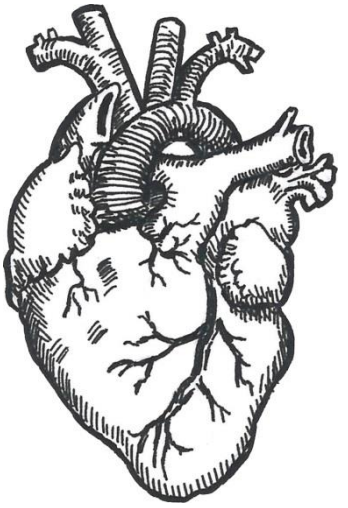
Cardiac autonomic nervous system control in pediatric congenital heart disease



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PARANIMPHS

Annelies Nederend

Nienke Schutte

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Cardiac autonomic nervous system control in pediatric congenital heart disease

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door

Ineke Nederend

geboren te Bodegraven

promotoren:	prof.dr. J.C.N. de Geus
	prof.dr. N.A. Blom
copromotor :	dr. A.D.J. ten Harkel

Contents

Chapter 1

Introduction

Congenital heart disease (CHD) is the most common congenital defect, affecting approximately 1 per 100 newborns. In the past century, major developments have been made in pediatric cardiac surgery including the first ligation of a patent ductus arteriosus by Robert Edward Gross[2] in 1938, placement of the first aortopulmonary shunt by Alfred Blalock, Vivian Thomas and Helen Taussig[3] in 1945, the first correction of coarctation of the aorta in the same year by Robert Gross and Clarence Craford[4;5], the first cardiopulmonary bypass in 1954 and the first repair of a ventricular septal defect in 1955 by Lillehei[6]. Due to the surgical techniques available today, survival in this patient group is good and a growing number of children with a CHD lives into adulthood[7]. After the critical first months of life, often including one or more corrective surgeries and long hospital stays, most patients live a relatively uncomplicated childhood without major cardiac problems. They feel good during childhood and early adolescence and they may believe that they are 'cured' of their CHD: the ''honeymoon period'' (*figure 1*). However later in life, cardiac problems including arrhythmias and heart failure tend to reappear. The exact mechanism behind this long term sequelae, the timing and who is at risk are unknown. It is important to study this in order to know how to prevent or reverse this process. A potential actor in this process is the autonomic nervous system (ANS).

The ANS acts unconsciously to regulate bodily functions and to ensure homeostasis in our body. This system does so by adjusting amongst others heart rate and blood pressure to the demands of the environment. The ANS consists of two branches; the sympathetic (SNS) and the parasympathetic (PNS) branch. While the SNS is responsible for the so-called ''fight or flight'' response, preparing the body for action, the PNS is responsible for the ''rest and digest'' state of the body. Heart rate is the net result of one's intrinsic heart rate (i.e. heart rate without any influence of the ANS on the sinus node) and the SNS and PNS control. When in rest, the parasympathetic activity prevails while at maximal exercise, the sympathetic branch dominates and the parasympathetic activity is reduced to zero. The ANS can be very effective as a compensatory mechanism when cardiac function is hampered, for example in heart disease. However, when the system is compensating for a long time –in most cases by increased SNS activity and decreased PNS activity- it may cause damage to the heart. This phenomenon is known to cause progression of heart failure[8]. It is plausible that the ANS also plays a role in the late problems seen in patients with a (corrected) CHD as the function of this system may be altered because of altered haemodynamics due to the heart defect, damage of ANS nerves as a result of corrective surgery or it might be part of the pathology.

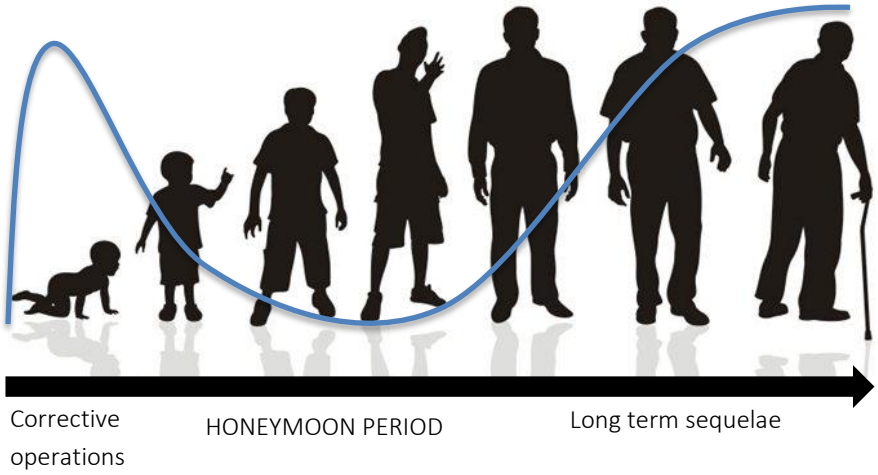

Figure 1

The activity of the cardiac ANS can be measured in several ways, ranging from highly invasive (e.g. spillover studies) to non-invasive (e.g. heart rate variability, chapter 3). One non-invasive method is using a combination of electrocardiography (ECG) and thorax impedance cardiography (ICG). The studies described in this thesis employ this method for the assessment of ambulatory cardiac ANS activity by use of the Vrije Universiteit Ambulatory Monitoring System (VU-AMS) device which is developed at the Vrije Universiteit[9]. Thoracic impedance can be derived by introducing a small alternating current through the thorax. The signal will show variation over time due to breathing (low frequency variation) and cardiac activity (high frequency variation). Simultaneously, an electrocardiogram is obtained which registers the electric activity of the cardiac muscle. By combining the ICG signal with the ECG, estimates of (changes in) cardiac SNS and PNS activity can be obtained. The ECG is used to extract heart rate variability (HRV). HRV coupled to the respiratory frequency, which can be derived from the thorax impedance, is a measure of cardiac PNS activity. Combining the ECG with the impedance cardiogram allows derivation of the pre-ejection period (PEP), a measure of cardiac SNS activity. Furthermore, ICG technology was originally developed by NASA to enable the non-invasive measurement of stroke volume (SV) and derived hemodynamics during manned flight [10].

In this thesis, I focus on two congenital heart defects: ventricular septal defect (VSD) and coarctation of the aorta (CoA). These are amongst the most common defects and different surgical techniques are applied for repair. Whereas a VSD is usually repaired via median sternotomy and cardiopulmonary bypass, CoA is repaired via left thoracotomy

without cardiopulmonary bypass. These different surgical techniques are expected to have a different effect on the ANS. A VSD is caused by incomplete closure of the intraventricular septum (*figure 2*, left). As a result, oxygen-rich blood in the left ventricle will shunt to the right ventricle. Depending on the size and the exact location of the defect, it may cause problems after birth. In hearts with a VSD, the defect typically causes a left-to-right shunt due to higher pressure in the left- compared to the right heart. The subsequent increased blood return to the left heart might cause enlargement of the left atrium and ventricle. Increased pulmonary pressure may cause feeding difficulties and failure to thrive. CoA is the local narrowing of the aorta, typically near the ductus arteriosus and just distal from the left subclavian artery (*figure 2*, right). As a result, the left ventricle will have to work harder to pump the blood out to the body. Also, blood flow towards the lower part of the body is compromised, causing an increased blood pressure in the upper part of the body and a decreased blood pressure in the lower extremities. The exact location and severity of the coarctation will determine the severity of the symptoms the patient presents with.

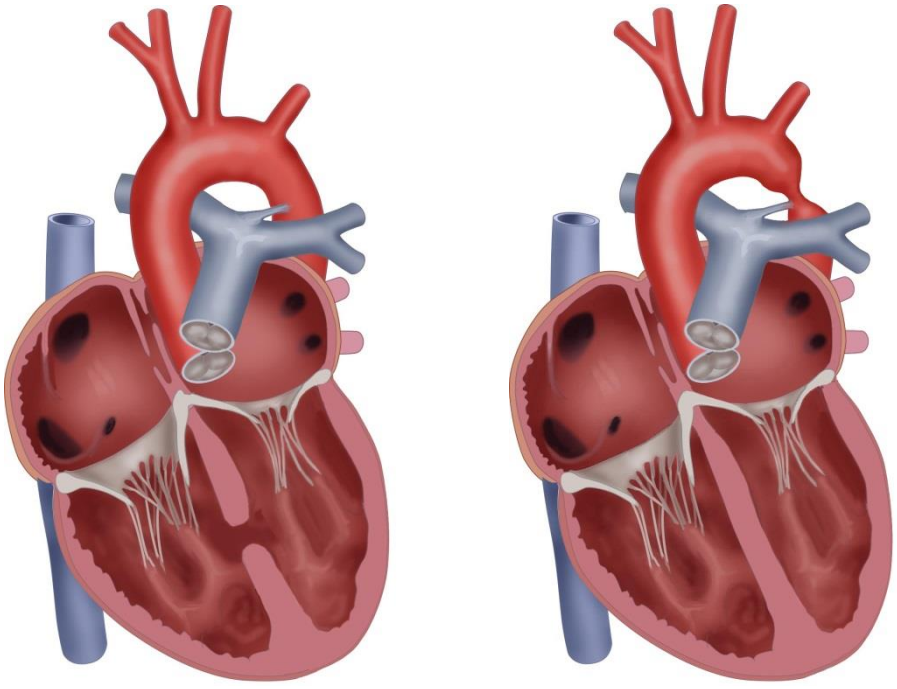

Figure 2 schematic of a ventricular septal defect (left) and coarctation of the aorta (right)

The aim of this thesis is to investigate the feasibility and validity of ambulatory recording of cardiac ANS activity and cardiac output using the ECG and ICG in healthy children and in children with a repaired CHD. Assuming valid recording of these signals, a second aim is to examine whether altered cardiac SNS and PNS activity is found in CHD patients in childhood.

Outline of the thesis

In chapter 2, the research design and data collection of the studies in chapter 4, 6 and 7 is described in detail. Chapter 3 provides an overview of the literature on cardiac ANS control in children with a CHD. This narrative review starts out with a description of the physiology of the cardiac ANS, ways to measure it and the normal maturation of the ANS. Thereafter, studies on ANS activity in children with an atrial- or ventricular septal defect, transposition of the great arteries, univentricular heart, coarctation of the aorta and tetralogy of Fallot are discussed. In the study described in chapter 4, the aim was to improve stroke volume estimation from spot electrode based impedance cardiography. Hereto, transthoracic echocardiography and impedance cardiography were measured simultaneously in a group of healthy volunteers and in a group of children after congenital heart disease repair in order to validate stroke volume measurements of the VU-AMS. The Kubicek equation which is currently used for stroke volume estimation in VU-DAMS (software package for data analysis), is based on band electrodes while nowadays spot electrodes are employed. The spot electrodes significantly decrease obtrusiveness and measurement burden on the subjects, thereby increasing feasibility of longer (e.g. 24-hour) recordings which arguably have a higher clinical relevance. However, the spot electrodes have a lower resistance compared to the band electrodes which has repercussions for the equation. As a first step, the problem of ambiguous ICG scoring was addressed; optimal methods were developed to select the correct point from multiple potential B-, C- and X-point candidates in the ICG signals. As a second step, stroke volume estimation was improved by adjusting the Kubicek formula for the difference in basic thoracic impedance. Chapter 5 describes a study in twins, in which we investigated heart rate recovery (HRR, the decrease in heart rate after exercise cessation), respiratory sinus arrhythmia (RSA) during exercise recovery, resting heart rate, resting RSA and exercise behavior in order to understand the origin of the individual differences in cardiac PNS functioning. HRR is largely dependent of PNS activity. The prognostic power of this measure is well known; a low HRR is associated with increased cardiac mortality. Twin studies enable the decomposition of total variance into innate biological factors, common environmental factors and unique environmental factors. Moreover, the extent to which a phenotypic correlation is explained by genetic factors can be estimated. In this study, multivariate twin modelling is used to estimate the heritability

of all measured variables and to compute the genetic contribution to their covariance. In chapter 6, differences in ambulatory cardiac ANS control and differences in exercise HRR between healthy controls and patients after VSD repair and the relationship with cardiac function are investigated. Chapter 7 studies differences in ambulatory cardiac ANS control and exercise HRR between healthy controls and patients after CoA repair and the relationship with cardiac function. In chapter 8, the main results of this thesis are summarized and recommendations are made for future research using ICG for ANS assessment and on research regarding cardiac ANS function in CHD patients.

Chapter 2

Research design and data collection

All data collection took place at the LUMC medical centre at the pediatric outpatient clinic, the department of radiology and the department of cardiology. Data was collected between February 2014 and March 2016. All study procedures were reviewed and approved by the Medical Ethics Review Committee of the LUMC medical centre (P13.198 & P14.095).

PARTICIPANTS

Healthy controls

Healthy controls between 1 and 18 years of age were recruited to take part in the study. Children from 1-4 years were recruited from the outpatient department of pediatric cardiology; those who had an innocent murmur or other complaints that turned out to be unrelated to a cardiac disorder were asked to participate. Healthy controls aged 4-18 were passively recruited through an advertisement at multiple schools in the vicinity of the hospital. The advertisement stated general information about the purpose and procedure of the study and that travel expenses would be reimbursed. When interested, parents could request the detailed study information package (see appendices at http://www.vu-ams.nl/research/phd-theses/) containing an information letter for the parents/caregivers, a letter for the child and an informed consent from which was sent by email or post. At least 24 hours later, the parent or caregiver was contacted in order to give the opportunity to ask additional questions and, if they volunteered to participate, to make an appointment. Chronic disease or medication use was questioned at first contact and were exclusion criteria. When the echocardiogram showed a structurally and functionally normal heart, the volunteer was included in the study. Descriptive characteristics of the healthy control group can be found in *table 1*.

Additionally, two separate control groups were recruited in order to study differences in exercise capacity and exercise heart rate dynamics and to study the heritability of heart rate recovery and vagal control. A group of healthy children aged between 8 and 18 years old was recruited for the comparison of exercise capacity and exercise heart rate dynamics. Exercise testing was performed at the Erasmus University Medical Centre as described in detail elsewhere [11]. In order to study the heritability of exercise heart rate recovery and vagal rebound, twin pairs between the age of 16 and 18 and their siblings within an age range of 12-25 years were recruited via the Netherlands Twin Register [12]. This procedure is described in chapter 5 of this thesis.

This chapter will describe data collection of the first mentioned control group for cardiac ANS activity in detail. The latter two control groups will not be discussed in detail here.

Patients

Patients after isolated ventricular septal defect (VSD) repair and patients after isolated coarctation of the aorta (CoA) repair from the outpatient clinic aged 8-18 years old were asked to participate via telephone. When interested, parents received an information letter for the parents/caregivers, a letter for the child and an informed consent (see appendices at http://www.vu-ams.nl/research/phd-theses/) which was sent by email or post. At least 24 hours later, the parent was called in order to give the opportunity to ask additional questions and, if they volunteered to participate, to make an appointment. The visit was part of regular clinical follow-up for these patients except for the 24 hour ambulatory monitoring which was done purely for research purposes. Children with chromosomal disorders were excluded. Descriptive characteristics of the patients can be found in *table 1*.

INFORMED CONSENT

All participants and both (one in the case of single-parent families) of their parents/guardians provided written informed consent (see appendices at http://www.vu-ams.nl/research/phd-theses/).

PROCEDURE

First, the procedure was explained to the participants and their parents when present. Next, participants were weighted, length was measured and they were queried on their medication use, voluntary exercise behaviour, gymnastics at school and walking and biking behaviour. Medication use of the patients was verified in the electronic patient file. Then, electrodes for thoracic impedance monitoring were attached, connected to the device and the participant was laid down for the transthoracic echocardiogram (TTE), which took on average 20 minutes. Thoracic impedance and echocardiography were recorded simultaneously (*figure 1*). After the echocardiogram, blood pressure was measured in sitting position. The participant was instructed to sit quietly for 4 minutes, while blood pressure was measured after 1 and 3 minutes. Thereafter, the images of the echocardiogram were shown and explained to the participant. The participant went home wearing the ambulatory impedance monitor which they took off after 24 hours. Healthy controls send the device back via mail using the pre-stamped return envelope we had provided.

Table 1 descriptive characteristics

	All controls	VSD patients	Matching controls VSD group	CoA patients	Matching controls CoA group
N	128	34	67	32	61
Male (%)	52	56	48	59	59
Age (y)	9.7 (5.2)	11.8 (2.8)	11.9 (3.0)	13.3 (2.9)	13.3 (3.1)
Length (cm)	139.2 (31.6)	150.5 (15.0)	153.3 (16.2)	159.0 (14.9)	160.9 (16.9)
Weight (kg)	38.3 (20.4)	42.7 (13.2)	44.2 (14.2)	52.0 (16.7)	50.9 (16.9)
BSA	1.2 (0.5)	1.3 (0.3)	1.4 (0.3)	1.5 (0.3)	1.5 (0.3)
Age at repair	-		-		-
Range	-	29d – 7.3y	-	3d – 5.8y	-
Median,IQR	-	0.5y,0.2-2.2	-	0.4y,0.0-1.3	-
Time after reair (y)	-	10.0 (3.0)	-	12.3 (3.5)	-

Mean(SD). D=days. Y=years. IQR=Inter Quartile Range. CoA=Coarctation of the Aorta. VSD=Ventricular Septal Defect. BSA=Body Surface Area (Dubois[13]).

Table 2. Education level of the study population.

	All controls	VSD patients	Matching controls VSD	CoA patients	Matching controls CoA
Not in school (N)	10 (8%)	0	0	0	0
Kindergarden (N)	18 (14%)	0	0	0	0
Primary school (N)	53 (41%)	19 (56%)	35 (52%)	12 (38%)	24 (39%)
High school (N)	45 (35%)	13 (38%)	32 (48%)	17 (53%)	35 (58%)
Vocational education (N)	2 (2%)	2 (6%)	0	3 (9%)	2 (3%)

Patients revisited the hospital the next day to hand in the device and perform a cardiopulmonary exercise test and for CoA patients also a cardiac Magnetic Resonance Imaging (MRI) study. *Figure 2* depicts the timeline of the protocol for the different groups. Standard physical examination by a pediatric cardiologist was done before the echocardiography in all patients and as during normal follow up, the pediatric cardiologist discussed all results with the patient and their parents after the visit. An overview of the study protocol for the different groups is depicted in *figure 2*. All subjects (both patients and healthy volunteers) were provided with a specially designed USB stick in the form of a bracelet containing their echocardiograph and animated movies of ''Kris the Crocodile''. The movies are of educative nature and feature the adventures of Kris, who is born with a congenital heart disease. Movies were made in collaboration with the Dutch Heart Foundation. *Figure 3* shows a screenshot of one of the movies, full movies can be found online at www.hartstichting.nl/hartvrienden/kris-krokodil.

Figure 1. Simultaneous recording of VU-AMS and echocardiography.

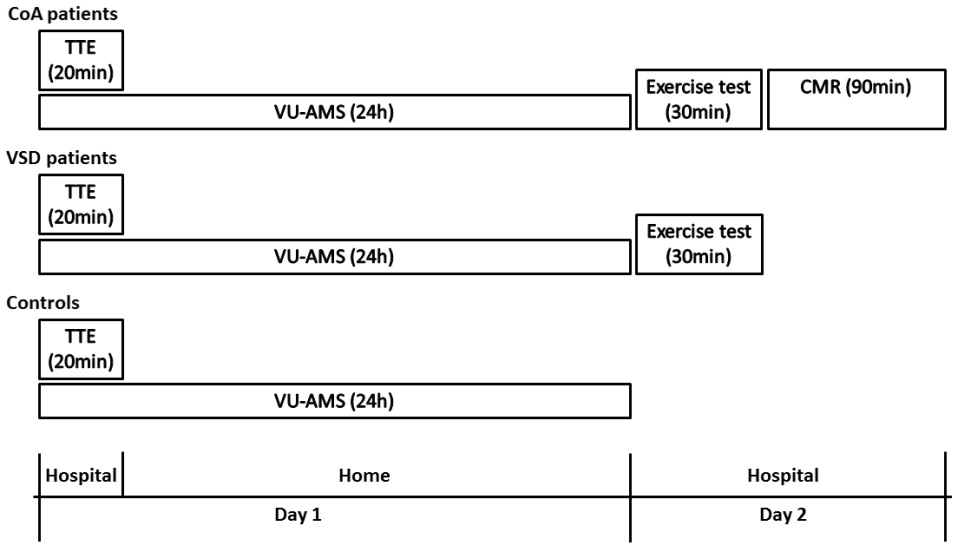

Figure 2. Timeline of the protocol.

Figure 3. Screenshot from animation movie ''Kris the Crocodile''.

VOLUNTARY EXERCISE BEHAVIOUR AND WEEKLY PHYSICAL ACTIVITY

Participants >4 years of age were queried on their voluntary exercise behaviour and weekly physical activity by the use of a short lifestyle interview. Parents were present during this interview and were, especially in the youngest children, asked to verify the answers. For each exercise activity (e.g. swimming, fitness, tennis, jogging, soccer) they were asked for how many years they have been doing that particular activity, how many months per year, how many times per week, and how many minutes each time. Participants had to have been active for at least 6 months and do it more than three months per year for the activity to be included as we were interested in regular exercise behaviour. Hence, ski holidays, sailing camps, and similar were excluded. Each exercise activity was converted into an MET (metabolic equivalent task) score[14]. One MET represents the amount of energy needed for sitting quietly. This MET score was multiplied by the frequency per week and duration per time of the exercise activity. MET scores of all endorsed exercise activities were summed to get a score (METhours/week) for voluntary exercise behaviour. Physical activity related to transportation (cycling, walking) and compulsory physical education classes were also queried. Conversion to a METhours score was done analogous to the voluntary exercise behaviour. Weekly physical activity was calculated as voluntary exercise behaviour (METhours/week) plus physical activity from walking, biking and physical education. *Table 3* summarizes voluntary exercise behaviour and weekly physical activity in the three groups, all divided into three age groups.

ELECTROCARDIOGRAM REGISTRATION AND THORACIC IMPEDANCE

ECG and ICG registration was done using the 5fs version of the VU Ambulatory Monitoring System (VU-AMS; VU University, Amsterdam, The Netherlands, www.vu-ams.nl), developed to study autonomic nervous system control in naturalistic settings[15;16]. One lead ECG was derived from 3 pregelled Ag/AgCl (Kendal H124SG) spot electrodes on the chest. ECG was sampled at 1kHz. Thoracic impedance (Z) was sampled at 250Hz and was conducted by introducing a small alternating current (50kHz, 350 µA) through the thorax, also by the use of spot electrodes. The measuring electrodes were placed just above and below the sternum. Current electrodes were placed 3 cm above and below the measuring electrodes (placement depicted in *chapter 6*) on the back of the thorax. The device was worn on the hip in a black bag (with a small stuffed animal for toddlers) attached to a belt. Variables extracted from the ECG and impedance cardiography (ICG) are summarized in *table 4*.

Table 3. Voluntary exercise behaviour and weekly physical activity

	Age group	Controls	VSD Patients	CoA patients
Voluntary exercise behaviour	4-8y	5.4, 0.9-15.1	-	-
(METhours/week)	8-12y	15.0, 6.2-27.6	24.0, 10.0-31.8	10.8, 0-28.8
	12-18y	23.4, 5.1-43.2	8.0,0-25.7	7.5, 0-27.8
Weekly physical activity	4-8y	18.8, 7.2-28.9	-	-
(METhours/week)	8-12y	33.8, 25.1-44.8	38.7, 25.8-44.5	29.3, 24.5-47.5
	12-18y	56.3, 40.3-78.9	46.7, 27.7-61.1	37.45, 28.4-58.3

Median, IQR.

Figure 4. Participant wearing the VU-AMS.

Table 4. Cardiac ANS variables extracted from the ECG and/or ICG used in this project

Measure *Parameter (unit)*	From	Parameter definition	Comment
Heart rate *HR (bpm)*	ECG	Number of heart beats per minute	Influenced by sympathetic and parasympathetic activity
Respiratory Sinus Arrhythmia *RSA (ms)*	ECG & dZ	Difference between the longest inter beat interval during exhalation and the shortest inter beat interval during inhalation	Relatively pure measure of cardiac parasympathetic control
Root mean square of successive differences *RMSSD (ms)*	ECG	Root mean square of successive differences	Relatively pure measure of cardiac parasympathetic control
Pre-ejection period *PEP (ms)*	ECG & ICG (=dZ/dt)	Time difference between the start of electrical depolarization and start of left ventricular outflow	Relatively pure measure of cardiac sympathetic control
Left ventricular ejection time *LVET (ms)*	ECG & ICG (=dZ/dt)	Time from the start to stop of left ventricular outflow	
Respiration rate *RR (breaths per minute)*	dZ	Number of breaths per minute	
Stroke volume *SV (ml)*	ECG & ICG (=dZ/dt)	Amount of blood pumped out per heart beat	
Cardiac output *CO (L)*	ECG & ICG (=dZ/dt)	Stroke volume * heart rate	

ECHOCARDIOGRAM

Transthoracic echocardiograms (TTE; Vivid 9, GE healthcare, Norway) were conducted by a pediatric cardiologist or an experienced technician and evaluated by one researcher, supervised by a pediatric cardiologist. Conventional echocardiography, consisting of M-mode, 2D echocardiography and echo-Doppler measurements was performed. Additionally, 3D echocardiography of the left ventricle was performed. Tissue Doppler Images (TDI) were recorded with a frame rate of 1s, strain images 50-90 frames per second. Variables extracted from TTE are summarized in *table 5*.

A specially designed time synchronization device (*figure 5*) was connected to the ultrasound machine (orange plug) and the VU-AMS (yellow plug). This device transmits a binary time code which makes it possible to exactly synchronize the ICG (VU-AMS) and TTE (GE) signals afterwards.

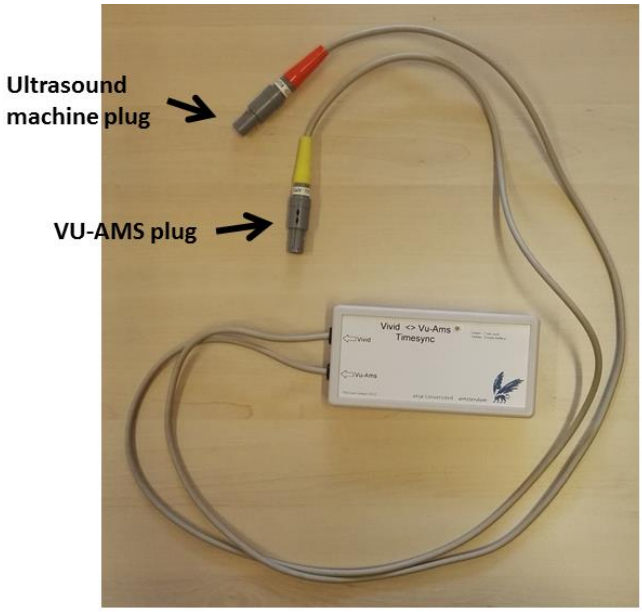

Figure 5. specially designed time synchronization device.

CARDIAC MAGNETIC RESONANCE IMAGING

Cardiac magnetic resonance imaging (MRI) was done in a 3T scanner (Ingenia, Philips Healthcare). Pulse wave velocity (PWV) and left ventricular (LV) wall mass were assessed in all patients. Analysis were done offline using in-house developed MASS software (Leiden, The Netherlands). PWV was determined from two high-temporal 1-directional velocity encoded time-resolved MRI acquisitions, planned perpendicular to the aorta, one at the ascending aorta at the level of the pulmonary trunk and one at the abdominal aorta 3 cm below the diaphragm. Flow mapping was performed to obtain velocity-time curves. The PWV was determined over both the proximal aorta (ascending aorta plus aortic arch and thoracic descending aorta) as well as the distal descending aorta. The validated foot-to-foot transit-time method was used to define PWV [17]. LV wall mass was assessed from a cine multi-slice short-axis data set acquired with steady-state free-precession gradient echo. Epi- and endocardial contours were drawn in every slice. Subsequently, the areas were subtracted, and the resulting areas were multiplied with slice thickness, number of slices and the density of myocardium. Contours were drawn end-diastole and end-systole by one researcher and supervised by one radiologist. The average from the end-diastolic mass and end-systolic mass was used for analysis. Variables extracted from MRI are summarized in *table 6*.

