is beschreven bij volwassen Fontan patiënten en autonome functie een rol kan spelen bij de achteruitgang van de Fontan circulatie, is het interessant voor toekomstig onderzoek om de ontwikkeling van de cardiale autonome zenuwactiviteit bij Fontan patiënten te onderzoeken.

Evaluatie van de Fontan circulatie

Meerdere pathofysiologische mechanismen kunnen de Fontan circulatie beïnvloeden. Een combinatie van maximale inspanningstesten en het gebruik van volumebelastingtesten, door middel van het acuut verlagen of verhogen van het centraal circulerend volume, kan meer inzicht geven in deze mechanismen.

Maximale inspanningstest

Tijdens een maximale inspanningstest is een adequate toename van de cardiale output (hartminuutvolume) noodzakelijk, welke wordt beïnvloed door de hartslag, preload, contractiliteit van de ventrikel en de afterload. Het is bekend dat bij Fontan patiënten de inspanningscapaciteit verminderd is. Zoals aangetoond in **hoofdstuk 4** kan het percentage van de voorspelde inspanningscapaciteit variëren van 54-72% in één cohort, afhankelijk van de gebruikte parameter, wat vergelijkbaar is met eerdere literatuur.

De belangrijkste beperkende factor van de Fontan circulatie is het onvermogen om de veneuze retour naar het hart te vergroten door het ontbreken van een ventrikel voor de pulmonale circulatie. Bij onze patiëntenpopulatie observeerden we een adequate toename van de hartslag, dat wil zeggen een goede chronotrope competentie, terwijl het slagvolume tijdens piekinspanningen afnam. We vonden dat een verminderde diastolische functie, die van invloed is op de preload, en een verhoogde arteriële stijfheid, die van invloed is op de afterload, gecorreleerd waren met verminderde inspanningscapaciteit. Het is bekend dat diastolische disfunctie en verhoogde arteriële stijfheid al op jonge leeftijd aanwezig zijn bij Fontan patiënten en dat beide in de loop van de tijd kunnen verergeren en daardoor kunnen bijdragen aan een verslechtering van het inspanningsvermogen. In tegenstelling tot andere onderzoeken was de systolische functie, dat wil zeggen de contractiliteit, in onze studie niet gecorreleerd met de inspanningsprestatie. Dit kan worden verklaard door de selectiecriteria van ons studiecohort, waarbij patiënten met een systolische disfunctie werden uitgesloten. Hoewel we de relatie tussen inspanningscapaciteit en verschillende cardiovasculaire parameters hebben onderzocht, hebben we geen andere parameters bestudeerd, zoals de invloed van resterende obstructies in de Fontan tunnel of longslagaders; of verhoogde pulmonale vasculaire weerstand die zal leiden tot energieverlies en verminderde pulmonale bloedstroom.

Acute volumebelastingtesten

Terwijl acute volumebelastingtesten vroeger vaak werden uitgevoerd middels invasieve (laboratorium) studies, worden ze de laatste decennia ook via niet-invasieve studies uitgevoerd. Dit kan door middel van passief heffen van de benen, om zo toename van het centraal circulerend volume na te bootsen, en middels een passieve kieptafel test, welke acute depletie van het centraal circulerend volume weerspiegelt.

Het passief heffen van de benen, als test om de hemodynamische reactie op verhoging van preload te evalueren, is eerder nog niet toegepast bij Fontan

patiënten. Het passief heffen van de benen is een niet-invasieve en betrouwbare test om de reactie op een vulling te voorspellen. In dit proefschrift, in **hoofdstuk 5**, werd de hemodynamische reactie op passief heffen van de benen onderzocht bij Fontan patiënten en gezonde controles. Over het algemeen was de hemodynamische respons van Fontan patiënten vergelijkbaar met de respons van gezonde controles. Bovendien hadden Fontan patiënten die niet reageerden met een toename van het slagvolume niet meer negatieve effecten van het passief benen heffen dan controles. Echter, in een eerdere katheterisatiestudie was een toename van de preload gerelateerd aan een toename van de eind diastolische druk, waardoor de transpulmonale gradiënt, het slagvolume en uiteindelijk de cardiale output bij een deel van de patiënten afnamen. Aangezien de respons op een verhoging van de preload kan worden beïnvloed door verschillen in vullingscondities, kan dit de verschillende resultaten van onze studie in vergelijking met de literatuur verklaren.