Table 5. Echocardiographic variables used in this project

Measure *Parameter (unit)*	From	Parameter definition	Comment
Stroke volume $SV_{biplane}$ *(ml)*	Apical 2- and 4-chamber view	Difference between end-diastolic and end-systolic left ventricular volume	
SV_{VTI} *(ml)*	Parasternal long axis (aorta diameter) and PW Doppler LVOT at aortic valve	multiplying the aortic cross sectional area with the velocity time integral from a pulsed wave Doppler flow signal over the left ventricular outflow tract	
SV_{3D} *(ml)*	Apical 4 chamber view	Difference between end-diastolic and end-systolic volume (calculated by the use of the divergence Theorem [17])	
Left ventricular ejection time *LVET (ms)*	PW Doppler LVOT at aortic valve	Time between the start and end of left ventricular outflow	
Left ventricular longitudinal global peak strain *Peak strain (%)*	Apical 4-chamber view using speckle tracking strain analysis	Peak from the time-strain curve	A lower peak strain equals worse contraction
Peak myocardial velocity *Peak systolic velocity (S') peak early (E') and late (A') diastolic velocities (in cm/s)*	Myocardial velocity curves were obtained for the left and right ventricular wall and the intraventricular septum	Peak velocities are measured in cm/s from the spectral Doppler tracings	S' is the maximal velocity of the myocardium during systole. E' is the maximal velocity during the early filling phase. A' is the maximal velocity during the late ventricular filling phase

CARDIOPULMONARY EXERCISE TEST

In all patients, cardiopulmonary exercise testing was performed at the LUMC Leiden (*figure 6*). The test was performed in upright position on a bike ergometer (GE healthcare type R3x 10416054 SA version V6.61). Maximal oxygen uptake was measured using breath by breath analysis (VIASYS Healthcare Type masterscreen CPX). The protocol consisted of an increasing workload per minute; starting wattage and increment per minute was defined by the physician. Patients were encouraged by the technician, physician and a researcher to exercise until exhaustion. After cessation of the test, the patient was instructed to stay seated and pedal at <40 rounds per minute with no resistance for at least 2 minutes. ECG was detached when the patient's heart rate returned to resting value. Variables extracted from the exercise test are summarized in *table 7*.

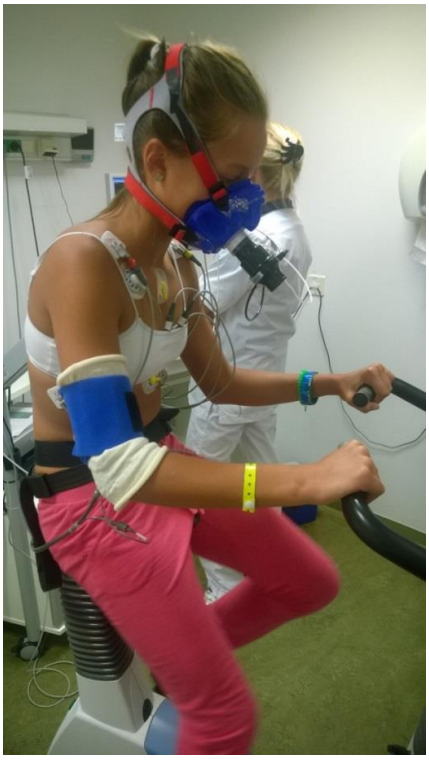

Figure 6. Cardiopulmonary exercise test

Table 6. MRI variables used in this project

Measure *Parameter (unit)*	From	Parameter definition	Comment
Aortic pulse wave velocity *PWV (m/s)*	Two high-temporal 1-directional velocity encoded time-resolved acquisitions	Propagation speed of the blood flow through the aorta: $\Delta x / \Delta t$ where x is the length of the vessel and t is the travel time	Measure of vessel stiffness; higher PWV equals stiffer a vessel
Left ventricular wall mass *LV mass (g)*	Cine multi-slice short-axis images	Subtraction of epi- and endocardial areas at end-systole and end-diastole	Provides information on presence of hypertrophy

Table 7. Exercise variables used in this project

Measure *Parameter (unit)*	From	Parameter definition	Comment
Peak oxygen uptake VO_{2peak} *(mL/kg/min)*	Breath by breath gas analyser	Highest measured oxygen consumption at the last phase of the exercise test	Measure of aerobic fitness
Peak heart rate HR_{peak} *(bpm)*	ECG	Highest heart rate achieved during exercise	Measure of chronotropic competence
Heart rate recovery *HRR60 (bpm)*	ECG	Peak heart rate minus the heart rate 1 minute after exercise cessation	Measure of PNS
Heart rate reserve $HR_{reserve}$ *(bpm)*	ECG	Difference between the peak heart rate and the resting heart rate (measured seated on the bike before the test)	Measure of chronotropic competence

Chapter 3

Postnatal cardiac autonomic nervous control in pediatric congenital heart disease

Ineke Nederend

Monique R.M. Jongbloed

Nico A. Blom

Eco J.C. de Geus

Arend D.J. ten Harkel

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ABSTRACT

Congenital heart disease is the most common congenital defect. During childhood, survival is generally good but, in adulthood, late complications are not uncommon. Abnormal autonomic control in children with congenital heart disease may contribute considerably to the pathophysiology of these long term sequelae. This narrative review of 34 studies aims to summarize current knowledge on function of the autonomic nervous system in children with a congenital heart defect. Large scale studies that measure both branches of the nervous system for prolonged periods of time in well-defined patient cohorts in various phases of childhood and adolescence are currently lacking. Pending such studies, there is not yet a good grasp on the extent and direction of sympathetic and parasympathetic autonomic function in pediatric congenital heart disease. Longitudinal studies in homogenous patient groups linking autonomic nervous system function and clinical outcome are warranted.

INTRODUCTION

Congenital heart defects are the most common congenital defects, affecting around 90 per 10,000 newborns [18]. Nowadays, for most of these defects, adequate catheter interventions, surgical correction or repair is available, which has resulted in a dramatic improvement of survival. Ninety percent of children born with a congenital heart disease (CHD) survive into adulthood [19]. Despite good survival in childhood, a substantial amount of patients with a CHD who reach adulthood suffer late complications such as pulmonary hypertension [20] and arrhythmias and die from heart failure, sudden cardiac death or other cardiac problems [21;22]. It is not known how heart function is kept at bay during childhood, which patients develop problems later in life and how to prevent or reverse this process. Chronic changes in cardiac autonomic control to compensate for hemodynamic alterations due to CHD or due to surgical intervention may play a role. Thus, assessment of cardiac autonomic function may provide insight in future disease progression. Increased sympathetic activity and decreased parasympathetic activity (which can be due to multiple causes) is strongly associated with myocardial dysfunction. Enhanced sympathetic activity plays a significant role in the progression of heart failure and it is very plausible that it also plays a role in the long term sequelae in congenital heart disease, including eventual fibrosis.

A fairly large body of research exists on cardiac autonomic function in adult heart disease. Altered autonomic function is found in various patient groups; for example, in cardiac failure [23] and patients after coronary artery bypass [24], decreased heart rate variability (HRV) is found. Altered function of this system in cardiac patients usually entails increased sympathetic and decreased parasympathetic activity and is associated with an increased risk of cardiac events and sudden cardiac death in patients with known cardiovascular disease [25;26], but also in persons without a history of cardiovascular disease [27]. However, it is still uncertain whether this dysfunction is part of the pathophysiology, mainly a compensation mechanism, caused by surgical procedures or a combination of these. Recent work has shown that autonomic regulation in fetuses with CHD already differs from healthy fetuses [28]. In a healthy fetus, mean heart rate will decrease and heart rate variability (HRV) will increase with gestational age [28;29]. In affected fetuses, decreased HR was found to be paired to decreased HRV. Subgroup analysis revealed that especially fetuses with hypoplastic left heart syndrome showed decreased HRV, and this was not observed in the groups with transposition of the great arteries and tetralogy of Fallot. Differences were detectable as early as 19 weeks of gestational age. A possible mechanism of the altered autonomic nervous system (ANS) development in CHD might be alterations in cerebral blood flow [30] and therewith brain development [31] caused by structural changes in circulation. Brain volume and head circumference is smaller in fetuses with CHD compared

to controls [32;33] and cerebrovascular resistance is lower [34]. In healthy fetuses, autonomic control seems to be associated with maternal exercise behavior [35] and this effect is maintained after birth [36].

As opposed to adults, children have little or no comorbidities. Therefore, they constitute a clean group to study ANS function and unravel its role in etiology. In this narrative review, we aim to summarize current knowledge on autonomic function in children with a congenital heart defect after birth. Before doing so, we will briefly describe the main structure and function of the ANS, ways to measure ANS and healthy maturation of the ANS.

AUTONOMIC NERVOUS SYSTEM

The term "autonomic nervous system" was coined by Langley in 1898. The main function of the ANS is to ensure homeostasis and coordinate bodily functions in response to a dynamic external and internal environment. The ANS can be subdivided into two branches; the sympathetic and the parasympathetic branch. Coarsely taken, the first is responsible for the "fight or flight" response whereas the second branch has a key role in the "rest and digest" state of the body. At any moment, both these branches are active at the same time but the balance of the two depends on the specific demand of the particular situation requiring a sympathetic or parasympathetic (or equal) dominance of the two systems. The sympathetic nervous system (SNS) is responsible for increasing heart rate, contractility of the cardiac muscle, blood pressure, epinephrine secretion, sweat production and breathing frequency in order to make the body ready for action. The preganglionic sympathetic fibers leave the central nervous system from the thoracic and lumbar regions of the spinal cord. These preganglionic fibers employ acetylcholine (ACh) as primary neurotransmitter and synapse onto the sympathetic ganglia (sympathetic trunk) which is located close to the spinal cord. The longer postganglionic neurons employ norepinephrine (NE) as their primary neurotransmitter. NE acts on α-1 adrenergic or β-1 or β-2 adrenergic receptors. Preganglionic neurons also directly innervate the adrenal medulla, which releases NE and epinephrine (E) into the blood stream. Circulating NE is converted into E which has affinity for binding to β-2 receptors causing vasodilatation and increase of heart rate and contractility. α-1 receptors cause vasoconstriction by acting on smooth muscles. β-1 and β-2 receptor activation will cause increased contractility of the ventricles and will increase heart rate by accelerating phase 4 of the pacemaker action potential. An increased activity of the SNS is an important and powerful mechanism of the body to compensate altered hemodynamics, e.g., due to heart disease. However, chronic exposure to enhanced levels of NE concentrations can in time cause maladaptive and even detrimental effects to the cardiovascular system and organs [8]. Based on this knowledge,

prescription of beta blockers is currently the cornerstone of treatment of heart failure. Additionally, Angiotensin Converting Enzyme inhibitors and diuretics to counteract the SNS-mediated increase in angiotensin I, aldosterone and fluid retention are used to tackle this "heart failure cascade" and thereby lead to significantly improved survival [37].

The parasympathetic nervous system (PNS) effectuates the opposite of the SNS; *i.e.*, a decrease in heart rate, breathing frequency, *etc.* The vagus nerve (cranial nerve X) originates from the medulla oblongata, which serves as the center for cardiovascular reflexes. The long preganglionic fibers of the PNS (originating from the vagus nerve) are the primary source of parasympathetic innervation of the heart (and other organs). Parasympathetic ganglia generally lie close or even in the effector organs. ACh is the primary neurotransmitter of both the pre- and postganglionic PNS fibers. Receptors on the postganglionic fibers are of the nicotinic subtype whereas the receptor subtypes on the target organ are one of the five muscarinic acetylcholine receptor subtypes (M1–M5). The predominant cardiac subtype is M2. Parasympathetic innervation is more dense in the atria, the sinoatrial and in the atrioventricular node than in the ventricles [38]. The functional role of this ventricular innervation has not yet been elucidated. Baroreceptors register blood pressure (stretch of the arterial wall) and send this information to the medulla oblongata which will respond in order to maintain pressure. Arterial baroreceptors are located in the carotid sinuses and aortic arch, and cardiopulmonary baroreceptors in the atria, ventricles and pulmonary vessels. Higher pressure will increase baroreceptor firing and decrease sympathetic activity, resulting in a decrease in blood pressure. Baroreceptor-mediated blood pressure control mainly buffers short term blood pressure variations. In the long term, blood pressure is regulated by the endocrine system. A schematic of autonomic cardiovascular control can be found in *figure 1*.

POSTNATAL MEASUREMENT OF CARDIAC AUTONOMIC CONTROL

A technique for assessing cardiac sympathetic innervation is a metaiodobenzyl-guanidine (MIBG) scan. This scintigraphy employs a radiolabeled molecule, similar to noradrenaline; MIBG labeled to iodine-123 (I^{123}-MIBG). Cardiac sympathetic innervation can be quantified as the ratio of MIBG uptake in the heart to MIBG uptake in a reference area, e.g., mediastinum (H/M ratio) [39]. A low H/M ratio indicates low sympathetic innervation. MIBG scintigraphy is a cardiac neurotransmission imaging technique to assess presynaptic reuptake and storage whereas at the effector level, cardiac ANS activity is assessed. The local sympathetic innervation as measured by MIBG uptake is only a part of that limb as circulating catecholamines also play a role.

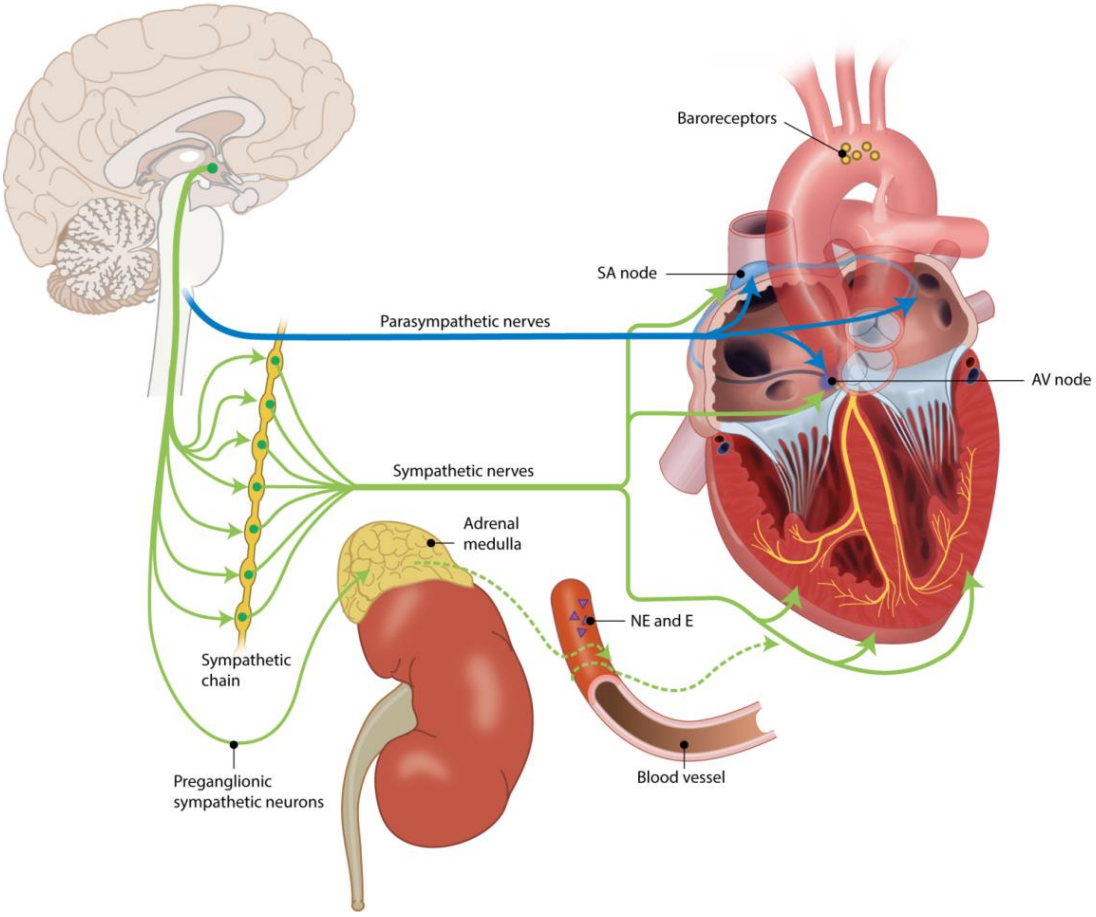

Figure 1. Schematic of autonomic cardiovascular control

Autonomic nervous system function is complex and difficult to measure. Gold standards for measuring cardiac ANS activity are unfortunately also the most invasive methods. Invasive techniques include measurement of norepinephrine (NE) regional spillover, microneurography (direct measurement of action potentials in the nerve), microdialysis (measurement of acetylcholine (Ach) in the dialysate sample in which the probe is designed to mimic a blood capillary and measures passive diffusion in the tissue) and pharmacological blockade (heart rate and diastolic blood pressure are measured both before and after pharmacological blockade of SNS or PNS (by e.g., metropolol/propranolol or atropine) [40]. These techniques are highly to moderately invasive and therefore not preferable, certainly not in pediatric studies.

There are also several non-invasive techniques available for the measurement of cardiac autonomic nervous control; including analysis of heart rate and HRV which will be discussed in more detail below. Another non-invasive measurement is baroreflex sensitivity (BRS) by measuring beat-to-beat changes in heart rate and blood pressure e.g., by a finger cuff (Finapres). BRS can also be evaluated in a lower body negative pressure test. The patient's lower body is placed in a tube where negative pressure is applied which reduces venous return while changes in blood pressure and heart rate are monitored. The valsalva maneuver follows the same reasoning but in this test venous return is reduced by increasing the intrathoracic pressure by performing forced expiration to an obstruction. By the use of two skin electrodes, (hand palm) skin conductance level can be used as measure for SNS activity. Sweat glands will increase productivity with increased SNS. The subsequent increase in conductance of the skin is used as a measure of SNS activity as sweat glands are innervated by the sympathetic but not the parasympathetic nerves. The focus in research has been largely on heart rate and blood pressure. However, these variables represent an unknown mixture of sympathetic and parasympathetic input. Since health outcomes for sympathetic and parasympathetic hyperactivity are different, it is important to distinguish between the two.

Pediatric studies most often employ measurement of HRV for the measurement of ANS activity. For most HRV analysis, an electrocardiogram is the only necessity. The existence of beat-to-beat variation in pulse has been known from 1733 when Stephen Hales described that heart rate varies with respiration [41]. Ever since those days, physicians have had a great interest in heart rate variability and its relationship with disease. After the invention of the "physician's pulse watch" by Floyer in the early 1700s, changes in pulse rate could be accurately assessed. This portable clock enabled him to tabulate both respiration and pulse. The first clinical relevance of HRV was described in 1965 by Hon and Lee [42] who noted that a change in HRV preceded fetal distress. HRV is now a frequently used tool to evaluate autonomic control [43] although some groups argue that HRV primarily depends on heart rate and therefore cannot be used in any simple way to assess nervous activity on the heart [44].

An overview of the most used HRV variables is given in *Table 1*. In general, HRV provides measures for PNS but not SNS activity. HRV can be divided into time domain and frequency domain analysis. For these HRV analysis, normal-to-normal (NN) interbeat intervals are used. That is, only heartbeats originating from the sinus node are used and ectopic beats should be removed. Respiratory sinus arrhythmia (RSA) provides a good PNS measure and can be measured with only electrocardiography (RMSSD, HF) or by combining electrocardiography with respiratory signal recording (pvRSA; see *figure 3*). Coupled to respiration rate, firing of motor neurons in the nerves ambiguous and the sympathetic

nuclei is phasically inhibited and exited. This phenomenon is caused by connections between the nuclei that control the respiratory generator in the pre-Bötzinger and Bötzinger complexes and parasympathetic and sympathetic motor neurons. These connections modulate the release of neurotransmitters in such a way that during inspiration the release of NE is increased and the release of Ach is decreased. During expiration the opposite occurs; release of NE is decreased and the release of Ach is increased. As a result, heart rate increases with inspiration and decreases with expiration and RSA is generated [45]. Changes in hyperpolarization of the sinus node in response to parasympathetic outflow occur within hundreds of milliseconds. However sympathetic outflow modulates the depolarization speed only on the scale of seconds and therefore is too slow to follow the phasic respiratory-coupled changes. Hence, the SNS does not contribute to RSA [46]. In the case of high PNS activity, thus many action potentials per second, the effect of the phasic inhibition and excitation will be more pronounced compared to when there is less PNS activity. As a result, RSA will be higher when there is more PNS activity. Respiration rate and tidal volume have to be considered when interpreting (changes in) RSA as those also have an independent influence on HRV. However, during approximately similar respiration, HRV provides a measure for parasympathetic outflow to the heart. Other HRV measures (*TP*, *ULF*, *VLF*, *LF*) are comparable to respiratory sinus arrhythmia, but these lower frequencies in heart rate are also influenced by sympathetic input. A higher respiratory sinus arrhythmia indicates higher PNS activity. The LF/HF ratio is a frequently used measure of SNS activity but has met with much controversy [40]. When employing impedance cardiography in addition to the electrocardiogram, a non-invasive measure of SNS can be derived: the pre-ejection period (PEP) [47]. The impedance cardiogram is computed by sending an alternating current through the thorax. The impedance (*i.e.*, complex resistance) will change over time due to respiration and the amount of blood in the thorax. The first derivative of this signal (*i.e.*, change in impedance) is the impedance cardiogram (*Figure 2*, upper graph) and can be used to derive systolic time intervals.

Table 1. Heart rate variability (HRV) based measures. Ms; milliseconds.

HRV Variable (Measure)	Principle
Time domain measures	
Mean RR	Average of all NN intervals
SDNN (ms)	Standard deviation of all NN intervals (inter beat interval of two successive sinus beats)
SDANN (ms)	Standard deviation of the average NN intervals over a short period (typically 5 min) over the entire recording
RMSSD (ms)	Root mean square of successive differences between adjacent NN intervals
Coefficient of variation	Ratio of standard deviation of NN intervals and the mean of RR intervals
NN50	Number of pairs of successive NN intervals that differ >50 ms
pNN50	Proportion of NN50/total number of measured NN intervals
pvRSA (ms)	Respiratory sinus arrhythmia determined by peak-valley method. The shortest NN interval during inspiration is subtracted from the longest NN interval during exhalation (see *Figure 3*)
Frequency domain measures	
HF (ms^2)	High frequency power. Power in the respiratory frequency range, typically from 0.15–0.4 Hz
HFnu	HF/(LF + HF)
LF (ms^2)	Low frequency power. Power in the low frequency range, typically from 0.04–0.15 Hz
LFnu	LF/(LF + HF)
LF/HF	Ratio of low frequency power to high frequency power
VLF (ms^2)	Very low frequency power. Power in the very low frequency range, typically from 0.003–0.04 Hz
ULF (ms^2)	Ultra-low frequency power. Power in the ultra-low frequency range, typically <0.003 HZ
TP (ms^2)	ULF + VLF + LF + HF

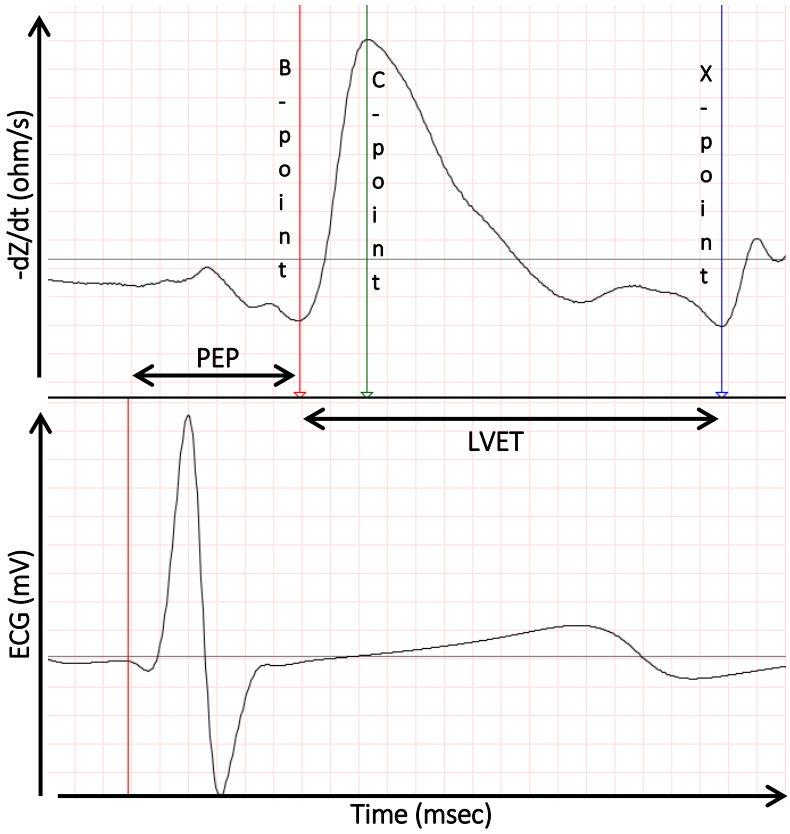

Figure 2. Systolic time intervals including PEP. PEP; pre-ejection period. LVET; left ventricular ejection time

The PEP is a measure of contractility and is defined as the time delay between the start of the electrical depolarization of the ventricles (Q-onset) and the start of the actual outflow of blood into the aorta (B-point) (*Figure 2*). Functional ventricular parasympathetic innervation is only proven in canine models. There is increasing evidence for parasympathetic innervation in human ventricles but the functionality remains to be elucidated [38]. Therefore, the PEP provides us with a pure measure of SNS activity. A shorter PEP indicates higher SNS activity. The T-wave amplitude, by reflecting sympathetic effects on repolarization can be used in addition to the PEP in order to characterize sympathetic activity non-invasively [48]. A lower T-wave indicates higher SNS activity.

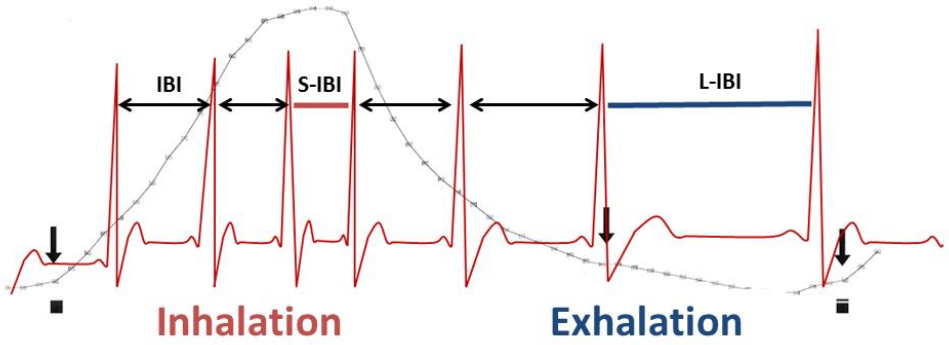

Figure 3. Respiratory sinus arrhythmia. Electrocardiogram in red, respiration in grey. IBI (black double headed arrows); inter-beat interval; the time between two consecutive R-peaks in the ECG. RSA; respiratory sinus arrhythmia; calculated as longest heart period during exhalation (L-IBI) minus the shortest heart period during inhalation (S-IBI)

CARDIAC AUTONOMIC NERVOUS SYSTEM IN HEALTHY MATURATION

In normal maturation of the ANS, several factors are of influence. In a recent review, Eyre *et al.* [49] give an overview of studies concerning the effect of age, gender and weight status. Most, but not all, studies in children found a positive correlation between age and HRV (in both time and frequency domain) measures, indicating a progressive increase in cardiac PNS activity with age. This pattern of development seems to be most progressive in infancy, continues more gradually in early childhood and even more so in late childhood. Heragu *et al.* [50] (not included in the review) also found a significant age-related increase in HRV in 45 healthy children aged 3 weeks to 16 years. The effect found of gender on ANS in childhood is not consistent [49]. Some studies report no gender differences [51], whereas others report higher HRV in males compared to females [52;53]. Resting values for heart rate, pre ejection period and respiratory sinus arrhythmia in different body positions are described in 3097 children aged 5–7 years old by van Dijk *et al.* [54]. Significant sex differences were found for all three measures (heart rate and pre-ejection period being higher for girls and respiratory sinus arrhythmia lower for girls) but not in all body postures. A better understanding of the specific time course and nature of the normal development of ANS in childhood would be of great importance in order to better discriminate between health and disease and to be able to intervene as early as possible.

CARDIAC AUTONOMIC CONTROL IN PEDIATRIC CONGENITAL HEART DISSEASE

To summarize the current knowledge on cardiac autonomic control in pediatric congenital heart disease we below present a narrative review of the extant literature on this topic. The Pubmed database was used as the primary source for the literature search. Search terms used were "autonomic nervous system" [MeSH] AND "congenital heart disease" [MeSH]. The MeHS entry terms were also entered as free text in order to find missed recent papers that were not yet indexed in the Pubmed database. English papers from 1965 onwards only were included and additional filters on age (child: birth–18 years) and language (English) were employed. We focused on structural defects; research concerning inherited rhythm disorders or channelopathies were excluded. Also, case studies were excluded. The search yielded a total of 197 papers. After screening on title and abstract, 55 were identified as potentially relevant and five were added after screening of reference lists. Thirty-four were included in the final review. Twenty-six studies were excluded because they included adults, did not concern congenital heart defects or did not measure ANS activity. *Table 2* concisely summarizes the preoperative, immediate postoperative and long term postoperative findings per CHD. *Table 3* gives an overview of all studies included; six studies on transposition of the great arteries, three on Tetralogy of Fallot, six on atrial- or ventricular septal defect, four on univentricular heart, five on coarctation and 10 on various CHD.

In cardiac disease, ANS control may adapt in order to compensate for changed hemodynamics. This change is usually characterized by an increased sympathetic and/or a decreased parasympathetic tone and works as a powerful compensation mechanism. Activation of arterial- and cardiopulmonary baroreceptors will change due to altered hemodynamics in the heart and blood vessels and may, with prolonged exposure to increased pressures, reset to a higher value. For instance, in response to heart failure, ANS activity can change. Since the early 90s, the idea has arisen that the deterioration of heart failure had in part a neurohormonal explanation [55]. Before this time, progress in heart failure was believed to be due solely to hemodynamic stress, triggered by the initial injury, e.g., in prolonged left ventricular failure, pressure and preload of the left atrium will rise. As a result, the resistance in the pulmonary vascular system (the afterload of the right ventricle) will increase and may eventually lead to right ventricular failure. The neurohormonal explanation includes increased activity of the SNS and the renin-angiotensin system. This is a powerful compensatory mechanism to deal with altered hemodynamics in rest and/or during exercise, depending on the severity of heart failure, but prolonged exposure of NE in chronic heart failure can cause damage to myocytes and cause β-receptor down regulation that will ultimately diminish heart function [56]. In cardiac failure, resting levels of the SNS measured by regional NE spillover can increase to as much

as 50 times which is comparable to the SNS response to maximal exercise in healthy man [57]. Sympathetic over activation may cause leakage of the ryanodine receptor 2 channel, located on the sarcoplasmic reticulum. The subsequent calcium leakage causes a decrease in cardiac contractility [58]. Furthermore, evidence suggests that an increased sympathetic tone is arrhythmogenic [59] while high parasympathetic levels have a cardioprotective effect [60]. Parasympathetic tone increases the electrical stability of the heart [61-64] but if it is delayed after potentials as a result of the calcium leakage through ryanodine channels, it may potentially cause arrhythmia. The maladaptive responses that can be directly related to increased SNS activity have contributed to the insight that beta-blockers are beneficial in the treatment of heart failure. By decreasing plasma E and NE levels, these agents likely modulate the maladaptive sympathetic responses and slow the heart failure cascade.

Different mechanisms are involved in various heart defects and the alterations in hemodynamics and ANS control are expected to be specific to the defect at hand. Also, the type of eventual intervention employed for repair (e.g., transcutaneous or via sternotomy) may have a substantial influence on the ANS. Altered ANS function may be due to pulmonary hypertension, systemic hypertension, heart failure, pressure- or volume overload following a (or multiple) cardiac malformation(s). Consequently, hemodynamics and thus ANS activity might also change following repair. Unfortunately, some studies do not take this into account and group a variety of different defects and patients before and after repair together for analysis of ANS function (see *Table 3*). Also, potential changes in pulmonary functional status must be taken into account. Especially following sternotomy, pulmonary function may be worsened [65] and tidal volume and breathing frequency might change and effect HRV.

Ventricular and atrial septal defect

Atrial and ventricular septal defects (ASD and VSD respectively) are relatively common with a prevalence of 13 and 41 per 10,000 live births respectively [66]. The hemodynamic changes that accompany these defects are dependent on the size and location of the defect, the resistance in pulmonary and systemic vasculature and the atrial and ventricular pressures. In uncomplicated ASD, oxygenated blood is shunted from the left to the right atrium. The resulting volume overload may induce enlargement of the right atrium, right ventricle and pulmonary artery. The ventricular volume overload may cause a decrease in HRV via baroreceptors and stretch of the sinoatrial node [67]. Also, the respiratory fluctuation in atrial pressure may be altered or at least be proportionally less compared to healthy subjects, causing a reduction in HRV [68;69]. Several authors studied pediatric ASD patients preoperatively [70-72] and found decreased HRV (both time and frequency domain) compared to healthy controls. Additionally, Massin and colleagues [71] found a negative correlation

between HRV and right atrial pressure and between HRV and end diastolic right ventricular pressure. Finley *et al.* [69] and Bialkowski *et al.* [72] studied their patients a few months postoperatively. Compared to controls, Finley found higher supine SDNN (indicating higher PNS tone) after repair compared to before but this difference disappeared while in upright position. Bialkowski and colleagues compared patients before and after transcutaneous or surgical ASD repair and found that in the transcutaneous repaired, HRV increased early (1 month) after repair and increased further after 3 months. In contrast, in the surgical group, HRV reduced to below preoperative values shortly after but recovered to higher compared to preoperative values 3 months after.

In uncomplicated VSD, the defect also causes a left-to-right shunt, potentially causing enlargement of the left atrium and left ventricle because of increased blood return to the left side of the heart. The left ventricular volume overload may via baroreceptors change autonomic control and decrease HRV [67]. Hata *et al.* [68] compared ASD and VSD patients preoperatively. Compared to ASD patients, HF power in respiratory frequency band was significantly higher in VSD patients indicating higher parasympathetic tone in VSD patients. No difference was found in respiratory rate, LF, TP nor LF/HF. Kul Yum [73] studied HRV in a group of VSD patients during catheterization and found lower HF and LF in a subgroup of patients with high pulmonary artery pressure compared to patients with normal pressure. After having been on cardiopulmonary bypass for the correction of the septal defect, cardiac ANS activity and HRV can be altered compared to healthy persons. In a subgroup (28 ASD and 3 VSD) analysis, Ohuchi and colleagues [74] found a reduction of HF (log transformed) and baroreceptor sensitivity 1 month after surgery, indicating a drop in parasympathetic tone. One year after surgery, they were back to preoperative values.

In conclusion, preoperatively, HRV seems to be reduced in ASD patients, indicating lower PNS activity compared to healthy controls. There is not enough evidence from VSD cohorts to draw a conclusion on this issue. Postoperatively, HRV seems to normalize, although long term evidence is lacking and, also, age of intervention might play a role as the time of exposure to high pressures may have an influence.

Table 2. Summary of preoperative, immediate postoperative and long term postoperative findings per congenital heart disease (CHD).

	Preop	Immediate Postop		Remote Postop	
ASD	HRV ↓ #,*,1	Surgery	HRV ↓ (vs. preop)	Surgery	HRV ↑ (vs. preop)
		Cath	HRV ↑ (vs. preop)	Cath	HRV ↑ (vs. preop)
VSD	RSA ↑ (vs. ASD)	Cath	HRV ↓ in PH vs. NPP		No studies available
TGA	SNS innervation intact	Surgery	SNS denervation	Surgery	Reïnnervation SNS in 32% *,2
	HRV ↓ #		No difference or lower HRV #,*,3		PNS normalized; SNS ↓ #
Uni ventricular heart	No studies available	Surgery	No studies available	Surgery	PNS ↓; sympathetic denervation; SNS ↑ #
CoA	BRS & HRV ↓ #	Surgery	SNS ↑ (vs. cath)	Surgery	HRV and BRS normalized
		Cath	No studies available	Cath	No studies available
TOF	No studies available	Surgery	No studies available	Surgery	HRV ↓ #

*Immediate postop <1 year after intervention; PH = Pulmonary Hypertension; NPP = Normotensive Pulmonary Pressure; # Compared to healthy controls; *,1 Found in all studies measuring 24 h HRV but not in one study measuring 15 min HRV; *,2 In patients operated within the first 55 days of life, almost all showed positive MIBG uptake compared to only half of patients operated later in life; *,3 Patients did show a slower recovery of HRV after feeding*

Table 3. Overview of studies grouped by type of CHD. Preop; preoperatively. Postop; postoperatively. w; weeks. m; months. Y; years; pts; patients. P vs. C; patient versus control.

Author	Patient Group (N, Type CHD)	Age Patients	Pre or Postop	Control Group (N, Age)	Measure	Outcome Preoperatively	Outcome Postoperatively
Heragu et al. [50]	N = 36. Type of CHD not specified.	2 w–15 y	Preop & ± 1 w postop	N = 45. 3 w–16 y	24 h HRV	P vs. C: no difference in mean RR, SDNN, SDNN/RR, SDANN, LF and LF/HF. TP and HF ↓	All measures except for LF/HF ↓ compared to preop. LF/HF ↑ postop.
Massin et al. [75]	N = 258. Various types CHD.	2 d–14 y	Both	N = 210. 3 d–14 y	24 h HRV	SDNN, SDNNi, SDANNi, rMSSD, pNN50, LF, HF, LF/HF ↓ in pts in NYHA class II–IV.	SDNN, SDNNi, SDANNi, rMSSD, pNN50, LF, HF, LF/HF ↓ in pts in NYHA class II–IV.
Ohuchi et al. [74]	N = 143. ASD, VSD, RVOTR.	Mean 14.6 y	Preop & >1 m postop	N = 47. 15,6 ± 4	5 min HRV. Plasma NE. BRS, scintigraphy, blockade study.	-	P vs C: BRS, Log HF & Log LF, H/M↓. Plasma NE and =.
McGlone et al. [76]	N = 20. Various CHD	0–16 y	Preop & 1 d postop	No	1 h HRV preop, 24 h HVR postop	-	sNN50, SDNN, SDANN, RMSSD, SDNNi ↓ after surgery.
Currie et al. [77]	N = 12. TOF, CoA.	4 ± 1 y	Postop	N = 12. 5 ± 1 y	5 min HRV	-	P vs. C: SDRR, RMSSD, pNN50, log LF, LF, log HF, HF, LF/HF, DFA =.
Aletti et al. [78]	N = 15. Various CHD.	Mean 24 m	Preop	N = 10. 15 ± 10 m.	10 min HRV	P vs. C: TP and LF ↑. Mean RR, VLF, HF =.	-
Buchorn et al. [79]	N = 14. Various CHD.	2.6 ± 1.9 m	Preop	N = 70. 2.1 ± 2.7 m	24 h HRV. Plasma NE and E.	P (standard treatment) vs. C: SDNN, SDANN, rMSSD, VLF, HF, LF ↓. Mean RR, pNN50, LF/HF,	-

						TP =. P (propanolol) vs. C: Mean RR ↑, VLF ↓. All other =.	
Dzimir et al. [80]	N = 112. TOF, VSD, PST, COA, CPL.	Median 36 m	During catheriz ation	N = 14. median 48 m	Plasma E&NE. α- and β adrenoreceptor activity	PST vs. C: plasma E and NE ↑. All groups except CoA increased α-adrenoreceptor densities. LRS & PST vs C: B-adrenoreceptors ↓.	-
Goudjil et al. [81]	N = 22. PDA.	28 w	Preop	N = 22. 28 w	4 min HRV	P vs C: TP, LF, HF nu, HF/LF, SNDD ↓. HF, mean RR, RMSSD, SDSD =.	-
Kaltman et al. [82]	N = 60. Various CHD.	4.9 ± 3.3 y	Preop & 0–6 m postop	No	24 h HRV	Pts with biventricular vs. univentricular heart: mean HR, LF, LF, LF/HF =.	Pts with biventr vs. univentr heart: Mean HR ↓ LF ↑ at discharge. 3–6 m postop: Mean HR, LF, LF, LF/HF =.
Bakari et al. [70]	N = 28. ASD.	6.6 ± 2.1 y	Preop	N = 32. 6.4 ± 2.2 y	24 h HRV	P vs C: SDNN, SDANN, rMSSD, SD, SDNN index, PNN50, mean RR, TP, HF, LF/HF ↓.	-
Massin et al. [71]	N = 20. ASD.	Range 3–14 y	Preop	N = 210. 3 d–14 y	24 h HRV	P vs. C: mean RR, SDNNi, SDANNi, pNN50, LF, HF ↓.	-
Finley et al. [69]	N = 10. ASD.	Range 4–16 y	Preop & ± 5 m postop	N = 10. mean 6.1 y	10–15 min HRV and respiration standing and supine	P vs. C: Supine: mean RR, LF, resp rate =, SDNN, HF ↓. LF/HF ↑. Upright: mean RR, LF, HF, LF/H, resp rate =. SDNN ↓	P preop vs. P postop: Supine: mean RR, LF, HF, HF/LF =. SDNN ↑. Upright: mean RR, SDNN, LF, HF, HF/LF =.
Bialkowski et al. [72]	N = 19. ASD	2.5–14 y	Preop & 1 & 3 m postop	From earlier non-	24 h HRV	P vs. C: SDNN, SDANN, SDNN index, rMSSD, pNN50 ↓.	P (preop) vs. P (after transcutaneous intervention): SDNN and SDANN ↑ 1m after intervention. SDNN, SDANN,

				english publication			SDNN index, rMSSD, pNN50 ↑ 3m after intervention. P (preop) vs. P (after surgical repair): SDNN, SDANN, SDNN index, rMSSD, pNN50 ↓ 1m postop. SDNN, SDANN index ↑ 3m postop.
Hata et al. [68]	N = 43. ASD, VSD.	ASD 4.6 ± 3.6 y; VSD 4.1 ± 6.4y	Preop	No	HRV and respiration (time of recording not specified).	P (ASD) vs. P (VSD): HF, RSA ↓. Respiratory rate, LF, TP, LF/HF = .	-
Kul Yum et al. [73]	N = 32. VSD.	<12 months	Preop	No	5 min HRV during catherization	P (hypertemsive) vs. P (nonhypertensive) HR, SDNN =. LF and HF ↓.	-
Kondo et al. [83]	N = 51. TGA.	Mean 4.8 y	Preop & 1 m–10 y postop	N = 51. 4.2–6 m	Scintigraphy	All pts and controls showed positive MIBG uptake.	<1 m after ASO, all pts showed negative MIBG uptake. 15/47 pts negative MIBG uptake late postop.
Harrison et al. [84]	N = 15. TGA.	0–8 w	Preop & 2–8 w postop	N = 16. Age-matched.	2–4 h HRV	P vs. C: HF, LF ↓.	P vs. C: HF, LF =. Pts showed delayed recovery of HF after feeding.
Harrison et al. [85]	N = 15. TGA.	0–3 y	2 w and 3 y postop	N = 12. Age matched	15 m HRV	-	P vs. C: HF =. HF reactivity ↓.
Harrison et al. [86]	N = 15. TGA.	2–8 w	2–8 w postop	N = 16. Age matched	15 m HRV	-	P vs. C 2w postop: baseline HF, recovery HF ↓. P vs C 8w postop; baseline HF, recovery HF =.
Doksoz et al. [87]	N = 22. TGA.	Mean 59.5 ± 38.7 m	Postop	N = 22. Age 65,1 ± 39,4 m	24 h HRV	-	P vs. C: mean HR, max HR, min HR = . SDANN, VLF ↑. When awake, SDNN, rMSSD, pNN50, TP, VLF ↑. When

							asleep SDNN, rMSSD, pNN50, TP, VLF =.
Falkenberg et al. [88]	N = 8. TGA.	15.8 ± 1.5 y	15 y postop	N = 15. Age 19,7 ± 1 y	BRS and NE whole body and cardiac spillover	-	P vs. C: mean HR, BRS =. Total body NE spillover, regional spillover ↓.
Madan et al. [89]	N = 46. Univentricular heart.	BDG 5,2 ± 5.3 y Fontan 9.3 ± 5.9 y	Preop & 2 and 9 m postop	No	900 s HRV	P (BDG) vs. P (Fontan): coefficient of variance, SDNN, RNSSD, NN50, pNN50, VLF, HF, TP, LF/HF =.	P (BDG) vs. P (Fontan) 2 m postop: RMSSD ↑. P (BDG) 9m postop: coefficient of variance, TP, LF, VLF ↑. P (BDG) 9m postop: RMSSD, HF, LF, VLF ↑. P (BDG) vs. P (Fontan) 9 m postop: Coefficient of variation ↑.
Rydberg et al. [90]	N = 15. Univentricular heart.	Age not specified	Mean 7.2 y postop	No	24 h HRV. Poincaré plots.	-	P (with VA) vs. P (without VA): TP, VLF, LF, HF, HF/LF =. SD poincaré plots ↑.
Ohuchi et al. [91]	N = 63. Univentricular heart.	TCPC 12.8 ± 5.0 y, APC 14.2 ± 3.9 y	Postop	N = 44. 14.7 ± 3.9 y	Blockade study, scintigraphy, plasma NE	-	P vs. C: Log HF, log LF, BRS, H/M ↓. NE ↑.
Butera et al. [92]	N = 39. Univentricular heart.	Mean 12.2 ± 4.1 y	Mean 5.8 y postop	N = 18. 11.1 ± 2.5 y	24 h HRV	-	P vs. C: SDNN, RMSSD, pNN50, TP, LF, HF ↓.
Polson et al. [93]	N = 8. CoA.	Term neonates	Preop	N = 13. term neonates.	Spontaneous BRS, 15 min HRV, BPV	P vs. C: HR =. BRS ↓. SDNN, RMSSD, TP, LF,HF ↓. BPV HF ↑.	-
Kenny et al. [94]	N = 6. CoA.	5 y	Postop	N = 7. Age & sex matched	Spontaneous BRS, 15 min HRV, BPV		P vs. C: BRS, SDNN, TP, LF, HF, BPV =.

Kenny et al. [95]	N = 29. CoA.	14-18 y	<1 y posop	N = 20. 15.7 ± 0.3 y	BRS, HRV, BPV		P vs. C: HR, HF, LF, BPV =.
Beekman et al. [96]	N = 6. CoA.	Mean 16.8 ± 2.0 y	1–11 y postop	N = 7. 15.4 ± 4 y	BP, HR response to exercise, BRS	-	P vs. C: BRS ↓. BP response to exercise ↑. Resting HR, max HR =.
Choy et al. [97]	N = 15. CoA.	Mean 8,5 y	Preop & <1 y postop	No	BP, Plasma renin and catecholamine		P (surgical intervention) vs. P (catheterization): plasma renin, plasma catecholamines ↑.
Silvilairat et al. [98]	N = 30. TOF.	Median 14 y	2–16 y postop	No	24 h HRV	-	Positive correlation between LF and VO2peak and HF and VO2peak.
Butera et al. [99]	N = 23. TOF.	14 ± 6.6 y	Postop	N = 18. 11.2 ± 4.9 y	24 h HRV	-	P vs. C: SDNN, RMSSD, pNN50, TP, LF, HF, LF/HF ↓.
Wyller et al. [100]	N = 17. TOF.	Median 16 y	10–16 y postop	N = 56. Age range 13–18 y	24 h HRV, LBNP	-	P vs. C during rest: LF, HF, LF/HF, SDNN, pNN50, RMSSD =. P vs. C during LBNP: HR response ↓. TP, HF, SDNN, pNN50, RMSSD ↓ in controls and ↑ in pts.

Transposition of the great arteries

In transposition of the great arteries (TGA), the aorta arises from the right- and the pulmonary artery from the left ventricle, resulting in two parallel circulations leading to cyanotic heart disease. Reported prevalence is 2.3 per 10,000 live births [66]. Hemodynamics and therewith clinical presentation is dependent on the degree of mixing of the two parallel circulations via the ductus arteriosus, foramen ovale and presence of anomalies causing shunts (e.g., VSD). TGA requires neonatal surgical intervention. As sympathetic nerves course along the origin of the great arteries to find their way to the heart, they may be injured during surgery at this site. Operated patients are at increased risk of ventricular tachycardia due to scar tissue or repolarization instability following altered cardiac autonomic control or polymorphic ventricular tachycardia due to right ventricular dysfunction. Kondo *et al.* [83] show that cardiac sympathetic nerves are denervated in 4 patients shortly (<1 month) after arterial switch operation (ASO) for correction of TGA, measured by MIBG uptake. Before surgery, all children had positive MIBG uptake. As a control group, they also measured this in four children operated for a ventricular septal defect (VSD) and found normal innervation an all four shortly after surgery. Furthermore, 47 patients were measured late after ASO and 32% showed absent MIBG uptake. Cardiac nerves were re-innervated in almost all patients that were operated in early infancy (≤55 days). In contrast, this was seen in only half of the patients after late operation. Furthermore, they found that patients who did show re-innervation, were more likely to have normal exercise capacity. There is one more study that evaluated ANS function before reconstruction, they found decreased HF power in 15 patients, suggesting decreased parasympathetic activity [84]. A few weeks after surgery (up to 8 weeks), there was no difference in resting HF power (PNS activity) but patients did show a delayed recovery of HF power back to baseline after feeding. In another study by the same group, HRV was measured during a task (block stacking) 3 years after surgery. Again, mean resting values were not different between patients and controls but PNS reactivity (decrease in HF while doing the task) was lower [85]. In a third study on the same subject, authors do report a lower resting and recovery (HF power increase after feeding) HF power in patients 2 weeks after operation, at 8 weeks the difference had disappeared [86]. In a study on 22 patients without arrhythmia and operated in the neonatal period, a significantly higher SDNN, rMSSD, pNN50, TP and VLF was found in awake patients compared to controls at 12-188 months after surgery suggesting higher parasympathetic tone [87]. During sleep, no differences in HRV were detected. Authors conclude that patients have a predominant parasympathetic tone although HF is not significantly different between groups. Falkenberg *et al.* found that cardiac NE reuptake is impaired while parasympathetic function measured by baroreceptor sensitivity was not different between patients after ASO and controls [88]. In conclusion,

sympathetic activity seems to be intact preoperatively while parasympathetic activity is found to be reduced. Postoperatively, evidence on parasympathetic activity is equivocal. Sympathetic cardiac activity seems to be decreased shortly after repair but is able to recover late after surgery, especially when operation is conducted before the age of 55 days.

Univentricular heart

Different surgical procedures are available for the management of univentricular heart, all are palliative in nature. ANS control may be altered by abnormal load or heart failure before operation and after, ANS may be altered due to the surgical intervention, heart failure and changed hemodynamics. Although the overall incidence is low, arrhythmia is an important cause of morbidity in patients with a total cavopulmonary connection [101]. In the long term, arrhythmia often develops in these patients leading to severe heart failure and risk of sudden cardiac death. HRV could be an effective tool to detect this complication in an early stage [90]. As a result of surgery or as a result of pre-existing anatomical issues, there may be sinus node pathology that overlays the autonomic control. Madan *et al.* [89] compared ANS activity in patients at two different stages of univentricular palliation; bidirectional Glenn shunt (BDG) and total cavopulmonary connection (Fontan completion). Before operation, there was no difference in time and frequency domain HRV between the two groups. One month after surgery, RMSSD was higher in the BDG group compared to Fontan patients and 8 months later, the coefficient of variation was higher in this group. Unfortunately, this study group did not include a healthy control group. Ohuchi *et al.* [91] studied a large group of 63 patients after Fontan procedure and 44 controls. PNS activity was evaluated by HRV, BRS, and the change in heart rate after cholinergic blockade. SNS activity was evaluated by means of plasma NE, MIBG ratio and the change in heart rate after isoproterenol infusion. They found that all ANS indexes were lower in patients compared to controls except for heart rate change to isoproterenol. NE was higher in patients. Their results indicate a decreased PNS activity and sympathetic innervation. There was no relationship between ANS indices and age, age at operation, hemodynamics, type of operation nor time after operation. Maximal exercise testing revealed lower exercise capacity, higher resting heart rate and lower maximal heart rate and heart rate reserve in patients compared to controls. A significant correlation was found between heart rate reserve and all ANS indices except for H/M ratio. Butera *et al.* [92] studied 24 h holter monitoring of 39 patients with total or partial cavopulmonary or atriopulmonary connection and two control groups; 18 healthy volunteers and 16 patients after biventricular repair for their CHD (defects not specified). Patients after total cavopulmonary connection had most aberrant HRV and were significantly different from both healthy

controls and the CHD group with biventricular repair. In conclusion, no studies have been conducted on ANS function before surgery compared to healthy controls. Postoperatively, HRV is reduced in patients compared to controls. Both parasympathetic and sympathetic activity seem to be reduced in these patients.

Coarctation of the aorta

Coarctation of the aorta (CoA) is a local narrowing of the descending aorta, typically located distal from the left subclavian artery at the insertion of the ductus arteriosus and has a prevalence of 4.4 in 10,000 live births [66]. CoA results in an increased LV afterload, causing increased pressures and potentially dilation of the left heart. Also, it causes high blood pressure in the upper- and low blood pressure in the lower part of the body. ANS in uncorrected CoA can be altered because of low blood pressure to the lower part of the body, including to the kidneys. Consequently, the renin-angiotensin system will be activated to increase systemic blood pressure. On the other hand, the increased pressure, decreased elastic properties [102] and secondary flow patterns [103] in the aortic arch may alter function of baroreceptors which are largely expressed in the aortic arch. Also, increased load of the left heart may affect intracardiac ganglia. Repair of the CoA, especially following surgical intervention, may cause alteration in ANS function because of damage of nerves in the aortic arch. In term neonates with a simple coarctation, depressed baroreflex sensitivity, reduced HRV and increased blood pressure variability is found before intervention compared to healthy controls [93]. This cohort was studied again 5 years later after coactectomy and by that time, autonomic function seemed to have normalized [94]. One of the major clinical symptoms of these patients, even after successful repair of the coarctation is hypertension which is not fully understood. In patients after (successful) repair, hypertension continues to be a common complication with a median prevalence of 32.5% (range 25%–68%) [104]. Potential mechanisms explaining this postoperative hypertension include pathology of the vascular bed, local and systemic hemodynamic changes, shape of the aortic arch, impaired baroreceptor sensitivity and altered ANS activity. A potentially effective treatment being investigated for resistant hypertension is renal sympathetic denervation [105]. In a hypertensive group of six children who underwent repair of their coarctation at a later age (mean 9.9 years), diminished baroreceptor function was found 1–11 years after repair [96]. Kenny *et al.* [95] also found decreased BRS in hypertensive patients after repair compared to normotensive patients. There was no difference in BRS between normotensive patients and controls. Five years after surgery, BRS had normalized. However, the six patients included in this long term follow up were not hypertensive [94]. In a study comparing balloon angioplasty to surgical repair of the CoA, Choy *et al.* [97] found significant differences. Blood pressure in the group in which CoA was

relieved by means of balloon angioplasty was lower compared to the surgical group. Plasma renin activity and catecholamines increased in the surgical group but not in the balloon angioplasty group. However, these neurohormonal changes were only measured before and shortly (1 and 2 days) after intervention. These findings suggest that ANS activity is already altered before intervention and seems to normalize in normotensive patients but not in hypertensive patients. Type of intervention may have an influence on ANS function late after repair.

Tetralogy of Fallot

Tetralogy of Fallot (TOF) is a congenital heart defect that encompasses stenosis of the pulmonary artery, ventricular septal defect, overriding aorta and right ventricular hypertrophy. Prevalence is about 4–5 in 10,000. In a group of postoperative TOF patients, Butera *et al.* [99] found a significant reduction in parasympathetic control measured by HRV from 24 hour Holter recording, particularly in patients with ventricular arrhythmia. Wyller *et al.* [100] found no significant differences in resting autonomic regulation measured by HRV between postoperative TOF patients and controls. However they did find a difference between controls and patients in response to the lower body negative pressure test; patients showed less HR increase and HRV increased whereas in controls lower body negative pressure led to a decrease in HRV. Silvilairat *et al*. [98] found a significant positive correlation between HF and exercise capacity (measured by maximal oxygen uptake) and between LF and exercise capacity. As exercise capacity is a predictor of mortality in this patient group, HRV can potentially be used as a proxy. In conclusion, results on HRV are inconclusive. One group does find aberrant ANS function and another does not while age does not differ much between study groups.