Kieptafeltesten kunnen worden gebruikt om een acute orthostatische stressreactie te testen. Een goede reactie op orthostatische stress vereist activatie van het sympathische zenuwstelsel en gelijktijdige afname van vagale activiteit om de hartslag, systemische vasoconstrictie en veneuze retour te verhogen om een adequate bloeddruk en cerebrale bloedstroom te kunnen handhaven. Eerdere studies, meestal uitgevoerd bij volwassen Fontan patiënten, hebben verschillende resultaten laten zien met over het algemeen een verminderde cardiale autonome zenuwactiviteit en verminderde baroreflex gevoeligheid, resulterend in een verminderde autonome reactie op orthostatische stress. In tegenstelling tot deze onderzoeken toonden wij in **hoofdstuk 6** aan dat pediatrische patiënten een voldoende adequate autonome respons hadden tijdens kieptafeltesten met behoud van adequate bloeddruk en cardiale output. Voldoende autonome respons werd aangetoond door een toename in hartslag en aortastijfheid, beide indicatief voor intacte autonome regulatie, en door dezelfde mate van afname in cardiale parasympathische activiteit tussen patiënten en controles. Aangezien eerdere onderzoeken werden uitgevoerd bij volwassen patiënten, kan de leeftijd het verschil in resultaten hebben beïnvloed. Bovendien werd de cardiale output gehandhaafd, wat kan betekenen dat patiënten waarschijnlijk in staat zijn om zich aan te passen aan de Fontan circulatie. Deze patiënten lijken tijdens staan veneuze pooling te vermijden om zo ook een ernstige daling van de centrale veneuze druk te voorkomen. Hoewel pediatrische patiënten zich lijken aan te passen, laten onderzoeken bij volwassen Fontan patiënten een grote afname van de cardiale output zien als reactie op orthostatische stress, wat suggereert dat de aanpassingsmechanismen op de lange termijn mogelijk tekortschieten. Het is interessant om zowel patiënten die goed reageren als patiënten die dat niet doen verder te onderzoeken om het verschil

op te helderen en mogelijk risicofactoren te identificeren voor verslechtering van de circulatie in de loop van de tijd.

ACE remming

Omdat veel Fontan patiënten, zelfs zonder systolische ventrikeldisfunctie, momenteel behandeld worden met ACE remming zonder bewijs van effectiviteit, hebben we in dit proefschrift, in **hoofdstuk 6 en 7**, het effect van ACE remming op de Fontan circulatie onderzocht. De eerdergenoemde methoden om de circulatie te onderzoeken werden ook gebruikt om de effecten van een behandeling met enalapril van 3 maanden te evalueren.

Wij toonden aan dat een behandeling met enalapril gedurende 3 maanden geen effect had op de inspanningscapaciteit van de Fontan patiënten en ook de arteriële stijfheid en zowel de diastolische als systolische ventrikelfunctie niet verbeterde, wat vergelijkbaar was met de twee eerdere kleinere onderzoeken die zijn uitgevoerd bij Fontan patiënten. Enalapril verlaagde echter wel de systolische bloeddruk en de NT pro-BNP-spiegels. Lagere NT pro-BNP-spiegels zouden kunnen duiden op een lagere vullingsdruk, hoewel dit niet direct gemeten is in deze studie, is dit effect van enalapril eerder aangetoond in een kleine katheterisatiestudie bij univentriculaire hartpatiënten. Hoewel enalapril de bloeddruk in rust verlaagde, leidde het niet tot orthostatische hypotensie of een afname van de cardiale output tijdens kieptafeltesten. Tijdens de titratieperiode, waarin de dosering van enalapril werd verhoogd, kreeg een groot deel van de patiënten last van bijwerkingen (42%), zoals een aanzienlijke daling van de systolische bloeddruk, duizeligheid en syncope. Na de titratieperiode kregen patiënten een stabiele dosering, die beter werd verdragen. Met andere woorden, ACE remming heeft een vaatverwijdend effect bij Fontan patiënten wat leidt tot verlaging van de bloeddruk, maar na de titratieperiode zijn de meeste bijwerkingen verdwenen. Deze gewenning wordt verder ondersteund door de goede orthostatische tolerantie wanneer de patiënten zijn ingesteld op enalapril.