Massin *et al.* [75] described the biggest cohort and retrospectively studied a group of 258 children (56 of 258 children had been operated at least one year before) with various congenital heart defects. They grouped the cohort based on the New York Heart Association (NYHA) functional classes I–IV. Twenty four-hour holter analysis showed that all measures of both time and frequency domain HRV were decreased compared to controls in all groups except for NYHA class I. Interestingly, within the same NYHA class they found no differences between patients with different mechanisms causing their heart failure (e.g., volume overload, pressure overload, myocardial damage). There was no correlation between HRV and hemodynamics in this cohort. HRV is found to have a negative relationship with hospital stay duration [50;82] and reduced HRV can be restored with propranolol [79].

CONCLUSIONS

There is a scarcity of research on pediatric cardiac autonomic nervous system function in congenital heart defects, especially before intervention and in relation to clinical outcome. Therefore, it is still uncertain whether altered cardiac autonomic nervous system control in these patients is part of the pathophysiology, a compensation mechanism, result of surgical procedures or a combination of these. The biggest cohort described concerning ANS function in pediatric congenital heart disease included 258 patients [75]. Unfortunately, this study was one of nine that did not segregate groups of different types of CHD or operated *versus* non operated patients in their analysis. Both are expected to be of influence on the function of the ANS. Overall, ANS function seems to be altered both before and after operation in children with a CHD. ANS control after catheter interventions may be more favourable compared to surgical intervention. However, large scale studies that measure both SNS and PNS for prolonged periods of time (e.g., 24 h) in well-defined patient cohorts in various phases of childhood and adolescence are currently lacking. Pending such studies there is not yet a good grasp on the extent and direction of PNS and SNS dysfunction in CHD.

ANS dysfunction might well play a clinically meaningful role in long term sequelae of patients with congenital heart disease. As in progressive left sided heart failure, compensatory changes in cardiac autonomic control may in time cause deterioration of heart function in these patients. Longitudinal studies linking cardiac autonomic control and clinical outcomes are warranted in order to gain more insight into the potentially causative role of the ANS in the etiology of CHD and the potential benefits of intervention targeting the ANS.

Chapter 4

Impedance cardiography in healthy children and children with congenital heart disease: improving stroke volume assessment

Ineke Nederend
Arend D.J. ten Harkel
Nico A. Blom
Gary G. Berntson
Eco J.C. de Geus

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ABSTRACT

Introduction Stroke volume (SV) and cardiac output are important measures in the clinical evaluation of cardiac patients and are also frequently used in research applications. This study was aimed to improve SV scoring derived from spot-electrode based impedance cardiography (ICG) in a pediatric population of healthy volunteers and patients with a corrected congenital heart defect.

Methods 128 healthy volunteers and 66 patients participated. First, scoring methods for ambiguous ICG signals were optimized to improve agreement of B- and X-points with aortic valve opening/closure in simultaneously recorded transthoracic echocardiography (TTE). Building on the improved scoring of B- and X-points, the Kubicek equation for SV estimation was optimized by testing the agreement with the simultaneously recorded SV by TTE. Both steps were initially done in a subset of the sample of healthy children and then validated in the remaining subset of healthy children and in a sample of patients.

Results SV assessment by ICG in healthy children strongly improved (intra class correlation increased from 0.26 to 0.72) after replacing baseline thorax impedance (Z_0) in the Kubicek equation by an equation ($7.337 – 6.208 * dZ/dt_{max}$), where dZ/dt_{max} is the amplitude of the ICG signal at the C-point. Reliable SV assessment remained more difficult in patients compared to healthy controls.

Conclusions After proper adjustment of the Kubicek equation, SV assessed by the use of spot-electrode based ICG is comparable to that obtained from TTE. This approach is highly feasible in a pediatric population and can be used in an ambulatory setting.

INTRODUCTION

Congenital heart defects (CHD) affect around 9 per 1000 newborns [106] and is the most common congenital defect. Due to modern surgical techniques, survival in childhood is good [19], but complications in adulthood, including pulmonary hypertension [20;107] and arrhythmias [108;109], are not uncommon. Many patients eventually die from heart failure, sudden cardiac death or other cardiac problems [21;22]. However, the exact mechanisms through which these late problems develop is not yet fully understood, and the individual characteristics that could predict who is most at risk remain to be identified.

Stroke volume (SV) and the product of SV and heart rate (cardiac output; CO) are core measures in the clinical evaluation of cardiac patients [110] and are also frequently employed for research purposes. However, most of the methods used to measure SV are moderate to highly invasive which make them unsuitable for use in young patients and restricts their use to clinical settings.

Much effort has been put into measuring SV and CO in a non-invasive way [111;112] with impedance cardiography (ICG) emerging as the most promising method [10;113;114]. The burden imposed by ICG is sufficiently low for it to be used even in very young children [54;115]. As an additional advantage it can be employed in naturalistic settings for up to 24 hours, without the need for continuous supervision by a clinician [16;116;117]. For an ICG, no behavioural demands have to be made on the patient whereas Magnetic Resonance Imaging and transthoracic echocardiography require one to lay still and sometimes perform breath holds which can be problematic in young children.

The development of ICG technology was originally sponsored by NASA specifically to measure SV and derived hemodynamics during manned flight [10]. Although the original method employed circumferential band electrodes, alternative spot electrode configurations have more recently been introduced [118-120]. Various studies comparing ICG-derived SV to alternative SV scoring modalities in standardized clinical settings have found good criterion validity [112;121-125] even during exercise [126-128], although not all studies find good agreement [129-132]. In 1992 and 1999, meta-analyses were carried out on studies testing the agreement between ICG derived SV and from reference modalities showed an overall correlation coefficient of 0.81 and 0.82 [113;133]. A more recent meta-analysis comparing ICG to thermodilution reported a correlation coefficient of 0.79 [134]. Validation studies on pediatric cardiac patients [135;136], pediatric intensive care patients [137], obese children [138;139] and healthy neonates [140] show good agreement. However, Taylor et al. and Schubert et al. concluded that ICG did not perform properly in estimating SV in children during nor directly after surgery for their CHD [141-143]. ICG proved to be more challenging in

ambulatory settings [9;114;144] where temporal stability of ambulatory ICG derived SV was moderate at best, compared to excellent temporal stability found for ICG-derived systolic time intervals, even during resting periods that excluded potential movement artefacts [145]. This suggests that the approach to score the SV from a spot electrode-derived ICG is not yet optimal.

In the impedance cardiogram, which is the first derivative of the thoracic impedance (Z (Ω)) with respect to time (dZ/dt (Ω/s)), three points can be derived: 1. The 'B-point' that represents the moment of opening of the aortic valve, reflected in a notch in the ICG signal. 2. The 'C-point' corresponding to the peak blood flow in the aorta, reflected in a minimum in the ICG signal. 3. The 'X-point' that corresponds to the moment of closing of the aortic valve, reflected in an incisura in the idealized ICG signal (*figure 1, top panel*). It should be noted that the signal is typically drawn in reverse polarity; the dZ/dt minimum is shown as a maximum. By combining the ICG and the electrocardiogram (ECG), it is possible to derive the pre-ejection period (PEP) as the time between the start of ventricular depolarization (Q onset in the ECG) and the opening of the aortic valve (B-point in the ICG). A second systolic time interval, the left ventricular ejection time (LVET), is derived as the difference between the B- and X-points in the ICG. The LVET can be used in conjunction with the amplitude of the C-point to compute SV using the Kubicek equation [10]:

(1) $SV = \rho (L/Z_0)^2 \bullet LVET \bullet dZ/dt_{max}$

Where SV is the stroke volume (mL); ρ = blood resistivity (fixed at 135 Ω*cm); L = measured length between the measuring electrodes (cm); Z_0 = baseline thorax impedance (Ω); LVET = left ventricular ejection time (s); dZ/dt_{max} (Ω /s) = the amplitude (i.e. absolute value), measured from either the dZ/dt=0 baseline or the dZ/dt amplitude registered at the B-point [47]. Note that thorax impedance decreases during the systolic phase, but in keeping with typical visual depiction of cardiac signals, the ICG signal is usually drawn in reverse polarity and the dZ/dt minimum is shown as a dZ/dt maximum. This convention will be retained throughout this report and the dZ/dt minimum will be referred to as $dZ/dt_{max.}$

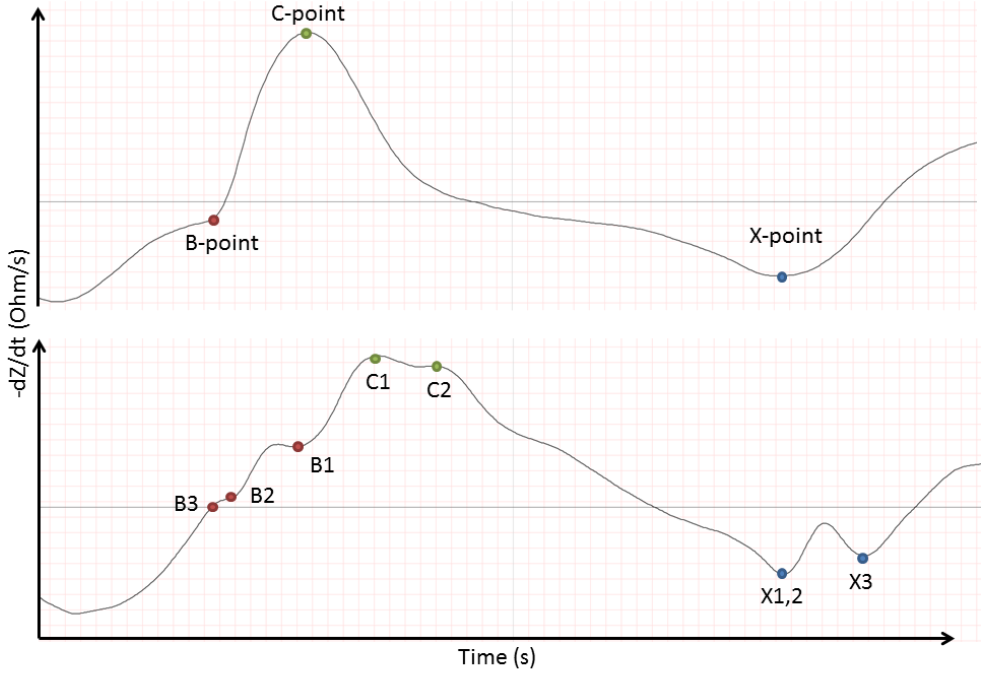

Figure 1. Top panel: unambiguous ICG including B- C- and X-point. Lower panel: complex ICG waveform with multiple cadidates for B- C- and X-points

Various groups that have intensively used the ICG in psychophysiological research, including our own, have noted that instead of an idealized ICG wave form (*figure 1, top panel*) many subjects present with a more complex ICG wave form (*figure 1, lower panel*) introducing ambiguity in the scoring of the B-point, as well as that of the C- and X-points [47;146-148]. Typically, the ambiguity in B-point scoring is caused by a double notch. B-point ambiguity is most clearly seen in cardiac patients, especially at older ages [149]. Ermishkin and colleagues hypothesize that the B-point is the intersection of two waves: the pre-ejection wave (caused by changes in heart geometry and that of surrounding vessels due to cardiac contraction in the isovolumetric phase) and the ejection wave (caused by increased volume in the aorta and surrounding vessels). Timing of the pre-ejection wave could explain the double notch in the ICG signal. The double C-point may be attributable to different aortic flow patterns[150], in healthy subjects during exercise but in cardiac patients may also be present in rest. The presence of multiple of these ICG landmark candidates can also be caused by the non-synchronous contraction of the ventricles, for example because of conduction disorders such as a bundle branch block, sometimes seen in patients with prior

operations on the intra-cardiac septum. Typically, the uncertainty in scoring of the X-point is attributable to a W-shaped (double U) waveform, where the trough in either the first or second 'U' may be the point corresponding to the closure of the aortic valve. The W-shape likely represents the successive closing of the aortic and the pulmonic valve [151;152] which in healthy subjects can occur during inspiration but can also be caused by an atrial septal defect or a bundle branch block. Exercising at increasing levels of intensity further modulates the shape of the ICG at the X-point [153].

The ambiguities in B- and X-points are further aggravated by respiration, postural and movement artefacts, and in our experience occur more often in ambulatory recordings than in recordings from laboratory settings. ICG signal quality can be improved by band pass filtering and ensemble averaging, but no amount of signal conditioning completely dispenses with the ambiguity of the critical landmarks in the ICG. Ambiguity in the B-point immediately affects the validity of the PEP and LVET, but it also distorts the dZ/dt-min amplitude that is used in SV estimation. Ambiguity in the X-point and C-point further distort SV estimation by influencing the left ventricular ejection time (LVET) and dZ/dt-min amplitude respectively. Empirical validation of B- and X-point scoring in the ICG against a criterion measure of aortic valve opening and closure is direly needed.

To obtain the ICG signal, current electrodes on the back send a small alternating current through the thorax and measuring electrodes on the chest detect changes in thoracic impedance related to the aortic blood flow. In the original development of the technique band electrodes around the neck and waist were used, but soon spot electrodes on the back and chest were found to yield ICG signals of comparable quality [118-120;154]. Compared to band electrodes, spot electrodes greatly decrease obtrusiveness and measurement burden on the subjects, increasing feasibility of longer recordings and improving ecological validity [155]. However, there are two important distinctions in the ICGs generated from spot and band electrodes. The baseline impedance of the thorax is much higher using band electrodes and L parameter (distance between the measuring electrodes) is often larger since the band electrodes have to be attached further apart than the spot electrodes in most spot electrode configurations [118;154]. This has repercussions for the Kubicek equation where SV is estimated using the product of the dZ/dt amplitude and the LVET, weighing for blood resistivity, the distance between the measuring electrodes, and the baseline thorax impedance. Typically, absolute SV seems to be overestimated if no correction of the Kubicek equation is made to account for the use of spot electrodes [118].

The main aim of this study was to improve SV assessment from a spot-electrode based ICG in a pediatric population of both healthy children and children with a corrected

CHD. We used a two-step approach that we present in two separate analyses based on the same experiment. Both sets of analyses consist of a discovery and a replication phase and employ the same group of healthy controls and patients. We present these analyses as study 1 (optimization of scoring methods for ambiguous ICG landmarks) and study 2 (tailoring the Kubicek SV equation to spot electrodes). An overview of the complete design is depicted in *figure 2*. In study 1, optimal methods to select the correct point from multiple potential B-, C- and X-points in the ICG signals were determined in a discovery set of healthy children by comparing the ICG-derived systolic time intervals to those derived from simultaneously recorded Doppler signal by transthoracic echocardiography (TTE). Ambiguity for the C-point is usually caused by the presence of a bimodal peak, where it is still unclear if the first or the second of such C-points corresponds to the fastest acceleration of aortic blood flow. As acceleration can be detected visually with reasonable fidelity in the echocardiogram of most subjects, our data will allow us to resolve this by visual inspection. Ambiguity in the B-point and X-point in the ICG can be resolved by TTE as they should coincide with the moments of opening and closure of the aortic valve which can be detected accurately in the echocardiogram. The method for the B- and X-point selection that yield the highest Intra Class Correlation (ICC) compared to echocardiography was taken forward in two replication datasets: a second group of healthy children and a group of patients with a corrected CHD (ventricular septal defect and coarctation of the aorta). ICCs for TTE- and ICG-derived PEP and LVET in these datasets will be used to test the criterion validity of the new B- and X-point selection method.

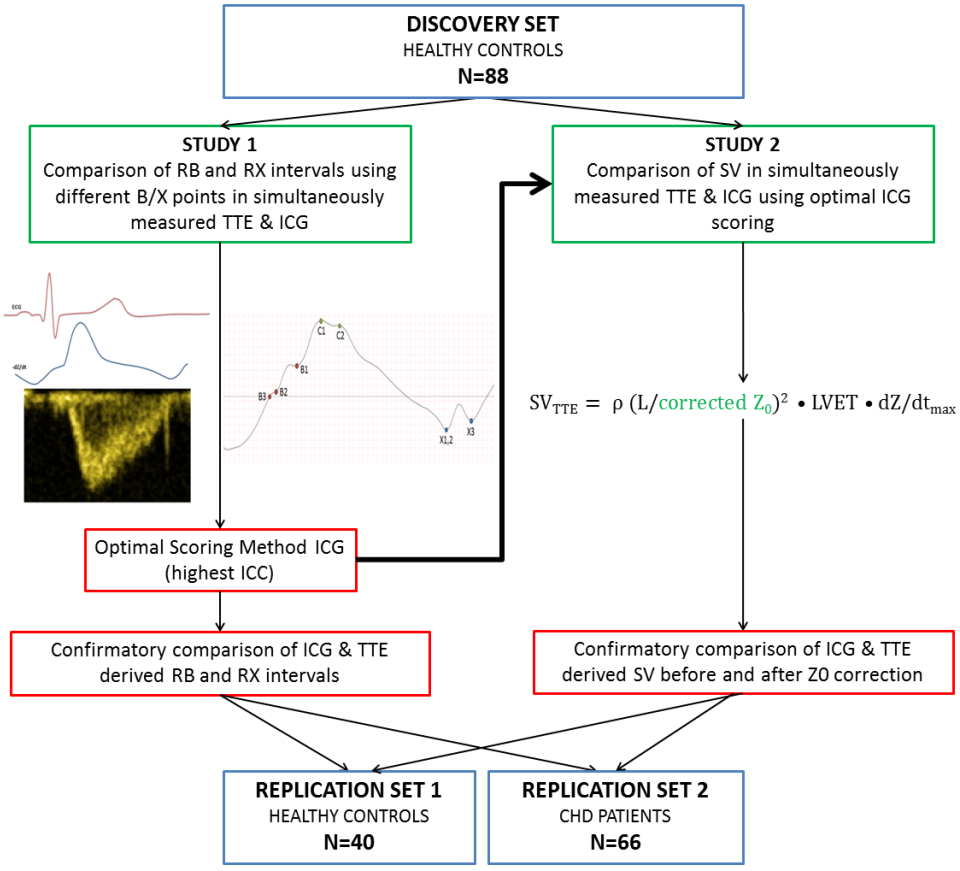

Figure 2. Study design

In study 2, using the optimal scoring methods for B-, C- and X-points from study 1, SV scoring was optimized by adjusting the Kubicek equation for the value of the baseline impedance (Z_0) in the spot electrode configuration. Based on the difference in the SV as measured by the ICG and the echocardiogram, a corrected Z_0 was computed in the discovery set that maximizes the ICC between SV derived from echocardiography and SV derived from ICG for each individual subject, additionally taking into account that subject's value for L, dZ/dt_{max}, age, sex, height and weight. The resulting Z_0 correction approach was applied to the two independent replication sets (healthy controls and children operated for their CHD). ICCs for SV was used to test the criterion validity of this new ICG-based SV-scoring approach in both healthy children and children with CHD, again by comparing to echocardiography. Because SV can be derived from the echocardiogram through different

methods, we repeated the SV validation using the three most often used methods; biplane, velocity time integral and 3D.

PARTICIPANTS AND PROCEDURE

Participants

Healthy controls between 1 and 18 years of age were recruited to take part in the studies. Children from 1-4 years were recruited from the outpatient department of pediatric cardiology. Those who had an innocent murmur or other complaints that turned out to be unrelated to a cardiac disorder were asked to participate. Healthy controls aged 4-18 were passively recruited through an advertisement at school. Chronic disease or medication use were exclusion criteria. When the echocardiogram showed a structurally and functionally normal heart, the volunteer was included in the study. Patients with repaired CHD from the outpatient clinic aged 8-18 years old were asked to participate. Patients after isolated ventricle septum defect (VSD) repair and patients after isolated coarctation (CoA) repair were included in the study. Children with chromosomal disorders were excluded. All participants and both (one in the case of single-parent families) of their parents/guardians provided written informed consent. All study procedures were reviewed and approved by the Medical Ethics Review Committee of the LUMC medical centre (P13.198 and P14.095). The 128 healthy control subjects were randomly divided into a discovery set (N=88) and a replication set (replication set 1, N=40). The group of CHD patients were allocated to replication set 2 (N=66).

Procedure

Data collection took place at the LUMC medical centre. First, the procedure was explained to the participants and their parents. Next, participants were weighted and length was measured. Then, electrodes for thoracic impedance monitoring were attached, connected to the device and the participant was laid down for the echocardiogram with simultaneous measurement of the ICG which took on average 20 minutes.

Electrocardiogram registration and thoracic impedance

ECG and ICG registration was done using the 5fs version of the VU Ambulatory Monitoring System (VU-AMS; VU University, Amsterdam, The Netherlands, www.vu-ams.nl) [15]. One lead ECG was derived from 3 pregelled Ag/AgCl (Kendal H124SG) spot electrodes on the chest. ECG was sampled at 1kHz and R peaks are automatically detected by the device. We measured thoracic impedance (Z) against a small alternating current (50 kHz, 350 μA)

induced by two spot electrodes at the back. Thoracic impedance was recorded with a sample frequency of at 250 Hz. The measuring electrodes were placed just above and below the sternum. Current electrodes were placed 3 cm above and below the measuring electrodes (placement depicted in *figure 3*) on the back of the thorax. A fixed period of 1 minute synchronous to the echocardiography acquisition was selected from the VU-AMS data. Ectopic beats were removed from the data. Ensemble averaged ICG and ECG over that period were used for analysis.

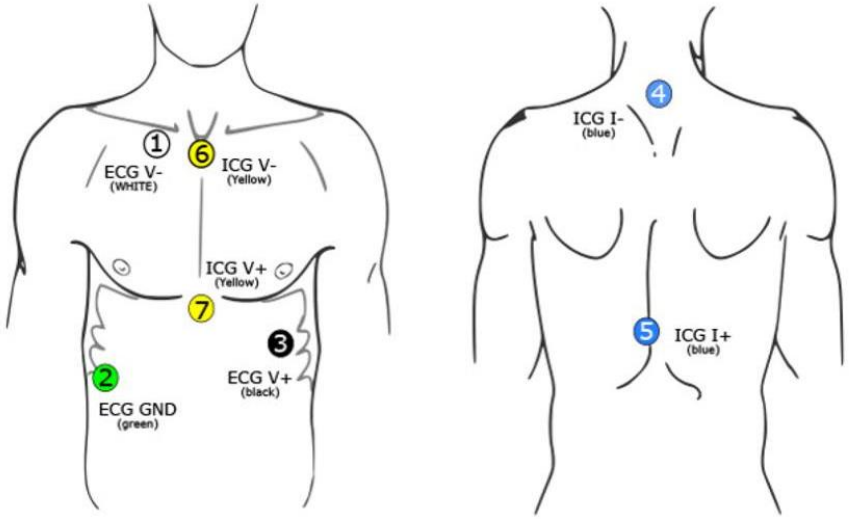

Figure 3. Placement of the seven spot electrodes

Transthoracic echocardiogram

Transthoracic echocardiograms (TTE) were conducted by a pediatric cardiologist or an experienced technician (Vivid 9, GE healthcare, Norway) and evaluated by one researcher, supervised by a pediatric cardiologist. EchoPac version 113 was used for analysis of the images.

STUDY 1 (optimization of scoring methods for ambiguous ICG landmarks)

METHODS

Echocardiogram

Systolic time intervals were assessed using a pulsed wave Doppler flow signal just after the aortic valve in a parasternal 5 chamber view. Time between the R peak and the opening

and closing of the aortic valve were measured (*figure 4*). This was repeated in three different heart beats for every subject, the average of those three measurements was used for analysis.

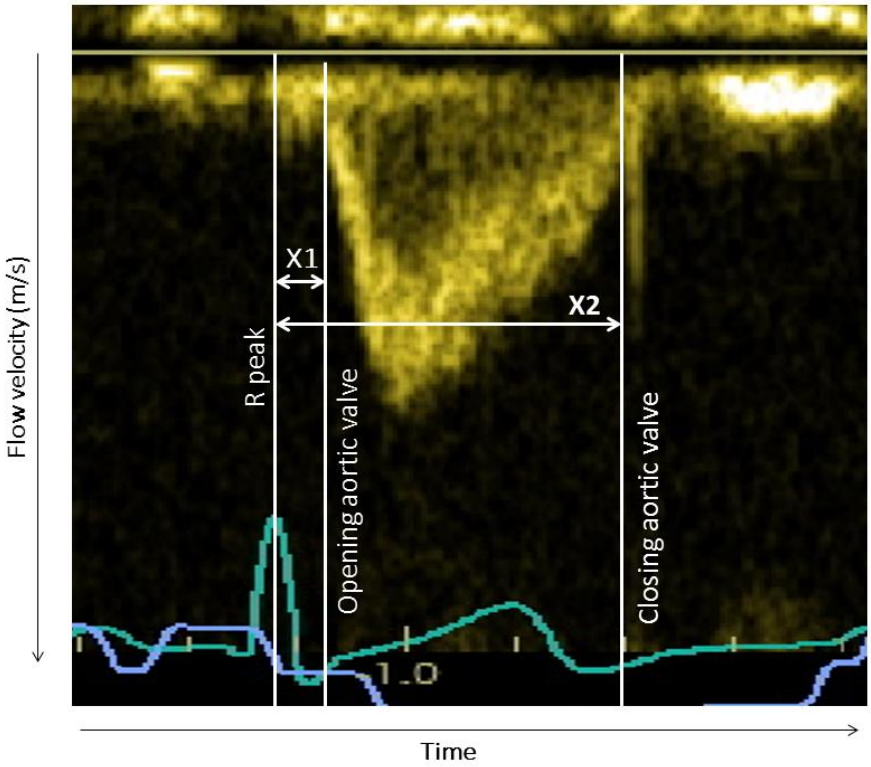

Figure 4. Time intervals measured by TTE. RB: time between R peak and opening of the aortic valve; RX: time between R peak and closing of the aortic valve

Optimizing B- point selection

Based on the literature and our previous experience, 3 candidates (*figure 1, lower panel*) were considered to potentially reflect the true B-point during interactive visual scoring. The candidate B-point had to occur in a 150 msec window after the R-peak.

Candidate B-points:

1. candidate before highest upstroke of dZ/dt (B1)
2. candidate closest to dZ/dt=0 (B2)
3. dZ/dt=0 (B3)

For each of these candidate B-points the interval (ms) between the R-peak and the B-point was calculated. If only candidate B-point 1 was discernible, no ambiguity was present and the intervals for the B-point for candidate 2 was set to an identical value as the interval for candidate B-point 1. *Figure 5* shows an example with 3 different intervals (red lines). We then compared the RB intervals for each of the three candidates to the criterion RB intervals extracted from the echocardiogram. For the extraction of these RB intervals we visually marked the moment of opening of the aortic valve from the Doppler signal (*figure 4*). We did this comparison across all subjects and separately for illustrative purposes using only subjects with an ambiguous B-point. The scoring method (B1, B2 or B3) with the highest ICC (across all subjects) between the intervals in the discovery set was subsequently applied in the replication sets.

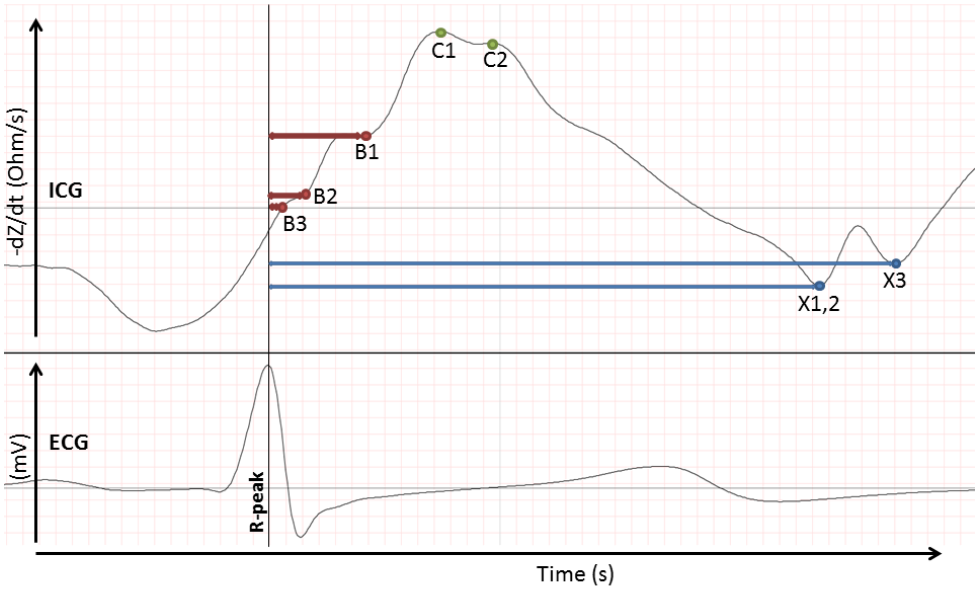

Figure 5. Time intervals measured in the ICG. RB intervals for the different potential B points (red lines) and RX intervals for the different potential X points (blue lines).

Optimizing C-point selection

The echocardiogram was visually inspected for the moment of maximal blood flow acceleration in the ascending aorta. The maximal acceleration is located at the steepest tangent to the Doppler flow signal.

Optimizing X- point selection

Based on the literature and our previous experience, 3 candidates (*figure 1, lower panel*) were considered to potentially reflect the true X-point during interactive visual scoring.

Candidate X-points:

1. lowest point in dZ/dt signal (X1)
2. first trough (X2)
3. second trough (X3)

In the example in *figure 1*, X1 and X2 coincide. It is also possible that X1 and X3 coincide. For each of these candidate X-points the interval (ms) between the R-peak and the X-point was calculated in the ICG, again in the discovery set (N=88). If only candidate X-point 1 was discernible, no ambiguity was present and the RX intervals for candidates 1, 2 and 3 were set to an identical value. *Figure 5* shows an example with 2 different intervals (blue lines); intervals for X1 and X2 are equal as this point is the lowest point in the signal and the first trough. We then compared the RX intervals for each of the three candidates to the criterion RX intervals extracted from the echocardiogram. For the extraction of these RX intervals we visually selected the moment of closing of the aortic valve from the Doppler signal (*figure 4*). We did this comparison across all subjects and separately for illustrative purposes using only subjects with an ambiguous X-point. The scoring method (X1, X2 or X3) with the highest ICC (across all subjects) between the intervals in the discovery set was subsequently applied in the replication sets.

As an additional check we created a physiologically plausible window around the T-wave in which the X point should fall. For each X-point candidate (X1, X2 and X3), it was checked whether they fell in this window. Because ventricular repolarization must occur before the ventricular pressure decreases sufficiently to allow the aortic valve to close, we expect the X point to always fall after the peak of the T wave. The QT interval duration is dependent on heart rate; QT-interval time corrected for prevailing heart rate can be described as $QT_{corrected}$=QT+0.154(1000-RR) where RR is the inter-beat-interval in ms [156]. Also, the general consensus is that the shortest QT is 360 ms in healthy persons [157], thus making the equation for the shortest QT interval expected 360-0.154(1000-RR). Since detecting an R-peak is much easier than detecting the Q-onset we subtract a QR interval of 55 ms in order to measure from the R-peak. Additionally, since the QT interval is measured to the end of the T-wave -which is roughly 100ms- and we want to set the window from the T-wave peak, we subtract an additional 50ms. Thus, the expected heart rate corrected RT_{wave} interval –and thus the start of our physiologically plausible X-point window- would be

(360-(55+50)-0.154(1000*RR)= 255-0.154(1000-RR). The end of the X-point window was defined by the use of the longest expected LVET based on a large published data set [158]; LVET during sleep was 314.9±28.1 in this study of 564 healthy individuals. The mean LVET plus 3 standard deviations (=400ms) was taken as the longest expected LVET. Again, in order to measure from the easily detected R-peak, the RB interval must be added which was studied by Lozano et al. who found RB=0.55RZ+4.5 where RZ is the time interval from R-peak to C point [147]. Summing the RB interval and the longest expected LVET, the end of our X-window is described as: 404.5+0.55RZ. In *figure 6*, the physiological plausible X-point window is visualized.

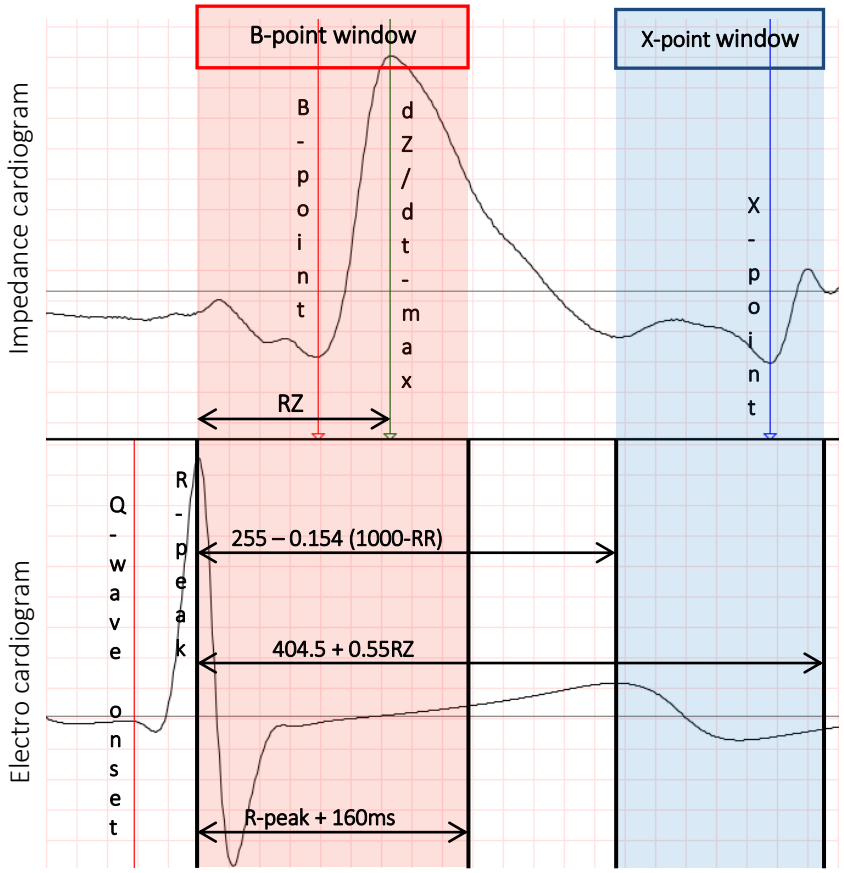

Figure 6. Physiologically plausible windows for the B-point and X-point. HR=heart rate; ms=milliseconds; RR=inter-beat interval

Statistical analyses

IBM SPSS statistics software (version 23.0, Armonk, NY) was used for statistical analysis. The random number generator was used to allocate healthy control subjects to the discovery or replication set. For the comparison of the different B and X points and SV, intra class correlation (ICC; two way mixed, consistency agreement, single measures) was calculated.

RESULTS

General descriptives

General descriptives of the healthy control group and the patient group can be found in *table 1*. Age, gender, length and weight were not different in the discovery set and replication set of healthy controls. Patients, however, were slightly older and therefore longer and heavier.

Table 1. General descriptives of the healthy controls (discovery and replication set 1) and of the CHD patients (replication set 2).

	Healthy Controls Discovery	Healthy Controls (Replication set 1)	CHD patients (Replication set 2)
N	88	40	VSD: N=34 CoA: N=32
Male (%)	56	45	43
Age (y)	9.1; 9.3	11.0; 8.2	12.3; 5.3
Length (cm)	140.0; 55	151.5; 43.8	156.0; 23.7
Weight (kg)	32.5; 34.1	39.1; 30.7	45.5; 24.8

Note. Median; IQR. CHD; congenital heart disease. VSD; ventricle septal defect. CoA; coarctation of the aorta

B-, C- and X-point scoring

Table 2 shows the number of cases in which we found 1, 2 or 3 candidate points. In six children no reliable B- or X-point could be detected. A majority of the children showed multiple candidates for the B- (57%) and X-points (69%) whereas for the C peak often only a single candidate was present (70%). Best agreement with echocardiography for the B-point across all subjects was found for candidate B1: the point just before the longest uninterrupted upstroke of dZ/dt (*table 3;* ICC=0.48 95%CI:0.27;0.65). The B1 point also performed best in the subset of subjects with ambiguous B-points. When applying this B1 scoring method to the replication set of healthy controls, agreement for the RB interval was 0.50 *(table 4*; 95%CI:0.22;0.70). In the CHD patients, ICC for the RB-interval with this B-point was 0.48 (*table 4*; 95%CI:0.25;0.66).

Table 2. Number of candidate B- and X-points in the discovery set.

	N	*%*
Missing	6	7
Single B-point (B1=B2)	32	39
Double B-point (B1+B2)	50	61
B3 *	64	78
Single X-point (X2=X3)	21	26
Double X-point (X2+X3)	61	74
X1 (always present)	82	100

**when B3 (dZ/dt=0) was located before the R-peak, it was set to missing*

In those instances where multiple C-points were present, visual inspection of the echocardiogram systematically favoured the peak nearest to the B-point (C1) as being closest to the point of maximal blood flow acceleration. This suggests that the moment of maximal blood flow acceleration in the aorta comes very soon after the opening of the aortic valve.

For the X-point, the best agreement across all subjects was found for candidate X2 (*table 3;* ICC=0.86 95%CI:0.76;0.91). The X2 point also performed best in the subset of

subjects with ambiguous X-points. Often, X2 and X3 co-occur, as part of a W-shaped (double U) waveform. Here, we found that the trough in the first U has the best correspondence to the closure of the valve in the echocardiogram. In replication set 1 (healthy controls), ICC for the RX interval based on X2 was 0.84 (*table 4*; 95%CI: 0.59; 0.93) and in replication set 2 (patient group) 0.82 (*table 4*; 95%CI:0.48;0.92)

We also computed the percentages of the candidate X points that fell into the physiological plausible X-point window for each of the X-point candidates. Both X2 and X3 fell 100% of the subjects within this window, but X1 was outside the window in 13.1% of the subjects. Of note, 100% of the 'true' X points measured by TTE fell into this window. On average, the X2-point fell 91±20 ms after T-wave peak.

Table 3. Intra class correlation and 95% confidence intervals (95% CI) between the RB and RX intervals measured using ICG (figure 5) and the RB and RX intervals from TTE (figure 4) for the different candidate B- and X-points.

	Subjects with multiple B- (N=50) or X-points (N=61)		All subjects (N=82) *	
	ICC	95% CI	ICC	95% CI
B1	0.53	0.29; 0.70	0.48	0.27; 0.65
B2	0.12	-0.08; 0.34	0.11	-0.06; 0.29
B3	0.28	-0.13; 0.53	0.25	0.15; 0.46
X1	0.64	0.46; 0.76	0.63	0.48; 0.75
X2	0.95	0.86; 0.98	0.86	0.76; 0.91
X3	0.50	-0.04; 0.83	0.55	-0.09; 0.82

** For subjects with no ambiguity B1=B2 and/or X1=X2=X3*

PEP and LVET

PEP is defined as the time delay between the ventricular depolarization of the ventricles (Q wave in the ECG) and the start of left ventricular ejection. Because Q-onset was hard to detect in the echocardiographic ECG, PEP_{TTE} was measured from R-onset to the onset of left ventricular ejection. PEP_{ICG} was therefore also measured from R-onset to the B-point. The agreement between PEP_{ICG} and PEP_{TTE} was moderate: ICC=0.57 in replication set 1, ICC=0.50 in replication set 2 (see *table 4*). Using the optimal B- and X–point candidates, agreement between the LVET from ICG and LVET from TTE was 0.69 (*table 4*; 95%CI:0.27;0.86) in replication set of healthy children and 0.59 (*table 4*; 95%CI:0.10;0.80) in the patient replication set. Pearson correlation between ICG and TTE for PEP and LVET in the entire group of subjects was r=0.62 (p<0.001) and r=0.80 (p<0.001) respectively.

Table 4. Intra class correlation between the RB interval, RX interval and LVET measured in the ICG and by TTE in the independent replications sets.

Healthy Controls (replication set 1)			
	N	ICC	95% CI
B point	35	0.50	0.22; 0.70
X point	37	0.84	0.59; 0.93
PEP	35	0.57	0.27; 0.76
LVET	37	0.69	0.27; 0.86
CHD patients (replication set 2)			
	N	ICC	95% CI
B point	64	0.48	0.25; 0.66
X point	65	0.82	0.48; 0.92
PEP	64	0.50	0.22; 0.67
LVET	64	0.59	0.10; 0.80

Note. PEP=pre ejection period. LVET=left ventricular ejection time

STUDY 2 (Tailoring the Kubicek SV equation to spot electrodes)

METHODS

Transthoracic echocardiogram

Stroke volume was assessed in three ways in the echocardiogram; 1. Velocity time integral (SV_{VTI}), 2. Biplane method ($SV_{biplane}$) and 3. Three-Dimensional echocardiography (SV_{3D}). SV_{VTI} was obtained by multiplying the aortic cross sectional area with the velocity time integral from a pulsed wave Doppler flow signal over the left ventricular outflow tract in a parasternal 5 chamber view. $SV_{biplane}$ was obtained on a 2D view by drawing the end-systolic and end-diastolic left endocardial contours in a 2- and 4 chamber parasternal view and subtracting end-systolic from end-diastolic volumes (modified Simpson method). SV_{3D} was conducted by subtracting end-systolic from end-diastolic volumes (calculated by the use of the divergence Theorem [1]). Additionally, an average SV ($SV_{average}$) was calculated for every subject across the three methods.

Optimizing stroke volume estimation

Stroke volume was estimated from the ICG using the optimal scoring method for B, C and X points. DZ/dt_{max} was measured in relation to the dZ/dt_{max}=0 line. For every subject in the discovery set, a corrected Z_0 was calculated by rearranging the Kubicek equation and filling in the 'true' SV measured by the echocardiogram (SV_{TTE}) :

$$(2) \qquad Z_{0\,corrected} = \sqrt{\frac{\rho L^2 \left(\frac{dZ}{dt}\right)_{max} LVET}{SV_{TTE}}}$$

Subsequently, we tested whether we could successfully predict this corrected Z_0 from known subject characteristics (actual Z_0, dZ/dt_{max}, gender, age, height, weight, BMI, blood pressure and L). This was repeated for the SV_{TTE} obtained with the three different echocardiographic methods ($SV_{biplane}$, SV_{VTI} and SV_{3D}) and we also computed a mean SV across these three methods ($SV_{average}$). This provided an intercept and beta-values for regression equations to obtain the corrected Z_0 for every echocardiographic method and $SV_{average}$. The resulting regression equations for the corrected Z_0 were subsequently applied to the replication sets and the agreement between the SV estimated by ICG (using the estimated corrected Z_0) and SV_{TTE} was calculated as an ICC.

Statistical analyses

IBM SPSS statistics software (version 23.0, Armonk, NY) was used for statistical analysis. Multiple regression analysis (backward method) was used to find the optimal regression equation for the corrected Z_0.

RESULTS

Agreement between the SV obtained with the three different TTE methods is shown in *table 5*. The optimal regression equation for $Z_{0\ corrected}$ was calculated for every SV calculation method separately ($SV_{biplane}$, SV_{VTI}, SV_{3D} and $SV_{average}$) and shown in *table 6*. The ratio between the calculated $Z_{0\ corrected}$ and the measured Z_0 was on average 1.36±0.26. Simply using a corrected Z_0 based on this ratio yielded ICCs that were always higher than the uncorrected SV but 4 to 56% lower than those using our equation with dZ/dt (data not shown). In the discovery set, the agreement between the uncorrected SV from ICG and the SV from the echocardiogram was low to moderate (*table 7)*. When employing the corrected Z_0, agreement between SV from ICG and SV from echocardiography strongly increased. In the independent replication set, the median ICC across different TTE methods increased from 0.22 to 0.70 in the healthy controls and from 0.14 to 0.37 in the CHD patients. In addition to the ICCs, Pearson correlations were calculated in order to facilitate comparison to different studies as most other studies report only the correlation. Finally, bias, 95% limits of agreement and mean absolute percentage error are shown in *table 8* and *figure 7* shows the Bland-Altman plot and SV from ICG against SV from TTE for $SV_{average}$.

Table 5. Intra Class Correlations of three different stroke volume measurements from TTE (Biplane, VTI and 3D).

	Controls	95%CI	Patients	95%CI
Biplane vs. VTI	0.57	-0.01; 0.81	0.35	-0.10; 0.68
Biplane vs. 3D	0.81	0.73; 0.87	0.71	0.54; 0.83
VTI vs. 3D	0.68	-0.02; 0.88	0.25	-0.10; 0.56

Table 6. Regression equation for the corrected Z_0 using the stroke volume measured by the three TTE and their average. Last column shows the explained variance of the regression

	$Z_{0\ corrected}$	R^2
$SV_{biplane}$	$-1.291 + 0.304 * Z_0 - 6.695 * dZ/dt_{max} + 0.442 * L$	0.65
SV_{VTI}	$4.619 + 0.227 * Z_0 - 5.363 * dZ/dt_{max}$	0.71
SV_{3D}	$7.978 - 6.359 * dZ/dt_{max}$	0.65
$SV_{average}$	$7.337 - 6.208 * dZ/dt_{max}$	0.70

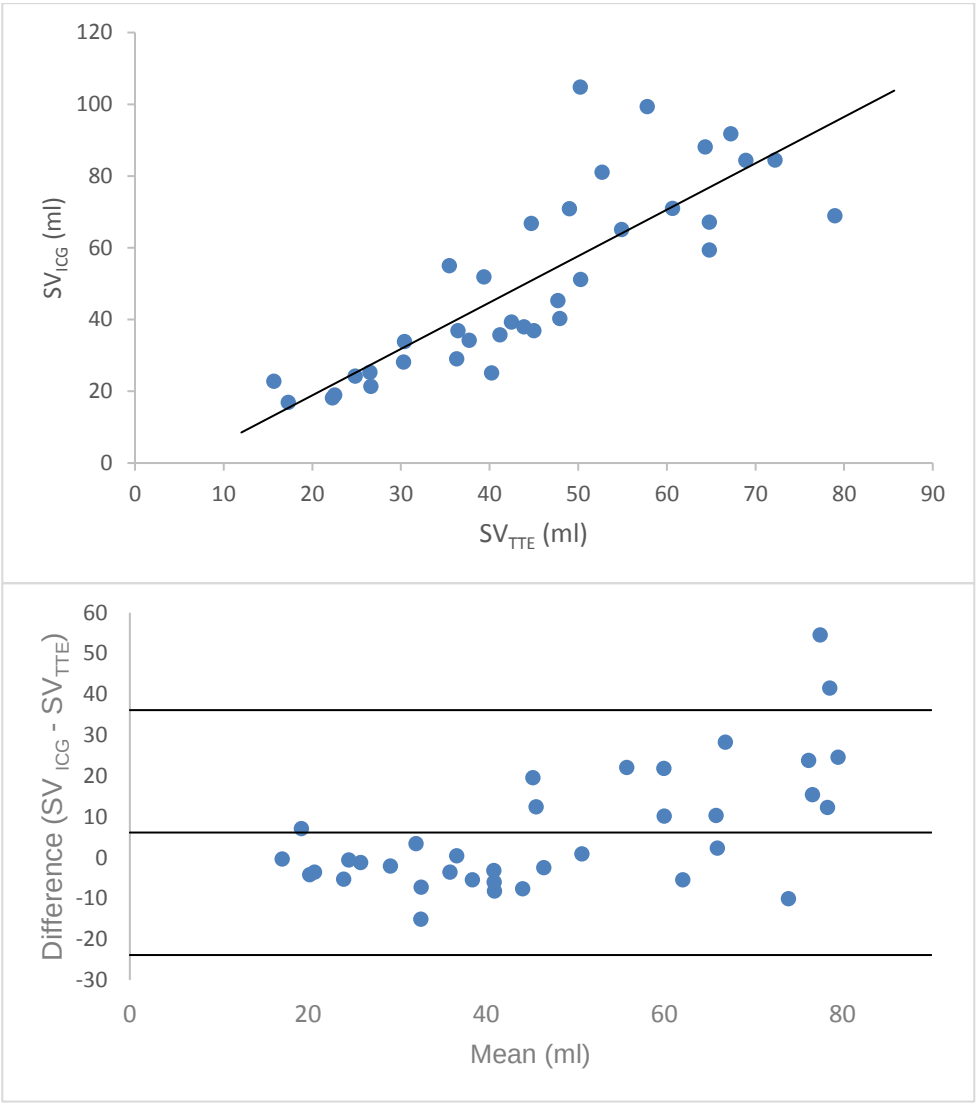

Figure 7. SV from TTE plotted against SV from ICG (upper panel) and Bland- Altman plot (lower panel) for $SV_{average}$ in replication set 1

Table 7. Intra Class Correlations (ICC), Pearson correlations (r) between SV estimated by ICG (SV_{ICG}) using the regression equation for the corrected Z_0 -specific to the SV method- and the SV measured by TTE (SV_{TTE}) for the discovery and replication sets. Final columns show mean SV_{TTE} and SV_{ICG} (corrected and uncorrected)

		$ICC_{uncorrected}$	$ICC_{corrected}$	*r*	$SV_{ICG\ uncorrected}$ (ml)	$SV_{ICG\ corrected}$ (ml)	SV_{TTE} (ml)
$SV_{biplane}$	Discovery	0.22 (95%CI:-.08;.48)	0.79 (95%CI:.68;.86)	0.81 (p<0.001)	86.1±51.4	34.9±12.7	35.4±16.2
	Controls	0.17 (95%CI:-.09;.45)	0.68 (95%CI:.38;.80)	0.68 (p<0.001)	88.5±51.8	37.0±12.9	37.4±17.4
	Patients	0.14 (95%CI:-.08;.37)	0.36 (95%CI:.08;.59)	0.50 (p<0.001)	94.7±44.0	39.7±9.8	48.1±16.6
SV_{VTI}	Discovery	0.48 (95%CI:.03;.73)	0.88 (95%CI:.81;.92)	0.89 (p<0.001)	86.1±51.4	54.9±29.0	52.0±25.9
	Controls	0.44 (95%CI:.02;.71)	0.81 (95%CI:.63;.90)	0.90 (p<0.001)	88.5±51.8	61.5±31.9	53.8±23.5
	Patients	0.15 (95%CI:.08;.36)	0.28 (95%CI:.05;.49)	0.32 (p=0.010)	94.7±44.0	64.5±21.7	75.8±26.5

		$ICC_{uncorrected}$	$ICC_{corrected}$	*r*	$SV_{ICG\ uncorrected}$ (ml)	$SV_{ICG\ corrected}$ (ml)	SV_{TTE} (ml)
SV_{3D}	Discovery	0.22 (95%CI:-.09;.50)	0.80 (95%CI:.69;.87)	0.81 (p<0.001)	86.1±51.4	40.3±20.8	40.6±17.9
	Controls	0.16 (95%CI:-.10;.44)	0.60 (95%CI:.32;.78)	0.66 (p<0.001)	88.5±51.8	45.4±22.8	41.4±18.5
	Patients	0.01 (95%CI:-.0;.30)	0.42 (95%CI:.17;.62)	0.43 (p=0.001)	94.7±44.0	48.0±16.1	46.3±12.9
$SV_{average}$	Discovery	0.33 (95%CI:-.07;.61)	0.86 (95%CI:.80;.91)	0.88 (p<0.001)	86.1±51.4	45.2±23.4	42.2±19.3
	Controls	0.26 (95%CI:-.08;.56)	0.72 (95%CI:.50;.85)	0.82 (p<0.001)	88.5±51.8	50.9±25.6	44.1±18.6
	Patients	0.13 (95%CI:-.07;.33)	0.37 (95%CI:.14;.56)	0.38 (p=0.001)	94.7±44.0	53.4±17.9	57.9±16.9

Table 8. Bias, 95% limits of agreement and MAPE for the different methods. Bias is calculated as: SV_{TTE}-SV_{ICG} (ml). MAPE=mean absolute percentage error

Healthy Controls (replication set 1)

	Mean SV_{ICG} (ml)	Bias (ml)	95% limits of agreement	MAPE (%)
$SV_{biplane}$	37.01	0.83	-21.12; 22.79	23.88
SV_{VTI}	61.48	-6.95	-37.62; 23.72	20.93
SV_{3D}	45.38	-5.93	-40.22; 28.35	31.61
$SV_{average}$	50.88	-6.14	-36.14; 23.86	23.86

CHD patients (replication set 2)

	Mean SV_{ICG} (ml)	Bias (ml)	95% limits of agreement	MAPE (%)
$SV_{biplane}$	39.70	8.99	-19.62; 37.61	22.50
SV_{VTI}	64.45	12.01	-44.24; 68.78	26.92
SV_{3D}	47.96	-0.44	-31.11; 30.24	22.97
$SV_{average}$	53.72	4.59	-33.90; 43.09	21.83

DISCUSSION

The aim of this study was to improve SV assessment from spot-electrode based ICG in a pediatric population of both healthy children and children with a corrected CHD. We tackled two sources of error in SV estimation: (1) ambiguity of the scoring of the B- C- and X-points, (2) the use of the Kubicek formula uncorrected for spot-band differences in baseline thorax impedance and uncorrected for the reduction in thorax volume enclosed by the measuring electrodes.

As a first step (study 1), scoring of the landmarks in the ICG defining the systolic time intervals was optimized in a discovery set (70% of the total dataset of healthy controls). In case of a biphasic C wave, the first peak was chosen based on the visual inspection of the echocardiogram in which it was clear that the moment of maximal blood flow acceleration in the aorta is always very soon after opening of the aortic valve. For the B-point (opening of the aortic valve), the point before the highest upstroke showed best agreement with echocardiography (B1 in the lower panel of *figure 1*). For the X-point (closing of the aortic valve) the first trough (X2 in the lower panel of *figure 1*) showed best agreement. Additionally, point X2 showed the highest percentage of beats falling into the physiological plausible X-window. Criterion validity for the optimized scoring method was obtained from two independent replication sets; replication set 1: the remaining 30% of the healthy controls and replication set 2: a group of patients operated for their CHD. In replication set 1, agreement was moderate to good (ICC B-point=.50; X-point=.84) and comparable results were found in the patient group (ICC B-point=.48; X-point = .82).

After optimizing the scoring of the B- C- and X-point in the ICG, we calculated a corrected Z_0 in the discovery set by filling in the 'true' SV from the simultaneously recorded echocardiogram into the Kubicek formula (study 2). This allowed us to estimate an ICG-based SV that is applicable for spot electrodes which are now common in both laboratory and ambulatory applications. The simple formula used for Z_0 correction can eliminate the differences in baseline values of Z0 and the distance between measuring electrodes existing between the spot and band configurations. In the discovery set, the corrected Z_0 was best estimated by a regression equation including electrode distance and/or dZ/dt_{max} as predictors. In the discovery set, the corrected Z_0 was best estimated by a regression equation including electrode distance and/or dZ/dt_{max} as predictors. Thus, interestingly, the actual Z_0 was removed from the equation. Removing Z_0 from the SV estimation equation solves any problems related to the sensitivity of baseline thorax impedance to electrode type and placement. Our results are well-aligned with previous applications of ICG based

CO-estimation without baseline thorax impedance [125;159]. Of note, however, is that under anaesthesia ICG-based CO estimation does not seem reliable [142;143].

We tested our improved SV assessment methods in two independent replication sets and showed moderate to good agreement for SV in healthy controls after correction (median ICC 0.70) but low to moderate in CHD patients (median ICC 0.37). In the healthy controls, highest ICC for SV was obtained when applying the Z_0 corrected for $SV_{VTI.}$ However, since there is no consensus favouring one method over another, we advise to use Z_0 corrected for $SV_{average}$ when estimating SV from ICG using spot electrodes in healthy subjects (Z_0 corrected = 7.337 – 6.208 * dZ/dt_{max}). The agreement found between SV_{ICG} and SV_{TTE} was always lower in patients compared to healthy controls. This was not surprising since the agreement between different methods of measuring stroke volume with TTE was also always lower in patients compared to controls. Of note, SV_{VTI} may have been biased in patients with CoA as they often -approximately 2/3 of patients- have a bicuspid valve which may make the velocity time integral method less reliable. Therefore, we advise to use the corrected Z_0 for SV_{3D} (Z_0 corrected = 7.978 – 6.359 * dZ/dt_{max}) in patients since this closely resembles that of $SV_{average}$ but not included the potentially biased SV_{VTI}. Also, this correction gains the highest ICC in patients (*table 7*).