Hoewel wij op de korte termijn geen gunstige effecten van enalapril zagen, zou remming van het cardiale renine-angiotensine-aldosteron systeem (RAAS) op de lange termijn de cardiale remodellering en fibrosering van cardiomyocyten kunnen beperken wat verdere achteruitgang van de ventriculaire functie zou kunnen voorkomen. Toekomstig onderzoek is echter nodig om te onderzoeken of ACE-remming of andere RAAS-remming op de lange termijn gunstige effecten kan hebben bij Fontan patiënten.

Plastische Bronchitis

Plastische bronchitis is een ernstige complicatie die kan ontstaan als de Fontan circulatie faalt. Plastische bronchitis wordt gekenmerkt door de vorming van mucofibrineuze afgietsels in de tracheobronchiale boom, wat leidt tot luchtwegobstructie en mogelijk verstikking. Systematisch literatuuronderzoek in **hoofdstuk 8** toonde aan dat het algehele sterftecijfer bij patiënten met één ventrikel 15.2% was, met een lager algeheel sterftecijfer van 10.5% in gevallen gepubliceerd na 2012. Mortaliteit was geassocieerd met de diagnose van plastische bronchitis binnen 12 maanden na de Fontan palliatie, wat erop zou kunnen wijzen dat een vroeg begin van plastische bronchitis een manifestatie kan zijn van een slechte Fontan circulatie. Verder werd een hogere leeftijd bij de Fontan operatie gevonden als een extra risicofactor, maar of deze bevinding is beïnvloed door andere factoren kon niet worden vastgesteld omdat veel andere mogelijke risicofactoren vaak ontbraken in de studies.

In de literatuur vonden we dat de meeste patiënten met plastische bronchitis werden behandeld met een combinatietherapie, bestaande uit het verwijderen van de luchtwegobstructie, anti-inflammatoire behandeling en behandeling om de hemodynamiek van de Fontan circulatie te verbeteren. Dit leidde uiteindelijk in de meeste gevallen tot een succesvolle behandeling van plastische bronchitis. Op basis van de gegevens uit onze systematische review hebben we daarom een behandelingsalgoritme ontwikkeld waarbij in de acute fase bronchoscopie voor directe verwijdering van een obstructie van de luchtwegen en verneveling met weefselplasminogeenactivator kunnen worden overwogen, naast diagnostische evaluatie. Na de acute fase kan de behandeling het beste bestaan uit een combinatie van alle drie de behandelstrategieën. Het verbeteren van de hemodynamiek kan in deze fase bestaan uit het optimaliseren van de Fontan circulatie door medicatie, chirurgie, antiaritmische therapie en/of het remmen van lymflekkage. Als de plastische bronchitis nog steeds niet verbetert, kan de hemodynamiek worden verbeterd door meer invasieve strategieën, d.w.z. decompressie van de circulatie door het creëren van een fenestratie of een Fontan takedown, en zelfs harttransplantatie kan worden overwogen. In onze casus, die ook in het artikel wordt gepresenteerd, was een tijdelijke Fontan-takedown levensreddend. In de periode dat de Fontan tunnel, de verbinding van de vena cava inferior naar de pulmonaal arteriën, niet meer aanwezig was kon de plastische bronchitis oplossen, herstelde de pulmonale circulatie zich en kon de Fontan circulatie later met succes worden hersteld. Tot slot raden we aan om patiënten profylactisch te behandelen

met antibiotica als preventieve therapie indien de bronchitis is verdwenen. Dit omdat een recidief is geassocieerd met luchtweginfecties.

Hoewel wij een goed overzicht hebben gegeven van alle gevallen in de literatuur, ontbraken veel gegevens van veel gevallen om preciezer te kunnen bepalen welke patiënten risico lopen op het ontwikkelen van plastische bronchitis en welke behandelingsoptie/-strategie het beste zal werken. Grote multicenter cohortonderzoeken zijn nodig om de exacte pathofysiologie te ontrafelen, om te bepalen welke patiënten risico lopen op het ontwikkelen van plastische bronchitis en welke therapeutische strategie het meest effectief is.

Conclusie en toekomstperspectieven

In dit proefschrift werden verschillende aspecten van de Fontan circulatie bestudeerd in een relatief homogene groep pediatrische patiënten, met een extra-cardiale Fontan tunnel en een goede of licht verminderde systolische ventriculaire functie, om niet alleen de effectiviteit en bijwerkingen van ACE-remming te evalueren, maar ook de beperkingen van de inspanningscapaciteit en hemodynamische reactie op acute volumebelastingtesten te bestuderen.