Although we here focus on SV estimation, ICG has also been widely used to noninvasively measure cardiac sympathetic activity by its effect on contractility [114;160;161]. Increased activity of the sympathetic branch of the autonomic nervous system to the heart is a powerful mechanism to compensate for altered hemodynamics due to heart disease, but it may in time have detrimental effects to the myocardium [8;55;162;163]. Enhanced sympathetic cardiac nervous activity is an important factor in the progression of heart failure and may also play a crucial role in the long term sequelae in CHD patients [164]. Studies comparing cardiac sympathetic activity in CHD patients with healthy controls could help explain how late cardiac complications arise in CHD, particularly if these studies could be done in real life settings as various studies have reported reduced exercise tolerance and functional status as reflected in reduced capacity to deal with activities of daily living [165;166].

There are several methods to measure cardiac sympathetic activity; e.g. scintigraphy of radiolabelled metaiodobenzylguanidine, measurement of norepinephrine regional spill over of plasma catecholamines, microneurography and pharmacological blockade [167;168]. Changes in PEP co-vary strongly with changes in contractility which led to the use of a change in PEP as a measure of the change in cardiac sympathetic activity [160;161;169-172].

This study found moderate agreement for PEP measured using TTE and ICG in healthy children (ICC=0.57, *table 4*) and CHD patients (ICC=0.50, *table 4*). LVET showed good agreement between ICG and TTE (ICC=0.69) in healthy children, and moderate agreement (ICC=0.59) in patients. In the entire group of subjects, Pearson correlation between ICG and TTE for PEP and LVET was r=0.62 (p<0.001) and r=0.80 (p<0.001) respectively. This is in accordance with a recent study by Lorne et al. comparing LVET measured by transoesophageal echocardiography and ICG [121] who found good agreement for LVET (r=0.69) as did Cybulski et al. who compared systolic time intervals between TTE and ICG and found good agreement for PEP (r=0.73) and LVET (r=0.84) in young adults in supine and tilted position [114]. The correlations for PEP and LVET found in our study were lower for PEP (r=0.62 for the entire group, N=183) and comparable for LVET (r=0.80 for the entire group, N=191). However, Fellahi et al. found only a weak agreement between LVET (r=0.27) measured by TTE and ICG [129] and Carvallo et al. conclude that ICG was inaccurate is assessing PEP (r=0.54-0.75 for different scoring methods) and LVET (r=0.19-0.36) compared to TTE [173].

A limitation of this study is that echocardiography and ICG were only measured in a supine position during rest. Therefore, it is unclear how the agreement is in different body positions and at higher heart rates. However, Welsman et al. and Pianosi et al. found increasing CO measured by ICG during a graded exercise test in children/young adults as expected, suggesting that ICG derived CO is able to detect changes in CO with changing physical activity [174;175]. Also, the current study used transthoracic echocardiography as a gold standard for SV measurement while this method itself has its own inaccuracy and therefore introduces additional error in the comparison [176]. This is also reflected in *table 5*; agreement between the different methods for SV calculation is not perfect – especially in patients. In a meta-analysis Critchley et al found an overall percentage error of 65% (25-225%) for Doppler methods compared to 37% (15-82%) for impedance [112]. Likewise, Chew et al. found an average error of around 30% [177]. Taken that into account, the results of this study are satisfactory. SV assessed by the use of ICG is non-inferior to other modalities available and therefore usable in pediatric populations. The fact that ICG is non-invasive and can be measured ambulatory is an important advantage of this method. However, caution is warranted when employing ICG in cardiac patients, as the reliability is less compared to healthy cohorts as shown in the current and other [113] studies. This, may be less of an issue for within-participant contrasts, but could strongly bias comparisons between groups. Further caution is required when different electrode configurations are used. It has been shown that the validity of the Kubicek equation is sensitive to the exact

electrode configuration [178] and we fully suspect that this remains true when using our new dZ/dt approach.

In conclusion, SV estimation by ICG showed moderate to good agreement in healthy children after adjustment of the Kubicek equation. When using spot electrodes, it is advised to use an adjusted Kubicek equation. In pediatric patients, agreement was slight to moderate. Stroke volume assessed by the use of ICG is non-inferior to other modalities available and therefore usable in pediatric populations. Additionally, it has the advantage of measurement in an ambulatory setting, which may increase clinical relevance. However, validity is somewhat less in patients compared to healthy controls. Therefore, ICG should be used as an addition to the clinical evaluation and cannot replace standard SV measurement in cardiac patients. Comparison of systolic time intervals including PEP and LVET measured by ICG and transthoracic echocardiography simultaneously, revealed moderate agreement in healthy children and pediatric CHD patients. Agreement was somewhat better in healthy children, especially in the case of LVET. A next step would be to relate ambulatory recorded SV, CO and cardiac sympathetic activity to clinical features in order to unravel the role of these parameters in the etiology of CHD and to establish whether non-invasive ambulatory ICG might be of additional value in the clinical evaluation of pediatric cardiac patients.

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Chapter 5

Heritability of heart rate recovery and vagal rebound after exercise

Ineke Nederend
Nienke M. Schutte
Meike Bartels
Arend D.J. ten Harkel
Eco J.C. de Geus

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ABSTRACT

Purpose The prognostic power of heart rate recovery (HRR) after exercise has been well established but the exact origin of individual differences in HRR remains unclear. This study aims to estimate the heritability of HRR and vagal rebound after maximal exercise in adolescents. Furthermore, the role of voluntary regular exercise behavior (EB) in HRR and vagal rebound is tested.

Methods 491 healthy adolescent twins and their siblings were recruited for maximal exercise testing, followed by a standardized cooldown with measurement of the electrocardiogram and respiratory frequency. Immediate and long-term HRR (HRR60 and HRR180) and vagal rebound (heart rate variability in the respiratory frequency range) were assessed one and three minutes after exercise. Multivariate twin modelling was used to estimate heritability of all measured variables and to compute the genetic contribution to their covariance.

Results Heritability of HRR60, HRR180 and immediate and long-term vagal rebound is 60% (95%CI:48-69), 65% (95%CI:54-73), 23% (95%CI:11-35) and 3% (95%CI:0-11) respectively. We find evidence for two separate genetic factors with one factor influencing overall cardiac vagal control, including resting heart rate and respiratory sinus arrhythmia, and a specific factor for cardiac vagal exercise recovery. EB was only modestly associated with resting heart rate (r=0.27) and HRR (rHRR60 = 0.10 ; rHRR180= 0.19) with very high genetic contribution to these associations (88-91%).

Conclusions Individual differences in HRR and immediate vagal rebound can to a large extent be explained by genetic factors. These innate cardiac vagal exercise recovery factors partly reflect the effects of heritable differences in EB.

INTRODUCTION

Low resting heart rate is associated with lower risk for cardiovascular and non-cardiovascular premature mortality [179-181]. Mechanistic explanations for this protective effect often invoke a key role for the autonomic nervous system, in particular the parasympathetic branch. The resting heart rate is predominantly determined by cardiac vagal control, which can be measured noninvasively by heart rate variability (HRV) [43;182]. High cardiac vagal control increases electrical stability of the heart [61;62;64;183] and various HRV measures have been shown to be predictive of cardiac mortality and morbidity.

High cardiac vagal control further seems paramount in explaining the predictive effects of heart rate recovery (HRR) after exercise testing. HRR can be divided into immediate (1 minute after exercise cessation) recovery and long term recovery (>1 minute after exercise cessation). During immediate recovery after maximal exercise, heart rate decreases mainly because of increased vagal control while sympathetic control remains practically unchanged [184-186]. Immediate HRR after exercise cessation is associated with lower cardiac mortality in various patient groups [187-194] but also in healthy individuals [195-200]. A recent meta-analysis showed that this relationship is stronger in persons >45 years of age compared to <45 years of age [192]. Furthermore, they showed that HRR was most robust in predicting all-cause mortality. In patients with heart failure, HRR after maximal exercise remains a significant predictor irrespective of beta blockers [201]. HRR after submaximal exercise also seems to have prognostic power in heart failure patients [202].

The prognostic importance of HRR is evident but the origin of the individual differences in HRR and vagal rebound after exercise remain unclear. The two main factors creating individual differences are innate biological differences and environmental factors. The latter can be subdivided in influences shared with other family members (common environmental influences; e.g. upbringing) and unique environmental influences (e.g. physical activity at work). Twin studies enable us to decompose the total variance in traits, e.g. HRR, into genetic, common environmental, and unique environmental components. Intrapair resemblance in HRR is compared between two types of twins; genetically identical (monozygotic, MZ) and non-identical (dizygotic, DZ) twins. When the MZ intrapair resemblance for HRR is higher than the DZ intrapair resemblance, this constitutes evidence for genetic influences on HRR. When the MZ resemblance for HRR is comparable to the DZ resemblance, this constitutes evidence for common environmental influences on HRR. The degree to which MZ twins are discordant for HRR indicates the influence of unique environmental influences on HRR. Structural equation modeling of the MZ and DZ twin

covariances allows an estimation of the relative contribution of genetic influences on HRR to its total variance, an estimate known as the heritability of HRR.

Regular exercise behavior has been cited as a potential causal source of differences in resting heart rate and cardiac vagal control and is therefore also expected to influence HRR and vagal rebound [203-205]. Because regular exercise behavior has been shown to be a heritable trait [206] it could be an important mediator of genetic effects on HRR and vagal rebound. Such hypotheses can be tested in twin models using a multivariate extension of the MZ and DZ twin (co)variance analysis. Work of Kupper et al. [207], for instance, has already shown that the correlation between resting heart rate and resting cardiac vagal control could for a large extent be explained by genetic factors. Moreover, a large amount (>80%) of the phenotypic correlation between exercise behavior and resting heart rate and between exercise behavior and resting cardiac vagal control could indeed be explained by genetic factors [208]. Whether a similar genetic contribution is seen when exploring the association between regular exercise behavior and HRR and vagal rebound after exercise remains unknown.

The first aim of the current study is to estimate the heritability of immediate HRR (60 seconds after exercise cessation) and also of HRR at 3 minutes after exercise and the degree of vagal rebound measured by heart rate variability in the respiratory frequency range (RSA) at these time periods. Multivariate genetic modeling was used to test two further hypotheses: (1) the genetic factors influencing post-exercise HRR and vagal rebound do not completely overlap with those influencing resting heart rate and vagal control; (2) heritability of this 'cardiac vagal exercise recovery' factor in part reflects the heritability of regular voluntary exercise behavior The latter hypothesis is optimally tested in a healthy adolescent population where exercise behavior is known to be highly heritable (72-80%) [209].

METHODS

Participants

Twin pairs aged between 16 and 18, enrolled in longitudinal survey studies of the Netherlands Twin Register [12] were invited to participate. Siblings within an age range of 12-25 years were also invited. Participants were excluded when having a history of cardiovascular or respiratory disease, or when being physically incapable of engaging in exercise activities. Eventually, 491 healthy adolescents participated in the study including 225 complete twin pairs: 58 monozygotic male pairs, 36 dizygotic male pairs, 56

monozygotic female pairs, 42 dizygotic female pairs, 33 dizygotic opposite sex pairs and 37 of their singleton siblings (56% female). Mean age at time of their visit was 17.1 ± 1.1 years (range 12 – 25). All participants and, in participants under 18, both (one in the case of single parent families) of their parents/guardians provided written informed consent. All study procedures were reviewed and approved by the Medical Ethics Review Committee of the VU University Medical Center Amsterdam (NL35634.029.10).

Electrocardiogram registration

Electrocardiogram (ECG) registration was done using VU-AMS device (Vrije Universiteit Ambulatory Monitoring System)[15]. R peaks were scored by an algorithm within the VU-AMS software package and, if present, Premature Ventricular Contractions (PVCs) were scored by a trained researcher under close supervision of a cardiologist. All further analyses were done using PVC-free signals. Heart rate was calculated as an average of a five second period. An overview of calculation of heart rate measures used is displayed in *table 1*.

Table 1. Description of variables

Variable	Description
Resting heart rate	Heart rate in number of beats per minute while sitting quietly
HRR60	Immediate heart rate recovery. Calculated as: maximal heart rate – heart rate at 1 minute after exercise
HRR180	Long term heart rate recovery. Calculated as: maximal heart rate – heart rate at 3 minutes after exercise
Resting RSA	Resting respiratory sinus arrhythmia. Calculated via peak valley method while sitting quietly by averaging RSA in all breaths during 6 minutes of quiet sitting
RSA60	Immediate vagal rebound. Respiratory sinus arrhythmia, an average of the RSA across all breaths in the first minute after exercise
RSA300	Long term vagal rebound. Respiratory sinus arrhythmia, an average of the RSA across all breaths in minute 2-5 after exercise

Thoracic impedance and heart rate variability

In order to obtain breathing frequency, thoracic impedance was measured also by using the VU-AMS device. Vagal rebound was assessed by HRV in the respiratory frequency range, extracted from the ECG and the respiratory signal [15;116;116;155]. Respiratory sinus arrhythmia (RSA) is defined as the longest heart period during expiration minus the shortest heart period during inspiration. RSA was computed on a breath-to-breath basis. Therefore, it is more robust than spectral decomposition methods against violation of the assumptions of stationarity. During exercise recovery such stationarity is likely low. When no difference in shortest and longest beats could be detected, RSA was set to be zero for that breath. An overview of calculation of RSA measures from the breath-to-breath data is displayed in *table 1*.

Exercise tests

Maximal exercise protocol was performed on a bike ergometer (Lode, type Corival) and consisted of an increasing workload per minute. Participants were encouraged by a researcher to exercise until exhaustion. Different recovery modes are in use [210]. Active or supine recovery is preferred since it facilitates venous return and to this end reduces the risk of arrhythmias, hypotension and syncope post exercise [211-213]. Also, since the participants were wearing equipment for the measurement of oxygen uptake on their back, supine recovery was unpractical. For these reasons, participants were obliged to remain seated on the bike ergometer for at least 5 minutes after cessation of the test for cool down. This cooldown was standardized as follows: after cessation of the test, the resistance was immediately decreased to 50W (girls) or 70W (boys) and participants were instructed to pedal at a comfortable rate between 30 and 60 rounds per minute. Resistance at recovery could be slightly adjusted on individual demand based on the maximally reached wattage during the test.

Voluntary exercise behaviour

Participants were queried on their voluntary exercise behavior (EB) as by the use of a short lifestyle interview described in detail elsewhere [209]. The questions in this interview were structured similarly to the longitudinal surveys in the Netherlands Twin Register. For each exercise activity (e.g. swimming, fitness, tennis, jogging, soccer) they were asked for how many years they have been doing that particular sport, how many months per year, how many times per week, and how many minutes each time. Participants had to have been active for at least 6 months and do it more than three months per year for the activity to be included as we were interested in regular exercise behavior. Hence, ski holidays, sailing

camps, and similar were excluded. Also, activity related to transportation (cycling, walking) were excluded. Compulsory physical education classes were also excluded as they do not reflect voluntary exercise behavior and exercise activities are poorly standardized across different high schools in the Netherlands. Each remaining exercise activity was converted into a MET (metabolic equivalent task) score [14]. One MET represents the amount of energy needed for sitting quietly. This MET score was multiplied by the duration of activities and summed to get a weekly MET score. For instance, an individual reporting 90 minutes of field hockey training (MET score = 8) and a 70 minute game each week would yield (2.67 hours*8=) 21.3 METhours /weekly.

Descriptive Statistics

A multivariate Saturated model in OpenMx [214] under R (R Core Team 2014) was used to estimate the phenotypic correlations and their 95% confidence intervals between EB, resting heart rate, resting RSA, HRR60, HRR180, RSA60 and RSA300 taking into account the nested structure of family data. To gain insight into the expected sources of variance in these measures and the underlying sources of phenotypic correlation between these measures MZ twin correlations and DZ/sibling correlations and cross-twin cross trait correlations were estimated.

Genetic modelling

Genetic structural equation modelling was done with the raw-data ML procedure for estimation of parameters. For all analyses, a threshold of $p<0.05$ was considered for statistical significance.

Because (non-twin) siblings share, like DZ twins, on average, 50% of their segregating genes and since the sample size was rather small, parameter estimates were constrained to be equal for males and females and for DZ twins and siblings. However, main effects of sex and age on mean levels of the phenotypes were considered in the model.

In multivariate models, the total phenotypic variance was decomposed into sources of additive genetic (co)variance (A), dominant genetic (co)variance (D) or common environmental (co)variance (C) and unique environmental (co)variance (E). Since C and D effects cannot be estimated simultaneously in the classical twin model, the ratio of the MZ correlations to the DZ correlations was used to determine which model (ACE or ADE) is most appropriate. Significance of variance components was tested by comparing the model including the specific component to a model in which the component is constraint to be equal to zero. This nested submodel was compared using hierarchic χ^2 tests. The χ^2statistic is computed by subtracting log-likelihood (–2LL) for a reduced model from the -2LL for the full model (χ^2= $-2LL_{\text{full model}} - -2LL_{\text{reduced model}}$). This χ^2statistic is distributed with degrees of

freedom (*df*) equal to the difference in the number of parameters estimated in the two models ($\Delta df = df_{\text{full model}} - df_{\text{reduced model}}$). If the difference test is significant the constraints on the reduced model cause a significant deterioration of the fit of the model and thus the component will be retained. *In a multivariate saturated model (in which all parameters are estimated freely)*, We started the genetic model fitting with a Cholesky decomposition [215] for these seven variables (model 1). This Cholesky decomposition reveals a first insight into the etiology of covariances between variables. The model is descriptive and not driven by a specific multivariate hypothesis. Next we tested for the significance of shared environmental influences by constraining these effects to be zero (model 2). This AE model is depicted in *Figure 1*.

To test our hypotheses, the significance of selected path loadings was tested by constraining them to zero. This was done in three steps: first, we tested whether all factors influencing resting heart rate and resting vagal tone (see *figure 1*; A2 and A3) also influenced HRR and vagal rebound after exercise. All path loadings with zero in their 95% confidence interval originating from genetic factor A2 and A3 (pathway *a22, a32, a42, a52, a62, a72, a33, a43, a53, a63 and a73* in *Figure 1*) were constrained to be zero and the fit of this reduced model (model 3) was compared to the Cholesky AE model (model 2). Next, to test the hypothesis that a single genetic factor influences post-exercise HRR and vagal rebound overlap, path loadings of A4 through A7 (pathway *a44, a54, a64, a74, a55, a65, a75, a66, a76 and a77* in *Figure 1*) with zero in their 95% confidence interval were constrained to zero and this reduced model (model 4) was compared with model 2. Finally, the overlap between the genetic factor influencing regular exercise behaviour and the cardiac vagal exercise recovery factors was explored by constraining the non-significant path loadings originating from A1 (pathway *a11, a21, a31, a41, a51, a61 and a71* in *Figure 1*) to zero. The fit of this model was then compared to model 2. In the final model only the significant pathways were retained and all heritability's were computed using this final model.

RESULTS

General descriptive

Means and standard deviations for resting heart rate, resting RSA, immediate HRR and vagal rebound, long term HRR and vagal rebound, EB and ventricular arrhythmia (VA) are shown in *table 2*. For the analysis of heart rate and HRV, further analyses were done using PVC-free signals. Subjects who had ectopic beats were not excluded from the analysis but ectopic beats were removed from the data. 381 (77%) adolescents showed no ventricular

ectopy during the entire exercise protocol. 94 (19%) had at least one but less than 10 premature ventricular contractions (PVCs) and 16 (3%) had more than 10 PVCs during the entire recording. There was no difference between males and females (p=.178). Within the group of adolescents with PVCs, these were polymorph in 10 (9%), bigiminal in 6 (5.5%) and 3 adolescents showed one or more couplets (2.7%). In the 16 adolescents whose ECG showed over 10 PVCs during the entire protocol, 13 showed one or more in rest, 10 during submaximal exercise and 6 during the maximal exercise test (see *table 2*). Athletes (defined as >50 MET EB per week, N=46) showed significantly more ventricular ectopy compared to non-athletes (p<.001). Variance in the presence of VA in this group could not be explained by genetic influences (data not shown).

Table 2. Descriptives.

	Males		Females	
	Mean	*SD*	*Mean*	*SD*
Age	17.0	1.1	17.2	1.1
Ventricular arrhythmia (#PVCs)	1.6	8.0	2.7	23.5
None (N, (%))	183 (76)		198 (79)	
<10 (N, (%))	50 (21)		44 (18)	
>10 (N, (%))	7 (3)		9 (4)	
Resting heart rate (bpm)*	72.4	11.3	75.7	11.1
Maximal heart rate (bpm)	195.5	9.7	194.4	8.9
HRR60 (bpm)*	25.4	7.8	22.1	7.2
HRR180 (bpm)*	58.6	10.5	53.4	10.5
Resting RSA (ms)	74.9	38.0	80.2	41.1
RSA60 (ms)*	8.2	2.1	7.0	1.9
RSA300 (ms)*	9.7	4.1	8.4	3.9
EB (METs/week) *	26.0	22.6	19.3	21.7

Note. PVC=Premature Ventricular Contraction. *significant difference between males and females

Heritability

Correlations for MZ twins were higher than DZ/sibling correlations for all phenotypes (see *table 3*), suggesting a genetic effect. The results of the model-fitting can be found in the *table 5*. Based on the best fitting model (*figure 2*) we estimated a heritability of 80% (95%CI: 73-85) for EB, 68% (95%CI: 58-76) for resting heart rate, 60% (95%CI: 48-67) for HRR60, 65% (95%CI: 54-73) for HRR180, 58% (95%CI: 45-69) for resting RSA, 23% (95%CI: 11-35) for RSA60 and 3% (95%CI: 0-11; not significant) for RSA300 (*see table 3*). The remaining variance in these variables could be explained by unique environmental factors.

Genetic contributions to covariances

Phenotypic correlations are shown in *table 4*. Resting heart rate was moderately correlated to resting RSA (r=-.39, 95% CI:-.47, -.30) and showed a small inverse correlation to HRR and vagal rebound (-0.17 < r < -0.29).

HRR was moderately correlated to vagal rebound, even when short- and long term recovery was cross-correlated (.24 < r < 0.58). EB was significantly correlated to resting heart rate and HRR180 only. *Table 4* therefore suggest the existence of a general cardiac vagal factor that influences resting heart rate and resting RSA but also HRR and vagal rebound; dark grey shaded area) and a specific cardiac vagal exercise recovery factor (including HRR60, HRR180, RSA60 and RSA300; light grey shaded area).

The existence of these factors were confirmed by the Cholesky model. Pathloadings *a62, a72, a43, a53, a63*, *a43, a53, a63 and a73, a65, a75, a76, a77, a31, a41, a61 and a71* were constrained to zero as zero was in the 95% confidence interval. The final model is shown in *Figure 2*. Factor A2 loaded on resting heart rate and resting vagal tone, as well as HRR60 and HRR180 and might be considered as a general cardiac vagal factor. The majority of the phenotypic correlations between these variables could be explained by genetic factors (72% - 99%). Factor A4 can be considered as a recovery factor, as significant path loadings were detected between this factor and all four recovery variables. 9% - 68% of the phenotypic correlations between these recovery variables could be explained by genetic factors. Finally, the same genetic factor (A1) that influenced regular exercise behavior also influenced resting heart rate and long term heart rate recovery. Almost all (88% - 91%) of the observable phenotypic correlation between these variables could be explained by this common genetic factor. Model fitting results can be found in *table 5*.

Table 3. MZ twin and DZ/sibling correlations were estimated in the multivariate saturated model and proportion of variance and confidence intervals due to A and E, were estimated from best fitting multivariate model

	r_{MZ}	$r_{DZ/sib}$	A	E
Resting heart rate	0.70 (0.60-0.78)	0.49 (0.36-0.59)	0.68 (0.58-0.76)	0.32 (0.24-0.42)
HRR60	0.61 (0.49-0.70)	0.38 (0.23-0.51)	0.60 (0.48-0.67)	0.40 (0.31-052)
HRR180	0.67 (0.56-0.75)	0.40 (0.27-0.51)	0.65 (0.54-0.73)	0.35 (0.27-0.46)
Resting RSA	0.64 (0.53-0.73)	0.19 (0.04-0.32)	0.58 (0.45-0.69)	0.42 (0.31-0.55)
RSA60	0.35 (0.19-0.49)	0.22 (0.06-0.37)	0.23 (0.11-0.35)	0.77 (0.65-0.89)
RSA300	0.24 (0.08-0.40)	0.15 (-0.04-0.33)	0.03 (0.00-0.11)	0.97 (0.89-1.00)
EB	0.80 (0.73-0.85)	0.48 (0.35-0.59)	0.80 (0.73-0.85)	0.20 (0.15-0.27)

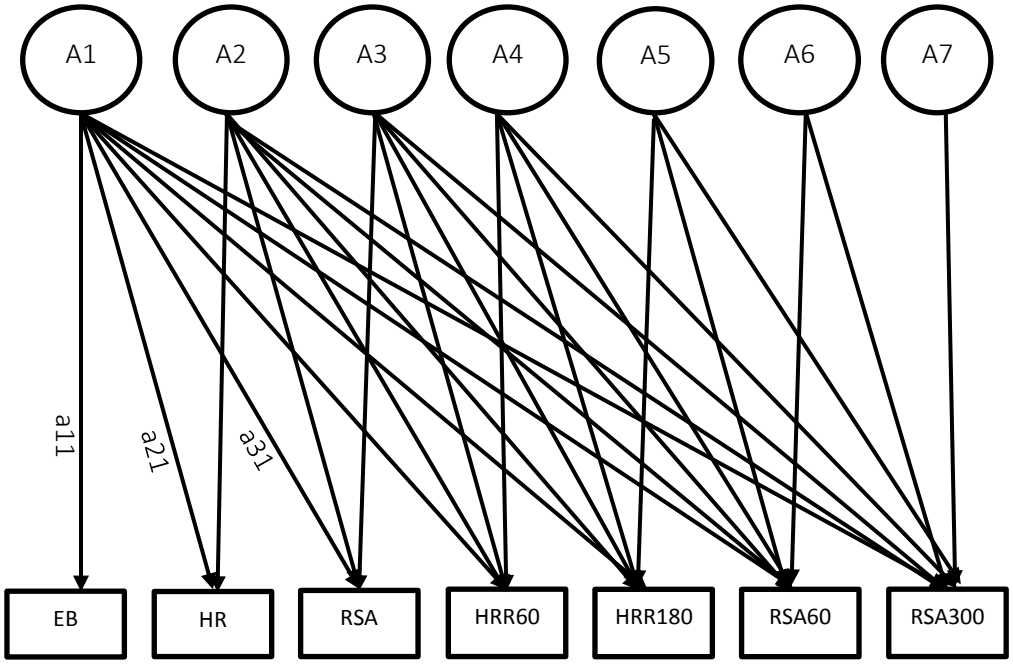

Figure 1. Full Seven variate Cholesky model (E not depicted in this figure). HR; resting heart rate. The path from factor A1 to EB is named a11, the path from A1 to HR a21; other path names follow the same reasoning

Table 4. Phenotypic correlations (95%CI) and in significant correlations % explained by overlapping genetic factors (*) estimated in multivariate model

	EB	Resting heart rate	Resting RSA	HRR60	HRR180	RSA60	RSA300
EB	1.00						
Resting heart rate	-0.27 (-.36, -.17) *91%	1.00					
Resting RSA	0.10 (.00, .20)	-0.39 (-.47, -.30) *72%	1.00				
HRR60	0.10 (-.00, .20)	-0.26 (-.36, -.16) *67%	0.13 (.04, .23) *99%	1.00			
HRR180	0.19 (.09, .29) *88%	-0.29 (-.39, -.20) *76%	0.14 (.04, .23) *78%	0.58 (.51, .65) *68%	1.00		
RSA60	0.10 (.00, .20)	-0.17 (-.26, -.07) *0%	0.11 (.01, .21) *0%	0.37(.28, .45) *56%	0.24 (.14, .33) *51%	1.00	
RSA300	0.06 (-.04, .16)	-0.18 (-.27, -.08) *0%	0.10 (.00, .19)	0.33 (.23, .41) *45%	0.33 (.24, .42) *27%	0.56 (.48, .62) *9%	1.00

Table 5. Model fitting results

Model	-2LL	*df*	Compared to	X^2	Δ*df*	*p*
1 Cholesky ACE	23466	3257				
2 Cholesky AE	23488	3285	1	22	28	.816
3 Cholesky AE - HR and resting vagal tone	23500	3291	2	12	6	.063
4 Cholesky AE - HRR and vagal rebound	23506	3295	3	6	4	.193
5 Cholesky AE - vagal exercise recovery and EB	23512	3299	4	6	4	.186