Wij toonden aan dat ACE remming op korte termijn geen positieve effecten had op de inspanningscapaciteit, cardiovasculaire en ventriculaire functie, maar naast de bijwerkingen tijdens de titratieperiode ook geen negatieve effecten had op de bloedsomloop zelf. Toen we de circulatie verder evalueerden, toonden we aan dat zelfs relatief gezonde jonge Fontan patiënten een verminderde inspanningscapaciteit hebben die gecorreleerd is met diastolische disfunctie en aortastijfheid. Tijdens inspanning vertoonden de patiënten een goede chronotrope competentie, wat duidt op een behouden cardiale autonome functie. De behouden autonome functie zou ook kunnen worden afgeleid uit de voldoende respons op acute depletie van het centraal circulerend volume, dat wil zeggen reactie op orthostatische stress. In tegenstelling tot de beperkte inspanningscapaciteit zijn pediatrische Fontan patiënten nog steeds in staat om goed te reageren op acute volumeveranderingen en lijken ze zich goed te kunnen aanpassen aan de Fontan circulatie.

Zoals aangetoond in dit proefschrift, moeten toekomstige therapeutische strategieën ook gericht zijn op het behandelen en voorkomen van diastolische disfunctie en aortastijfheid bij Fontan patiënten om zo de circulatie te optimaliseren en hopelijk verslechtering te voorkomen. Zowel diastolische disfunctie als verhoogde aortastijfheid zijn echter moeilijk te behandelen. Diastolische disfunctie

kan worden veroorzaakt door verminderde ventriculo-arteriële koppeling en myocardiale fibrose. De optimale behandeling zou de ventriculaire vullingsdruk laag houden en de ventriculaire trekkracht verbeteren, maar een dergelijk effectief lusotroop geneesmiddel ontbreekt nog. Bloeddrukverlagende medicijnen kunnen worden voorgeschreven om de systemische vaatweerstand te verlagen, zoals ACE remming. Echter, ondanks dat deze medicijnen de bloeddruk kunnen verlagen door een vasodilaterend effect, zijn ze niet effectief in het verbeteren van de compliantie van de aorta en verlagen van de aortale stijfheid, zoals aangetoond in dit proefschrift. Mogelijke behandelingen om de aortastijfheid te verbeteren met ontstekingsremmers of endotheline-A-receptorantagonisten hebben veelbelovende maar ook tegenstrijdige resultaten laten zien bij patiënten met een biventriculaire hartcirculatie. Elke behandeling kan echter slechts een deel van de oorzaak van de aortastijfheid beïnvloeden. Toekomstig onderzoek is nodig om eerst de exacte pathofysiologie te bepalen en vervolgens medische therapieën te ontwikkelen die zowel de diastolische functie als de aortastijfheid kunnen verbeteren.

Hoewel we diastolische disfunctie en aortastijfheid als beperkende factoren erkenden, hebben we niet alle mogelijke beperkende factoren bestudeerd en slechts een relatief specifieke groep patiënten onderzocht. De pulmonale circulatie, die wordt gezien als een van de belangrijkste beperkende factoren van Fontan circulatie, is bijvoorbeeld niet onderzocht. Desondanks is het verbeteren van de pulmonale circulatie door pulmonale vasodilatatoren het belangrijkste aandachtspunt geweest in een aantal recente grote gerandomiseerde onderzoeken en is een lichte toename in inspanningscapaciteit na behandeling met pulmonale vasodilatatoren aangetoond, wat veelbelovend is. Of dit verslechtering van de Fontan circulatie op de lange termijn voorkomt, moet echter verder worden onderzocht. Hoewel de systolische ventrikelfunctie geen beperkende factor was in onze populatie, is bekend dat de systolische ventrikelfunctie op termijn verslechtert en leidt tot hartfalen op de lange termijn. Daarom is het voorkomen van verslechtering van de systolische ventrikelfunctie en het behandelen van systolisch hartfalen nog steeds één van de therapeutische doelen. Uit dit proefschrift kunnen we echter concluderen dat er geen gunstig effect is van een kortdurende behandeling met ACE remming bij Fontan patiënten met een goede of licht verminderde systolische ventrikelfunctie. Of ACE remming een rol zou kunnen spelen bij de behandeling van patiënten met een significante systolische disfunctie moet nog worden vastgesteld.

De Fontan populatie bestaat uit een heterogene groep patiënten en daarom kan de oorzaak van de verslechtering van de circulatie en uiteindelijk Fontan falen per patiënt verschillen. Toekomstig onderzoek is niet alleen nodig om medische

therapieën te ontwikkelen voor de verschillende mogelijke beperkende factoren, maar ook om de beperkende factoren te kunnen herkennen en te bepalen welke patiënten het meeste baat hebben bij welke therapie.

A

Appendices

Abbreviations

AAIR	Atrial-pacing Atrial-sensing Inhibited-response Rate-adaptive
ABCD	Amsterdam Born Children and their Development
ACE	Angiotensin converting enzyme
AIXao	Augmentation index
ANS	Autonomic nervous system
APC	Atriopulmonary connection
CI	Cardiac index
E/A	Ratio of peak early and late conventional diastolic velocities
E/E'	Ratio of peak early conventional and Tissue Doppler diastolic velocity
ECC	Extracardiac conduit
etCO2	End-tidal CO2
FemNAT-CD	Neurobiology and Treatment of Adolescent Female Conduct Disorder
HF	High-frequency power
HP	Heart period
HR	Heart rate
HRV	Heart rate variability
HUTT	Head-up tilt testing
ICC	Intraclass correlation coefficient

ICG	Impedance cardiography
ILT	Intra-atrial lateral tunnel
IVC	Inferior vena cava
LF	Low-frequency power
LF:HF	Ratio of low-frequency and high frequency power
MINDS	Mother-Infant Neurodevelopment Study
NT-Pro BNP	N-terminal pro brain natriuretic peptide
NTR	Netherlands Twin Register
OUES	Oxygen uptake efficiency slope
PB	Plastic bronchitis
PEP	Pre-ejection period
PLE	Protein-losing enteropathy
PLR	Passive leg raising
PNS	Parasympathetic nervous system
PWVao	Aortic pulse wave velocity
RAAS	Renin-angiotensin-aldosterone system
RMSSD	Root mean square of successive differences between normal sinus beats
RSA	Respiratory sinus arrhythmia
SBP	Systolic blood pressure

SD	Standard deviation
SDNN	SD of the inter-beat interval of normal sinus beats
SNS	Sympathetic nervous system
SV	Single ventricle
SVI	Stroke volume index
TCPC	Total cavopulmonary connection
VE/VCO_2 slope	Slope of respiratory minute volume to CO_2
$VO_{2\ peak}$	Peak oxygen uptake
VTI	Velocity time integral
VU-AMS	VU University Ambulatory Monitoring System

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Curriculum Vitae

Lisette Harteveld was born on 21st of March 1990 in Voorburg, the Netherlands. After finishing secondary school cum laude at the Huygenslyceum in Voorburg, she began studying Medicine at the University of Leiden in 2008. During her Medical studies, in addition to studying, she spent time with the Leiden Medical Society Forestus, playing sports and participating in various committees and boards. In the 2011-2012 academic year, she interrupted her medical studies for one year to become a board member of the Medical Faculty of Leiden Students. At the beginning of her master's, she did a research internship at the Department of Paediatric Cardiology at the Willem Alexander Children's Hospital, Leiden University Medical Centre (LUMC), in Leiden, on risk factors for left ventricular dysfunction in paediatric patients with idiopathic frequent premature ventricular complexes and asymptomatic ventricular tachycardia. This research led to her first publication and a presentation during the annual conference of the Association for European Paediatric and Congenital Cardiology. After obtaining her master's degree cum laude in 2015, she started working as a medical resident in the Paediatric Intensive Care Unit at the LUMC in 2016. In 2016, she was offered a PhD position at the Department of Paediatric Cardiology, Willem Alexander Children's Hospital, LUMC, in Leiden, under the supervision of Prof. Dr. Nico Blom and Dr. Arend D.J. ten Harkel, which had led to this thesis. During her PhD, she was trained as an echocardiographer and, in addition to her PhD, worked part-time as an echocardiographer within the Department of Paediatric Cardiology, Willem Alexander Children's Hospital, LUMC in Leiden from 2016 to 2020. She also completed her PhD part-time; since 2020, in addition to her PhD research, she worked as a medical resident not in training in the department of paediatrics at the Willem Alexander Children's Hospital, LUMC, in Leiden and at the Spaarne Gasthuis in Haarlem.

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