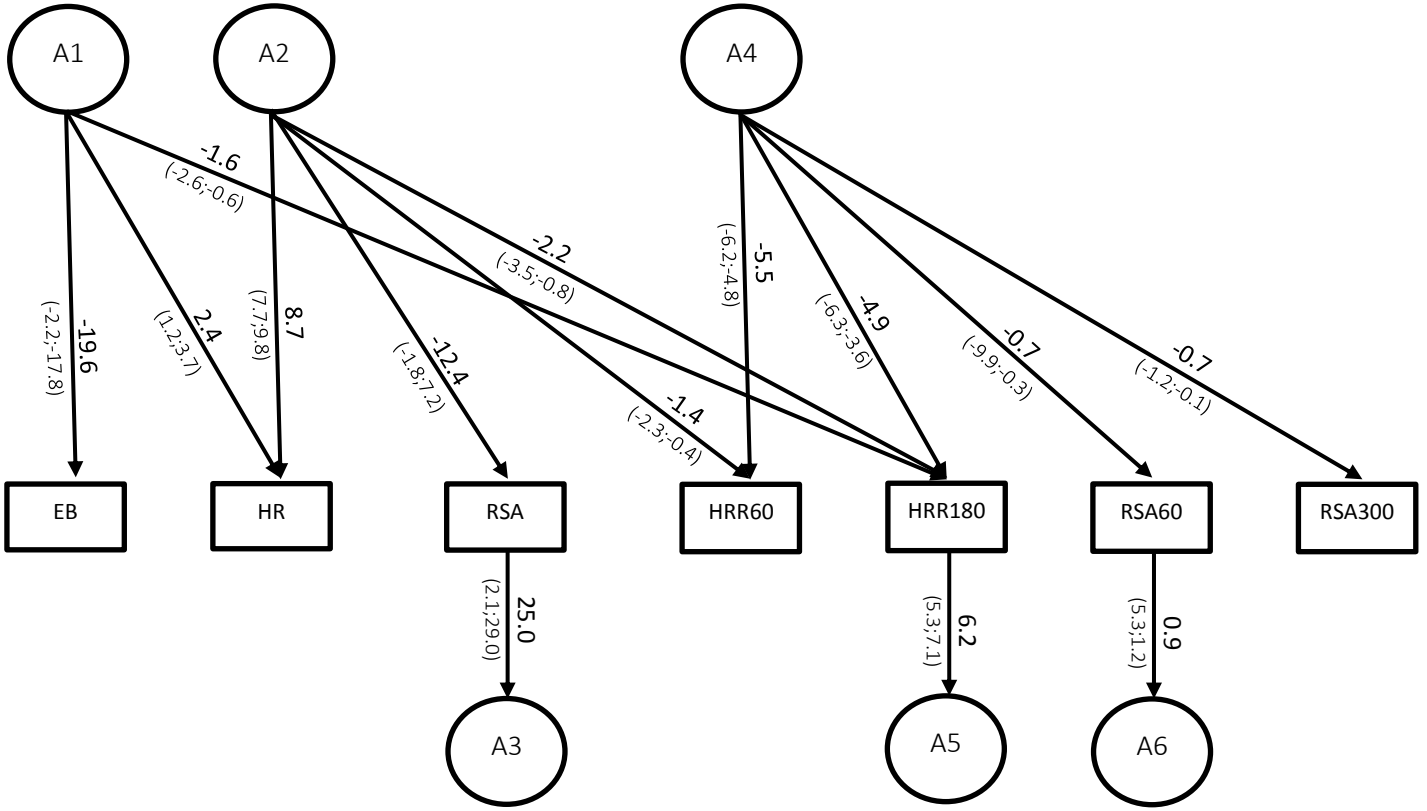

Figure 2. Reduced Cholesky model (E not depicted in this figure). HR; resting heart rate. Factor A1, A2 and A4 represent genetic influences which are partly shared. Tentatively, A1 can be labeled as an exercise/heart rate factor, A2 as a resting vagal tone and A4 as the cardiac vagal recovery factor. The numbers next to the arrows represent the unstandardized path coefficients and their 95% confidence intervals. Heritability can be computed by dividing the summed genetic variance by the total variance

DISCUSSION

Heart rate responses to (maximal) exercise exhibit important prognostic power for cardiovascular and total mortality. Immediate HRR (1 minute after exercise cessation) is an easy to measure and potentially very valuable predictor and a measure of cardiac vagal function in patients as well as in healthy individuals. This study in a group of healthy adolescent twins and their siblings estimated the heritability of HRR and vagal rebound after maximal exercise. In this group, estimated heritability of immediate HRR60 was 60% (95%CI: 48-67). The immediate vagal rebound, measured by RSA60 in this study, showed a heritability estimate of 23% (95%CI: 11-35). The long term vagal rebound showed heritability estimates of 65% (95%CI: 54-73) for HRR180 but only 3% (95%CI: 0-11) for RSA300. Another limitation of the present study is that maturational age may influence both heart rate and HRV and maturation is likely to be more synchronous in MZ than in DZ twins which may have inflated heritability. In contrast, the broad age range of the siblings could have acted to inflate the environmental effects.

Previous studies had already firmly established the heritability of resting heart rate with estimates varying from 26 to 68% [216-220]. Likewise resting RSA is substantially heritable with estimates ranging between 25 to 71% [167;221]. We replicate these findings in our sample (heart rate 68%, RSA 58%) with the heritability of resting heart rate very similar to a recent meta-analysis done by Wang et al. [220] and the heritability of resting RSA very similar to that found in a large non-overlapping study in adult Dutch twins (50%) [16]. To our knowledge, only one previous study has been done on the heritability of HRR and none on other vagal rebound measures. Ingelsson et al. [222] found a heritability of 34% for slow HRR (three minutes after cessation of exercise), which is much lower compared to our results (65%). This is probably due to the different methods used; participants did not exercise until exhaustion as in our study but until they reached 85% of their estimated maximal heart rate. Also, recovery was in supine position whereas our participants had an active recovery.

Previously, Kupper et al. [207] showed that the phenotypic correlations between heart rate and RSA measured in an ambulatory setting (ranging between .35 and .45, measured at different times of day) was for up to 52% determined by common genetic factors. We here replicate this finding for resting levels of heart rate and RSA in a more standardized setting. In our study the phenotypic correlation between resting heart rate and resting RSA was -.39, and 72% of this correlation could be explained by a common genetic factor influencing both resting heart rate and resting RSA. We now furthermore show that the general cardiac vagal factor influencing resting heart rate and RSA also influences HRR and vagal rebound to exercise. This could not simply have been assumed a

priori. Dewland et al. have suggested that different physiologic determinants probably underlie resting heart rate and HRR [223] and this was further reinforced by the findings of Duarte and colleagues [224]. Participants to their training study were divided based on their resting vagal control being high or low. Post-exercise vagal rebound showed no change in a control group, but training 3 days per week for 40 minutes at 75%-85% of their heart rate reserve led to a significant increase in maximal oxygen consumption and post-exercise vagal rebound. Resting vagal control, however, only increased for the group with low resting vagal control at baseline but not in the group with high resting vagal control at baseline. Phenotypic correlations between the heart rate and RSA variables in our study were in keeping with the existence of two different factors: a 'general cardiac vagal factor' (including resting heart rate, resting RSA and the vagal rebound effects after exercise; dark grey shaded area in *table 4*) and a more specific 'cardiac vagal exercise recovery' factor (including immediate HRR and vagal rebound and long term HRR and vagal rebound; light grey shaded area in *table 4*).

Voluntary exercise behavior is a highly heritable trait, 80% in our study corresponds well to an earlier study in an unrelated adolescent cohort [209]. Heritability of EB increases from childhood to adolescence [225] meaning that the likelihood of success of family based interventions is largest in children and less in adolescents. For adolescents, focus should shift to individually based interventions.

Regular exercise behavior and HRR have been reported to be significantly associated in patients [226;227] and in healthy persons [228]. In our study, the correlations between regular exercise and HRR60, HRR180, RSA60 and RSA300 were low (.06 - .19) and only HRR180 reached significance (r=.19). Albeit small, the correlation between EB and HRR180 was almost entirely explained by genetic factors (88%). Such a finding can be ascribed to (a combination of) three possible explanations. First, regular exercise behavior may causally influence long term cardiac vagal recovery, in which case the genes for exercise behavior are part of the heritability of cardiac vagal recovery. In this scenario, changing exercise behavior would positively influence this phenotype. Second, the reverse may also be true in that long-term cardiac vagal recovery may causally influence regular exercise behavior. Thirdly, there may be genetic pleiotropic effects that independently influence exercise behavior and vagal recovery. The latter two explanations are less likely, as in that case intervention on exercise behavior would not be expected to change vagal recovery. EB also correlated significantly with resting heart rate (r=-.27, 95% CI:-.36, -.17), of which 91% could be explained by genetic factors. The well-known phenomenon of bradycardia in athletes is mostly due to lowered intrinsic heart rate, although vagal control also plays a minor role [229;230]. This is in accordance with our current results, as EB shows a

significant correlation with resting heart rate and HRR180 but not with the other variables. A possible explanation for the bradycardia is increased stretch of the right atrium and thereby the sino-atrial and the atrio-ventricular node induced by the chronically increased stroke volume.

In conclusion, individual differences in HRR can to a large extent be explained by genetic differences. Because our results reveal partly different genetic determinants for general and post-exercise cardiac vagal control, we suggest that for heart rate and HRV, both resting and post-exercise values should be considered when evaluating differences in autonomic nervous system control as predictors of cardiac and all-cause mortality.

Chapter 6

Long term follow up after ventricular septal defect repair in children: cardiac autonomic control, cardiac function and exercise tolerance

Ineke Nederend
Eco J.C. de Geus
Nico A. Blom
Arend D.J. ten Harkel

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ABSTRACT

Objectives Survival after surgical repair of a ventricular septal defect (VSD) is good but as in almost all congenital heart disease, in adulthood late residua are frequent. The exact mechanisms, timing and who is at risk are not fully understood. Altered cardiac autonomic nervous system (ANS) activity might play a role in these long-term sequelae. The aim of this study was to extensively evaluate children late after VSD repair including cardiac ANS activity, cardiac function and exercise capacity.

Methods 33 patients after surgical VSD repair and 66 healthy age-matched controls underwent 24h monitoring of ANS control and cardiac output using impedance cardiography, detailed echocardiography and cardiopulmonary exercise testing.

Results Ambulatory cardiac ANS control was not different between patients and controls. Right ventricular function, exercise capacity and ambulatory cardiac output were decreased in patients compared to controls. No relationships were found between cardiac ANS activity and cardiac function.

Conclusions Late (average time after repair 9.9 years) after successful surgical correction of a VSD, right ventricular function and exercise capacity are impaired in VSD patients while cardiac ANS activity is not different between groups. Post-surgical outcome in these patients may be less benign than currently assumed, therefore follow-up should be continued into adulthood in order to detect adverse outcomes in a timely fashion.

INTRODUCTION

A ventricular septal defect (VSD) is classified as a simple defect and prognosis is generally believed to be excellent after surgical repair [231]. In childhood, most children indeed do well, however it is known that cardiac symptoms, reduced quality of life and cardiac events often resurface in adulthood [21;232] and survival is less than normal [233]. Even in this simple and 'easily cured' defect, evidence is starting to emerge on long-term sequelae in patients after VSD repair [234;235]. The time course of this late deterioration in VSD patients, which patients are at risk, and the prognostic factors are not known. Altered cardiac autonomic nervous system (ANS) activity has been shown to be associated with cardiovascular disease [26;56] and it is plausible that it also plays a role in late outcome in congenital heart disease. Longstanding preoperative and/or perioperative myocardial damage causing chronic alterations in ANS control may play a role in the long-term sequelae. The altered function of the ANS in cardiovascular disease is mainly characterized by decreased parasympathetic and increased sympathetic activity, potentially causing increased cardiac load, fibrosis and decreased electrical stability[61;62;183]. To date, no case-control studies have been performed on cardiac ANS activity in pediatric patients with a repaired VSD.

The aim of this study was to extensively evaluate cardiovascular status in children, late after surgical VSD repair. In addition to the standard clinical evaluation of cardiac function and exercise capacity, 24-hour ambulatory cardiac ANS activity and exercise heart rate dynamics were investigated. Also, the relationship between cardiac ANS control and cardiac function was explored. To this end, ambulatory impedance cardiography (ICG), detailed cardiac echocardiography and cardiopulmonary exercise testing were performed in patients after VSD repair and in a group of healthy controls.

METHODS

Participants

Patients with a surgical closure of an isolated VSD from the outpatient clinic aged 8-18 years old were asked to participate. All surgical procedures were performed through a median sternotomy on cardiopulmonary bypass. Children with chromosomal disorders were excluded. At the time of the study, none of the patients had a residual VSD. Additionally, two groups of healthy control subjects were recruited. The first group (healthy controls (1)) were age and sex matched to the patients and served as a control group for the cardiac function and autonomic nervous system activity. These volunteers were recruited through advertisement at schools in the vicinity of the hospital. The second

control group (healthy controls (2)) came from a previously described cohort [11] and served as a control group for exercise related heart rate dynamics. Chronic disease or medication use were exclusion criteria. When the echocardiogram showed a structurally and functionally normal heart, the volunteer was included in the study. All participants and both (one in the case of single-parent families) of their parents/guardians provided written informed consent. All study procedures were reviewed and approved by the Medical Ethics Review Committee of the LUMC medical centre (P13.198 and P14.095).

Ambulatory cardiac ANS measurement

ICG measured in combination with electrocardiography (ECG) is a non-invasive tool to measure cardiac ANS control and cardiac output (CO) and can be used in an ambulatory setting [164;221], which may have the highest clinical relevance. 24 hour ECG and ICG registration was done using the 5fs version of the VU Ambulatory Monitoring System (VU-AMS; VU University, Amsterdam, The Netherlands, www.vu-ams.nl)[221]. One lead ECG was derived from 3 pregelled Ag/AgCl (Kendal H124SG) spot electrodes on the chest. ECG was sampled at 1kHz. Thoracic impedance (Z) was sampled at 250Hz and was conducted by introducing a small alternating current (50kHz, 350 μA) through the thorax, also by the use of spot electrodes. The measuring electrodes were placed just above and below the sternum. Current electrodes were placed 3 cm above and below the measuring electrodes on the back of the thorax. Ectopic beats were removed from the data before analysis.

Sympathetic nervous system control was measured by the pre ejection period (PEP) as it is a measure of ventricular contractility [160]. PEP is defined as the time interval between the ventricular electrical depolarization (i.e. Q-wave onset in the ECG signal) and the start of left ventricular outflow (i.e. the B-point in the ICG signal). A shorter PEP reflects higher sympathetic control. Parasympathetic nervous system control was measured by respiratory sinus arrhythmia (RSA) [45]. RSA was calculated using the peak valley method [15] by combining the respiration signal, extracted from the thorax impedance signal (dZ) and the inter-beat-interval time series. This method scores RSA 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 breath. A higher RSA reflects higher parasympathetic control. Stroke volume was estimated using an adjusted Kubicek formula[10]. CO was calculated as heart rate * stroke volume.

By the use of the activity diary filled in by the participant in combination with the inbuilt accelerometer data from the VU-AMS device, the 24 hour recording was divided into

fixed periods, coded for activity. Minimum duration of a period was 5 minutes, maximum was 60 minutes. Ensemble averaged ICG and ECG over these periods were analysed. Ultimately, for each subject, the mean heart rate (HR), RSA, PEP and CO was calculated for sleeping (mean of all sleeping labels), sitting (mean of watching TV, reading, computer), active sitting (class, crafts, homework), light physical activity (walking, chores) and heavy physical activity (cycling, gymnastics, playing).

Echocardiogram

Transthoracic echocardiograms were conducted by a pediatric cardiologist or an experienced technician (Vivid 9, General Electric healthcare, Norway) and evaluated by one researcher, supervised by a pediatric cardiologist. Images were stored and analyzed offline using EchoPac software (General Electric Healthcare, version 113). Left ventricular longitudinal global peak strain (%) was obtained from the apical 4-chamber view using speckle tracking strain analysis as previously described[236]. Biventricular performance was characterized using pulsed wave Tissue Doppler Imaging (TDI), also obtained from apical 4-chamber view. Myocardial velocity curves were obtained for the left and right ventricular wall and the intraventricular septum. Peak systolic velocity (S') and peak early (E') and late (A') diastolic velocities (in cm/s) were assessed in three consecutive heart beats; the average was used for analysis.

Cardiopulmonary exercise test

In all patients, cardiopulmonary exercise testing was performed at the LUMC Leiden. The test was performed in upright position on a bike ergometer (GE healthcare type R3x 10416054 SA version V6.61). The protocol consisted of an increasing workload per minute; starting wattage and increment per minute was defined by the physician. Patients were encouraged by the technician, physician and a researcher to exercise until exhaustion. Peak maximal oxygen consumption (VO_{2peak}) was defined as the highest of two consecutive values of 10 second averages of VO_2. For the healthy control group that underwent echocardiography and 24h ICG monitoring but no exercise testing, VO_{2peak} was estimated using a prediction equation [237] in order to include VO_{2peak} as a covariate in the analysis of ANS. Exercise testing in the healthy controls (2) group was performed at Erasmus University Medical Centre as described elsewhere [11]. Peak heart rate was defined as the highest heart rate achieved during exercise. Heart rate reserve was calculated as the difference between the peak heart rate and the resting heart rate. Heart rate recovery was calculated as peak heart rate minus the heart rate 1 minute after exercise cessation.

Weekly physical activity

Participants were queried on their weekly physical activity by the use of a short lifestyle interview described in detail elsewhere[209]. In short, for each exercise activity (e.g. swimming, fitness, tennis, jogging, soccer) they were asked for how many years they have been doing that particular sport, how many months per year, how many times per week, and how many minutes each time. Also, activity related to transportation (cycling, walking) and compulsory physical education classes were queried. Each exercise activity was converted into an MET (metabolic equivalent task) score[14]. One MET represents the amount of energy needed for sitting quietly. This MET score was multiplied by the duration of activities and summed to get a weekly MET score.

Statistical analyses

IBM SPSS statistics software (version 23.0, Armonk, NY) was used for statistical analysis. We fitted a linear mixed model to study the differences in HR, PEP, RSA and CO between the healthy control and patient group. Because RSA was skewed, its natural logtransformation was used for further analysis (lnRSA). In the mixed model, we treated activity as a within-subject factor with 5 levels (sleep, inactive sitting, active sitting, moderate physical activity, heavy physical activity) and we treated group as a between-subject factor, with 2 levels (Healthy control and VSD patient). In the model, a random intercept was allowed over persons. Physical activity and exercise capacity were entered in the model as covariates as differences in ANS activity might partly be explained by physical activity and exercise capacity[224]. Additionally, breathing frequency was included as a covariate in the analysis of RSA. Differences in exercise capacity between groups was evaluated by means of an independent T-test. For comparisons of 10 echocardiographic parameters and 3 exercise heart rate dynamics parameters between groups, two MANOVAs were carried out. In case of significance of the omnibus group effect ($p<0.05$), post hoc testing on each separate variable used a Bonferroni correction of the overall p-value. Lastly, a correlation matrix was computed in the patient group for the echocardiographic parameters and ambulatory cardiac ANS parameters PEP and RSA during sleep in order to gain insight into the relationship between them.

RESULTS

Descriptives of the participants can be found in t*able 1*. Gender, age, length, weight and weekly physical activity were not different between groups. Follow up time was on average

10 years. All participants showed a normal ECG, except for a partial bundle branch block in 7 (21%) and a complete bundle branch block in 5 patients (15%). *Table 2* summarizes the uncorrected group means of HR, RSA, PEP and CO in each ambulatory activity. Results from the mixed linear model showed a main effect of activity for all four measures. No main effect of group was found for HR ($F(1,96)=0.003$, $p=0.956$), but there was a main effect of activity ($F(4,96)=666.97$, $p=<.001$). HR was higher in the activities of higher intensity. There also was a significant interaction between group and activity ($F(4,96)=3.01$, $p=0.022$). This indicates that the HR during activities of different intensities differed between patients and controls; heavy physical activity seems to provoke a bigger HR response in patients compared to controls. Sympathetic nervous system activity as measured by PEP showed no main effect of group ($F(1,82)=0.058$, $p=0.810$) and no group*activity interaction effect ($F(1,84)=1.294$, $p=0.279$). Parasympathetic activity as measured by RSA showed no main effect of group ($F(1,96)=0.326$, $p=0.570$) and no group*activity interaction ($F(4,101)=0.766$, $p=0.550$). CO showed a main effect of group ($F(1,98)=4.565$, $p=0.035$) but no group*activity interaction effect ($F(4,118)=0.248$, $p=0.911$). The VSD patients had a lower CO than controls throughout all conditions of the 24 hour recording.

Figure 1 displays an example of a 24 hour profile of PEP, RSA, CO and HR of a VSD patient and an age and sex matched control subject. In *figure 2* estimated marginal means for HR, CO, PEP and RSA averaged over five activity categories in the two groups are visualized.

MANOVA of the 10 parameters of cardiac function revealed a significant effect of group (Pillai's Trace, $V=0.420$, $F(10,73)=5.29$, $p<0.0001$). Test of between subject effects per parameter showed that the right ventricular function measured by tissue Doppler imaging was significantly lower in patients compared to controls (TDI RV S': $p<0.0001$, E': $p=0.0001$, A': $p<0.0001$) (*table 3*).

VO_{2peak} was significantly lower in patients compared to control groups ($p<0.001$). MANOVA of the 3 parameters of exercise heart rate dynamics revealed a significant effect of group (Pillai's Trace, $V=0.136$, $F(3,162)=8.456$, $p<0.0001$). Test of between subject effects per parameter showed that heart rate reserve was lower in patients compared to controls ($p<0.0001$, *table 4*).

In the patient group, no significant relationship was found between basal cardiac function and ambulatory cardiac ANS activity. The correlation matrix is shown in *table 5*. Correlations for the healthy control group showed similar results (data not shown).

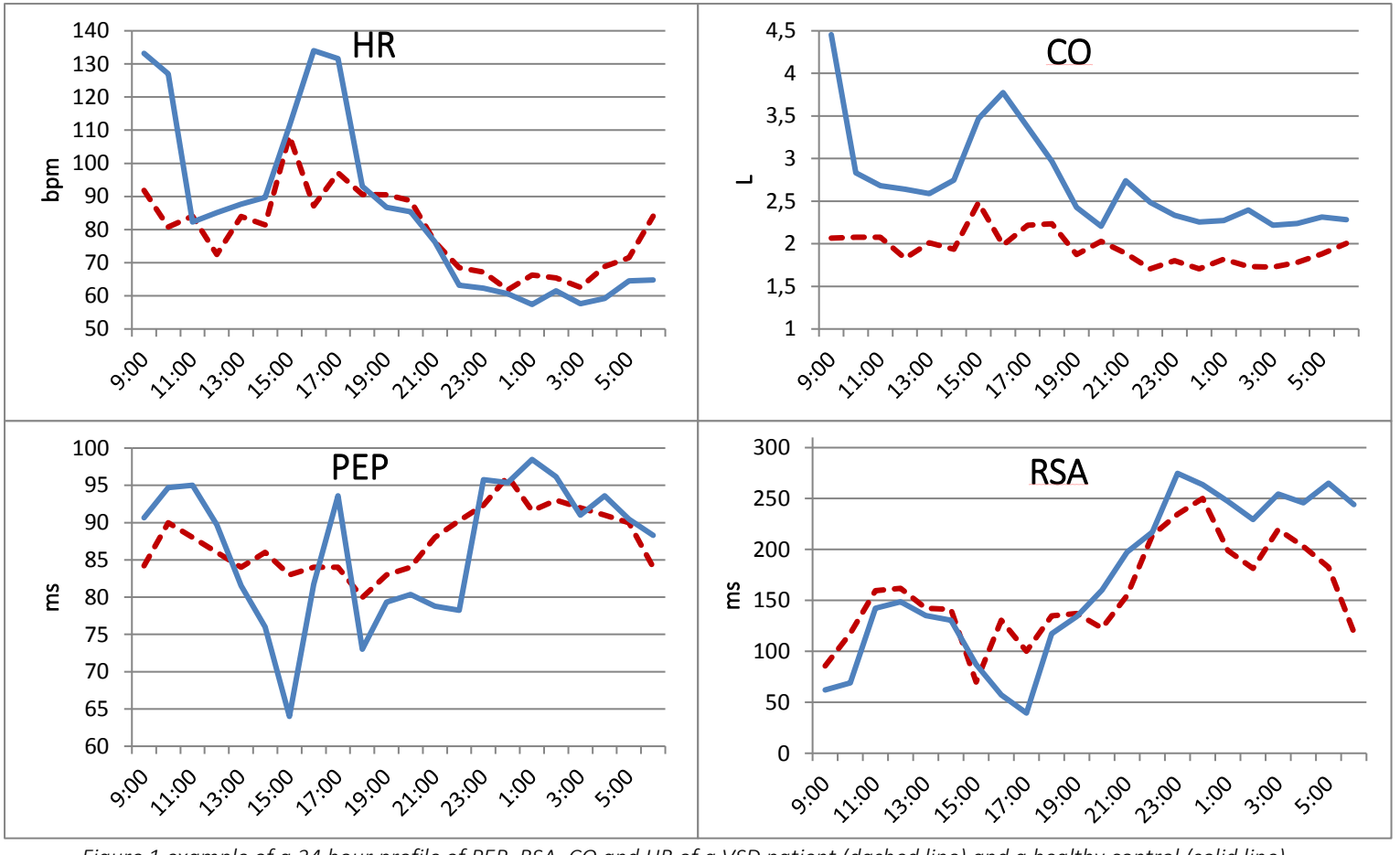

Figure 1 example of a 24 hour profile of PEP, RSA, CO and HR of a VSD patient (dashed line) and a healthy control (solid line)

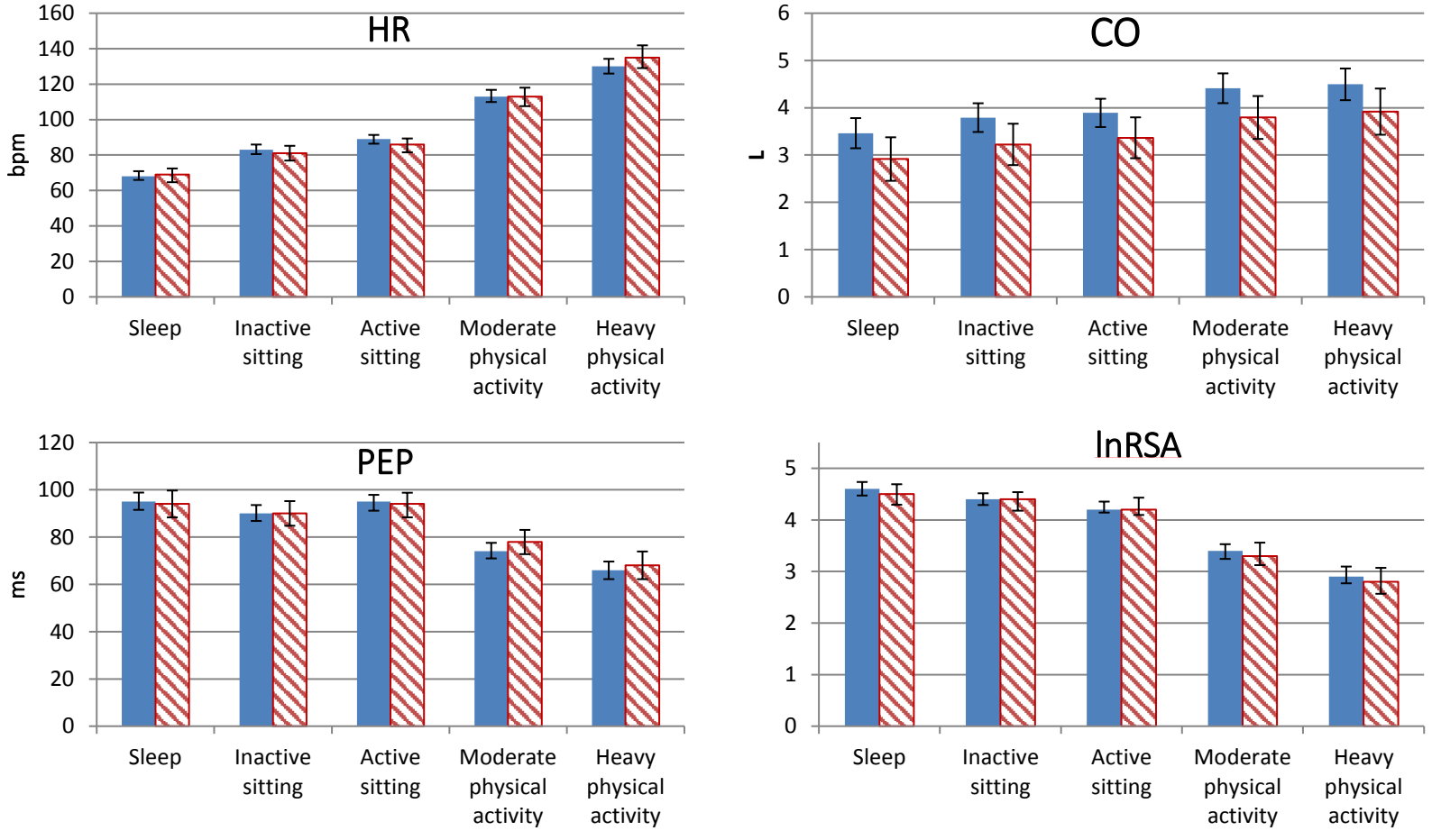

Figure 2 estimated marginal means for PEP, lnRSA, CO and HR of the VSD group (dashed bars) and the healthy control group (solid bars)

Table 1 subject characteristics

	VSD patients	Healthy controls (1)	Healthy controls (2)
N	33	66	140
Male (%)	52	52	49
Age (y)	11.8 (2.8)	11.9 (3.0)	11.8 (2.3)
Age at repair (y)	1.7 (2.3)	-	-
Time after repair (y)	9.9 (3.0)	-	-
Length (cm)	150.9 (15.1)	153.5 (16.0)	153.7 (13.0)
Weight (kg)	43.0 (13.2)	44.2 (14.2)	43.8 (12.1)
24 h HR (bpm)	87 (10)	88 (10)	-
24 h RSA (ms)	83 (367)	93 (34)	-
PVCs			
0 in 24 h (N)	15	42	-
1-50 in 24 h (N)	13	21	-
>51 in 24 h (N)	5	3	-

*Mean (SD). VSD= Ventricular Septal Defect. HR= heart rate. RSA= respiratory sinus arrhythmia. Y=years. PVCs=premature ventricular contractions. *significant difference between patients and healthy control groups.*

Table 2 Ambulatory measurement of HR, PEP, RSA and CO

	Activity	VSD patients	Heathy controls (1)
Heart rate (bpm)	Sleep	70 (8)	68 (8)
	Quiet sitting	82 (10)	83 (12)
	Active sitting	87 (12)	89 (10)
	Moderate PA	113 (14)	113 (14)
	Heavy PA	136 (17)	130 (15)
RSA (ms)	Sleep	102 (51)	123 (54)
	Quiet sitting	80 (38)	92 (45)
	Active sitting	79 (39)	80 (34)
	Moderate PA	33 (17)	35 (14)
	Heavy PA	21 (14)	24 (11)
PEP (ms)	Sleep	96 (16)	96 (16)
	Quiet sitting	91 (18)	89 (13)
	Active sitting	95 (18)	94 (14)
	Moderate PA	79 (14)	74 (12)
	Heavy PA	68 (12)	65 (8)
CO (L/min)	Sleep	2.92 (1.01)	3.49 (1.23)
	Quiet sitting*	3.22 (0.95)	3.76 (1.19)
	Active sitting*	3.37 (1.11)	3.92 (1.30)
	Moderate PA*	3.79 (1.18)	4.52 (1.54)
	Heavy PA	3.94 (1.36)	4.46 (1.63)

*Uncorrected mean (SD). PA=physical activity. HR=heart rate. RSA=respiratory sinus arrhythmia. PEP=pre-ejection period. CO=cardiac output. *significant difference between groups after Bonferroni correction for multiple comparisons.*

Table 3 echocardiographic parameters

	VSD patients	Heathy controls (1)
Longitudinal strain (%)	16.1 (3.5)	16.8 (2.7)
LV TDI S' (cm/s)	11.1 (2.8)	10.6 (2.7)
LV TDI E' (cm/s)	19.0 (4.5)	19.3 (3.9)
LV TDI A' (cm/s)	6.2 (1.7)	6.1 (2.0)
Septum TDI S' (cm/s)	7.3 (1.2)	7.2 (0.9)
Septum TDI E' (cm/s)	13.7 (2.5)	14.2 (2.1)
Septum TDI A'(cm/s)	5.9 (1.3)	5.6 (1.1)
RV TDI S' (cm/s)*	10.2 (2.3)	12.3 (1.9)
RV TDI E' (cm/s)*	12.3 (3.4)	15.0 (3.0)
RV TDI A' (cm/s)*	6.2 (2.1)	8.5 (2.1)

*Mean (SD). S=systole. E=early filling. A=atrial filling. TDI=Tissue Doppler Imaging. LV=left ventricle. RV=right ventricle. *significant difference between groups after Bonferroni correction for multiple comparisons*

Table 4 Exercise capacity, heart rate dynamics and physical activity level

	VSD patients	Heathy controls (2)
VO_{2peak} (ml/kg/min)*	39.0 (5.4)	44.3 (7.2)
HR_{peak} (bpm)	187 (12)	189 (9)
$HR_{reserve}$ (bpm)*	91 (14)	103 (14)
HRR_{60} (bpm)	35 (11)	35 (11)
Physical activity (METs/week)	39.5±16.7	46.2±22.4

*Mean (SD). VO_{2peak} =Peak oxygen consumption. HR_{peak}=peak heart rate. $HR_{reserve}$ = heart rate reserve. HRR_{60}=heart rate recovery 1 minute into recovery. *significant difference between groups after Bonferroni correction for multiple comparisons*

Table 5 correlation between cardiac ANS and cardiac function

	PEP (sleep)	RSA (sleep)
Longitudinal strain	.10 (95%CI: -.26;.44)	-.11 (95%CI:-.48;.27)
LV TDI S'	-.24 (95%CI:-.60;.12)	-.09 (95%CI:-.46;.27)
LV TDI E'	.02 (95%CI:-.35;.39)	-.11 (95%CI:-.47;.26)
LV TDI A'	-.21 (95%CI:-.57;.15)	-.10 (95%CI:-.47;.26)
Septum TDI S'	.10 (95%CI:-.28;.47)	-.29 (95%CI:-.64;.07)
Septum TDI E'	-.10 (95%CI:-.47;.27)	-.07 (95%CI:-.43;.30)
Septum TDI A'	-.27 (95%CI:-.62;.09)	-.23 (95%CI:-.59;.13)
RV TDI S'	-.02 (95%CI:-.39;.35)	-.06 (95%CI:-.43;.30)
RV TDI E'	.11 (95%CI:-.26;.47)	-.23 (95%CI:-.59;.12)
RV TDI A'	.12 (95%CI:-.25;.49)	-.18 (95%CI:-.54;.18)
CO (sleep)	.12 (95%CI:-.25;.49)	-.27 (95%CI:-.63;.10)

S=systole. E=early filling. A=atrial filling. TDI=Tissue Doppler Imaging. LV=left ventricle. RV=right ventricle. CO=cardiac output

DISCUSSION

The purpose of the current study was to evaluate cardiac ANS, cardiac function and exercise capacity in children after VSD repair. The main findings were that 10 years after surgical VSD repair, exercise tolerance, ambulatory cardiac output and RV function were significantly decreased in patients compared to age and sex matched controls. Cardiac ANS control does not seem to be altered, and basal cardiac ANS control is not related to cardiac function.

The current study is the first case-control study on ambulatory ANS activity in pediatric patients with a repaired VSD. In this study, no significant differences were found in terms of cardiac ANS control between patients and controls using ambulatory ECG and ICG measurement. However, a trend was seen towards a lower basal parasympathetic tone in patients compared to controls (i.e. lower RSA during sleep). It is well known that disturbed cardiac ANS control is related to adverse cardiovascular outcomes [26;56]. Based on the findings of this study, we conclude that an altered ANS function does not yet play a role during childhood in patients after VSD repair. In this study, on average 10 years after repair,

we do find reduced RV function and exercise capacity which may in time cause ANS alterations.

Altered cardiac ANS control may also be unmasked by a change in heart rate dynamics. Exercise related heart rate dynamics have been used to predict future cardiac events and mortality [187;192]. Heart rate recovery, peak heart rate and heart rate reserve are considered most useful. Several authors report a lower peak heart rate in patients after VSD repair [238]. In contrast, Norozi et al.[239] and the current study did not find a difference. Heiberg et al.[240] found a lower peak heart rate in patients with a right bundle branch block but not in those with normal ventricular conduction. In our sample, no difference was found between patients with and without conduction disorder (data not shown). Binkhorst et al.[238] report a lower peak heart rate in repaired but not in unrepaired VSD. Likewise, Maagaard et al.[241] found similar peak heart rates in their group of unrepaired VSD patients compared to a healthy control group, suggesting that the chronotropic limitation might be a result of the surgery. Heart rate recovery 60 seconds after exercise cessation was not different between the healthy controls and the patients. Heart rate reserve was higher in healthy controls compared to patients. However, resting heart rate from the ambulatory measurements and peak heart rate at peak exercise were not different. Therefore, the difference found may be caused by higher resting heart rate in the patients measured before the start of exercise. Exercise tests were performed on different locations where the pre-exercise protocol differed slightly; healthy controls were instructed to sit on the bike for 3 minutes before measuring the resting heart rate whereas this was not done in patients. To our knowledge, no other studies investigated heart rate recovery and heart rate reserve in pediatric patients after surgical VSD repair. In summary, the similar heart rate dynamics between patients and controls indorse the unchanged ambulant cardiac ANS activity in these patients. However, altered cardiac ANS function may still play a role in disease progression later in life in these patients. Therefore, we do advise to study cardiac ANS in adults with congenital heart disease.

This study found a significantly diminished exercise capacity in patients compared to controls *(table 4)*. Reybrock et al.[242] demonstrate a reduced exercise capacity shortly after repair. The most recent study on this subject by Heiberg and colleagues [243] describes a lower exercise capacity 20 years after VSD repair. According to several studies including ours, patients are equally physically active as their healthy peers [238;241;242]. Thus, physical activity cannot explain the difference in exercise capacity found in the current study. The diminished CO however may in part explain the difference in exercise capacity.

Immediately- and 1 year after operation, decreased RV function was found by Klitsie et al[244]. The current study shows that 10 years later, RV function is still reduced in these patients. Heiberg et al. also report reduced RV function 20 years after repair [245]. Potentially, exposure of the heart (especially the ventrally situated RV) during sternotomy has irreversible effects on the myocardium. Prognosis after surgical repair of a VSD is generally believed to be excellent but here we show that using advanced techniques, subtle differences in cardiac performance are present 10 years after repair. No relationship was found between cardiac function measured by echocardiography and cardiac ANS activity. Cardiac ANS activity does not seem to play a role in the decrease of RV function in childhood after VSD. To our knowledge, the current study is the first to describe 24 hour CO deviations in patients after VSD repair. A stepwise increase was seen with increasing physical activity in both patients and controls, however in all ambulatory conditions the CO of patients was consistently lower. It remains unclear whether these differences are related to long-term sequelae in these patients but CO is a core measurement in the clinical evaluation of cardiac patients[110].

A limitation of this study is the use of PEP as a measure of cardiac SNS activity; it is the best possible measure in an ambulatory setting but it is certainly not a gold standard. PEP is sensitive to changes in cardiac preload through the Frank-Starling mechanism that influences contractility independent of SNS activity. An increased cardiac afterload may also prolong PEP independent of SNS activity. As changes in pre- and afterload due to posture, temperature of the environment and physical activity are inherent to ambulatory monitoring in a naturalistic setting, PEP will therefore not perfectly reflect SNS activity. Another limitation is that the ambulatory ANS data was pooled in five conditions, which may introduce heterogeneity within these conditions. However, it is necessary to pool the ambulatory conditions in order to compare data between subjects. Lastly, the group of VSD patients in the current study was heterogeneous in terms of age at repair; 11 of 34 patients were operated on after the first year of life. This heterogeneity might in part mask differences in cardiac ANS function.

In conclusion, no significant differences were found in ambulatory cardiac ANS control between patients after surgical VSD repair and healthy controls. Additionally, exercise heart rate dynamics yielded the same conclusion towards ANS control. Ambulatory CO, exercise capacity and right ventricular function are decreased in patients after VSD repair compared to healthy controls but the impaired cardiac function is not related to altered basal cardiac ANS control. The unchanged ANS control is reassuring since multiple studies identified altered cardiac ANS function as a key factor in the risk of cardiac morbidity and mortality in cardiac patients. However, this study shows that even in this group of patients with a

simple congenital heart disease that is generally seen as 'cured' after surgical repair, the long-term effects might be not as benign as previously suggested. As the current study shows subclinical reduced RV function and reduced exercise capacity 10 years after surgical repair, this is expected to deteriorate further or remain constant. Therefore, patients should be advised to stay physically active and systematic follow up in a congenital heart disease centre should be continued into adulthood in order to detect adverse outcomes in a timely fashion.

Chapter 7

Cardiac autonomic nervous system activity, exercise capacity and cardiac function in children after coarctation repair

Ineke Nederend
Eco J.C. de Geus
Lucia J. Kroft
Jos J.M. Westenberg
Nico A. Blom
Arend D.J. ten Harkel

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ABSTRACT

Objective Coarctation of the aorta (CoA) is one of the most common congenital heart defects. Due to improved surgical techniques, most patients live into adulthood. However, late complications including hypertension, recoarctation and arrhythmias are common. The autonomic nervous system (ANS) might play a role in the pathology. The aim of this study was to extensively evaluate children after CoA repair including their cardiac ANS activity, cardiac function and exercise capacity.

Methods 31 children after CoA repair and 62 healthy controls aged between 8 and 18 years old participated in the study. By the use of ambulatory impedance cardiography, cardiac ANS activity and cardiac output were measured for 24h. Exercise capacity and exercise heart rate dynamics were measured by means of a cardiopulmonary exercise test and cardiac function was measured by the use of transthoracic echocardiography and cardiac magnetic resonance imaging.

Results No group differences were found in ambulatory cardiac ANS activity and exercise heart rate dynamics. Exercise capacity, ambulatory cardiac output and left ventricular function were significantly decreased in patients compared to controls.

Conclusions Left ventricular function, ambulatory cardiac output and exercise capacity are impaired in patients after CoA repair, despite unchanged cardiac ANS activity in this group. These results underscore the importance of clinical follow up, even in patients without residual stenosis.

INTRODUCTION

Coarctation of the aorta (CoA) is one of the most common congenital heart defects with a reported prevalence of 4/10.000 births approximately [66]. Today, survival is good due to available techniques for repair and improved postoperative care. The reported surgical mortality is 1.3% [246] and 60 year survival is 89% [247]. However, even after successful repair, late complications are common and may include recoarctation, aortic aneurysm, coronary artery disease, cerebrovascular events, heart failure and arterial hypertension. The latter being especially common: according to a review on hypertension after CoA repair, the median prevalence is 32.5% (range 25-68%)[104]. However the timing and pathophysiology of the long term complications are still largely unknown. Previous studies showed a decreased cardiac function as well as diminished exercise capacity after coarctation repair. Shortly after CoA repair, Klitsie et al. [248] found a decreased right- and left ventricular (RV, LV) function in patients. One year after repair, RV function has recovered while LV function is still decreased and it is unclear if LV function fully recovers later in life. In adult CoA patients, a reduced exercise capacity has been reported [249;250], which is related to the risk of hospitalization and death [251]. Altered cardiac autonomic nervous system (ANS) activity may play a role in the pathophysiology of long term complications. Cardiac ANS can be measured non-invasively using impedance cardiography (ICG) in combination with electrocardiography (ECG). This technique can be used in an ambulatory setting, which arguably has the highest clinical relevance.

The aim of this study was to extensively evaluate children after CoA repair, including ambulatory ANS activity, exercise heart rate dynamics, exercise capacity and cardiac function. Also, the relationship between cardiac ANS activity and cardiac function was investigated.

METHODS

Participants

Patients with a repaired isolated CoA from the outpatient clinic aged 8-18 years old were asked to participate. Children with chromosomal disorders were excluded. Additionally, two groups of healthy control subjects were recruited. The first group (healthy controls (1)) served as a control group for the cardiac function and autonomic nervous system activity and were recruited through advertisement at schools. These volunteers were age and sex matched to the patient group. The second control group (healthy controls (2)) came from a previously described cohort[11] and served as a control group for exercise related heart rate dynamics. Chronic disease or medication use were exclusion criteria. All participants and

both (one in the case of single-parent families) of their parents/guardians provided written informed consent. All study procedures were reviewed and approved by the Medical Ethics Review Committee of the LUMC medical centre (P13.198 and P14.095).

Ambulatory cardiac ANS measurement

24 hour ECG and ICG registration was done using the 5fs version of the VU Ambulatory Monitoring System (VU-AMS; VU University, The Netherlands [16]. One lead ECG was derived from 3 pregelled Ag/AgCl (Kendal H124SG) spot electrodes on the chest. Thoracic impedance (Z) was conducted by introducing a small alternating current (50kHz, 350 μA) through the thorax, also by the use of spot electrodes. The measuring electrodes were placed just above and below the sternum and current electrodes were placed on the back. Ectopic beats were removed from the data before analysis.

Sympathetic nervous system (SNS) activity was measured by the pre-ejection period (PEP) as it is a measure of ventricular contractility [160;161]. PEP is defined as the time interval between the ventricular electrical depolarization (i.e. Q-wave onset in the ECG signal) and the start of left ventricular outflow (i.e. the B-point in the ICG signal). A shorter PEP reflects higher sympathetic activity. Parasympathetic nervous system (PNS) activity was measured by respiratory sinus arrhythmia (RSA) [45]. RSA was calculated using the peak valley method [15] by combining the respiration signal, extracted from the thorax impedance signal (dZ) and the inter-beat-interval time series. This method scores RSA 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 breath. A higher RSA reflects higher PNS activity. Stroke volume was estimated using an adjusted Kubicek formula[10]. Cardiac output (CO) was calculated as heart rate * stroke volume.

By the use of the activity diary filled in by the participant in combination with the inbuilt accelerometer data from the VU-AMS device, the 24 hour recording was divided into fixed periods, coded for activity. Ensemble averaged ICG and ECG over these periods were analysed. Ultimately, for each subject, the mean heart rate (HR), RSA, PEP and CO was calculated for sleeping (mean of all sleeping labels), sitting (mean of watching TV, reading, computer), active sitting (class, crafts, homework), light physical activity (walking, chores) and heavy physical activity (cycling, gymnastics, playing).

Cardiac function

Transthoracic echocardiograms (TTE) were conducted by a pediatric cardiologist or an experienced technician (Vivid 9, General Electric healthcare, Norway). Images were stored and analyzed offline using EchoPac software (General Electric Healthcare, version 113). Left ventricular longitudinal global peak strain (%) was obtained from the apical 4-chamber view using speckle tracking strain analysis as previously described[236]. Biventricular performance was characterized using pulsed wave Tissue Doppler Imaging (TDI) also from an apical 4-chamber view. Myocardial velocity curves were obtained at the basal part of the left and right ventricular wall and the intraventricular septum. Peak systolic velocity (S') and peak early (E') diastolic velocities (in cm/s) were assessed in three consecutive heart beats; the average was used for analysis.

Cardiac magnetic resonance imaging (MRI) was done in a 3T scanner (Ingenia, Philips Healthcare). Pulse wave velocity (PWV) and left ventricular (LV) wall mass were assessed in all patients. Analysis were done offline using in-house developed MASS software (Leiden, The Netherlands). PWV was determined from two high-temporal 1-directional velocity encoded time-resolved MRI acquisitions, planned perpendicular to the aorta, one at the ascending aorta at the level of the pulmonary trunk and one at the abdominal aorta 3 cm below the diaphragm. Flow mapping was performed to obtain velocity-time curves. The PWV was determined over both the proximal aorta (ascending aorta plus aortic arch and thoracic descending aorta, see *figure 1*) as well as the distal descending aorta (see *figure 1*). The validated foot-to-foot transit-time method was used to define PWV [17]. LV wall mass was assessed from a cine multi-slice short-axis data set acquired with steady-state free-precession gradient echo. Epi- and endocardial contours were drawn in every slice. Subsequently, the areas were subtracted, and the resulting areas were multiplied with slice thickness, number of slices and the density of myocardium. Contours were drawn end-diastole and end-systole by one researcher and supervised by one radiologist. The average from the end-diastolic mass and end-systolic mass was used for analysis.

Cardiopulmonary exercise test and physical activity

In all patients, cardiopulmonary exercise testing was performed at the LUMC Leiden. The test was performed in upright position on a bike ergometer (GE healthcare type R3x 10416054 SA version V6.61). The protocol consisted of an increasing workload per minute; starting wattage and increment per minute was defined by the physician. Patients were encouraged to exercise until exhaustion. After cessation of the test, the patient was

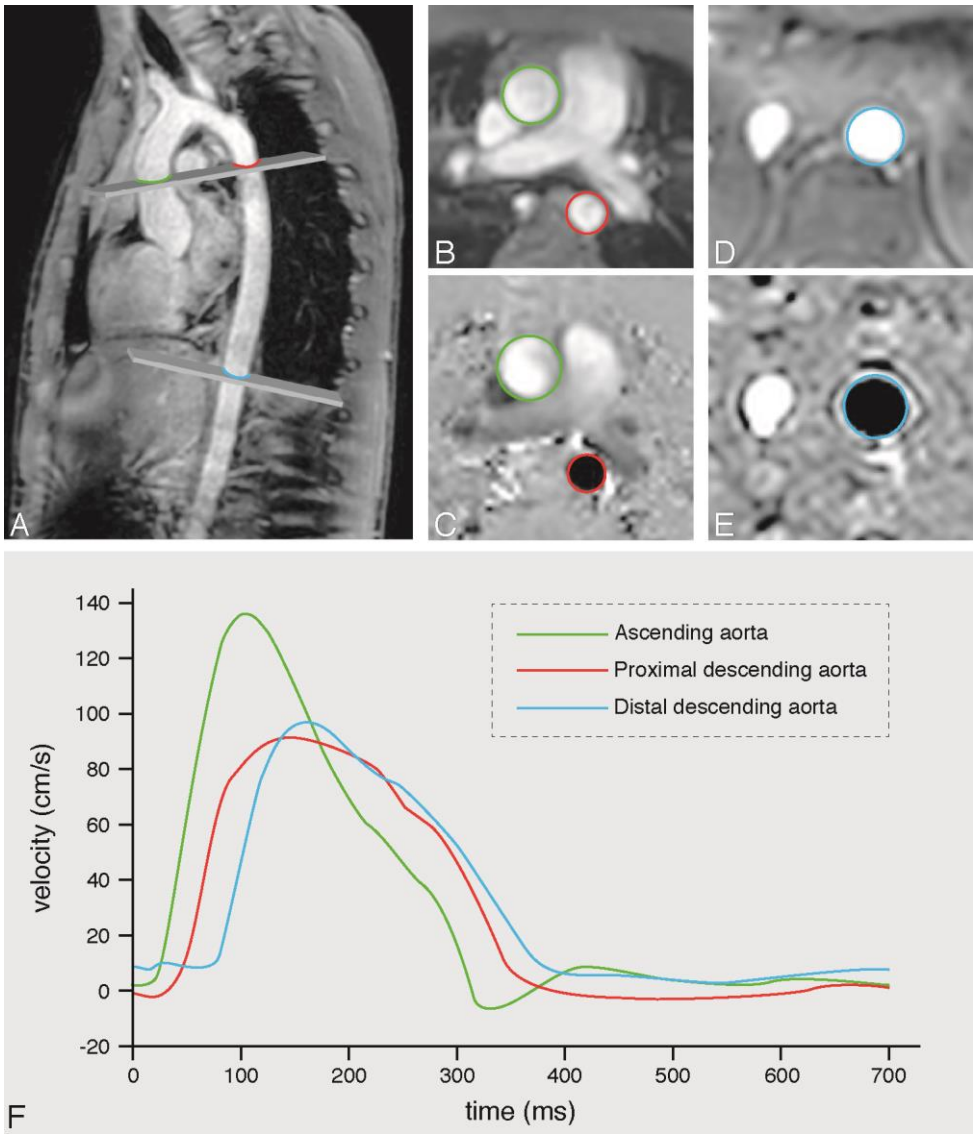

Figure 1 Pulse wave velocity assessment. Sagittal scout of the aorta, indicating the three sites (green, red and blue) for through-plane velocity encoded MRI acquisitions (A). Transverse image of the upper (B) and lower (C) acquisition plane and associated velocity images (D&E), used to create the velocity-time curves (F). PWV is assessed by dividing the segment length (for the proximal aorta from the green to the red site and for the distal aorta from the red to the blue site) by the transit time needed for the pulse wave to propagate between sites, automatically determined by the foot-to-foot method.

instructed to remain on the bike for active cooldown while resistance was decreased to zero. Peak maximal oxygen consumption (VO_{2peak}) was defined as the highest of two consecutive values of 10 second averages of VO_2. For the healthy control group that underwent echocardiography and 24h ICG monitoring but no exercise testing, VO_{2peak} was estimated using a prediction equation [237] in order to include VO_{2peak} as a covariate in the analysis of ANS. Exercise testing in the healthy controls (2) group were performed at Erasmus University Medical Centre as described elsewhere [11]. Heart rate recovery was calculated as peak heart rate minus the heart rate 1 minute after exercise cessation. Participants were queried on their weekly physical activity by the use of a short lifestyle interview described in detail elsewhere[209].

Statistical analyses

IBM SPSS statistics software (version 23.0, Armonk, NY) was used for statistical analysis. We fitted a linear mixed model to study the differences in HR, PEP, RSA and CO between the healthy control and patient group. Because RSA was skewed, its natural log transformation was used for further analysis. In the mixed model, we treated activity as a within-subject factor with 5 levels (sleep, inactive sitting, active sitting, moderate physical activity, heavy physical activity) and we treated group as a between-subject factor, with 2 levels (Healthy control and CoA patient). In the model, a random intercept was allowed over persons. Physical activity and exercise capacity were entered in the model as covariates as differences in ANS activity might partly be explained by physical activity level and exercise capacity[224]. Additionally, breathing frequency was included as a covariate in the analysis of RSA. Differences in exercise capacity and subject characteristics between groups was evaluated by means of an independent t-test. For comparisons of 7 cardiac function parameters and 2 exercise heart rate dynamics parameters between groups, two MANOVAs were carried out. In case of significance of the omnibus group effect ($p<0.05$), post hoc testing on each separate variable used a Bonferroni correction of the overall p-value. Lastly, a correlation matrix was computed for the cardiac function parameters and blood pressure and ambulatory cardiac ANS parameters PEP and RSA during sleep in the patient group.

RESULTS

Table 1 summarizes the subject characteristics, exercise capacity, exercise heart rate dynamics and physical activity level. Resting systolic blood pressure was significantly higher ($p=0.012$) in patients compared to controls. Follow up time was on average 12 years (range 3.8-17.6y). None of the patients had a clinically significant residual stenosis: maximal flow

velocity at the coarctation site was on average 2.3 m/s (SD=0.6). 19 (59%) had a bicuspid aortic valve. Three patients were using medication for blood pressure (labetalol or enalapril) at the time of the study. Group means of HR, RSA, PEP and CO in each of the five ambulatory activities are summarized in *table 2.*

Table 1 subject characteristics, exercise parameters and physical activity level

	CoA patients	Heathy controls (1)	Healthy controls (2)
N	32	61	138
Male (%)	59	59	51
Age (y)	13.3 (2.9)	13.2 (3.0)	13.0 (2.6)
Age at repair			
Range	3d – 5.8y	-	-
Median (IQR)	0.4y,0.0-1.3	-	-
Time after repair (y)	12.3 (3.5)	-	-
Length (cm)	159.0 (14.9)	160.9 (16.9)	159.7 (13.5)
Weight (kg)	52.0 (16.7)	50.9 (16.9)	48.6 (13.4)
SBP (mmHg)*	120 (13)	113 (12)	-
DBP (mmHg)	66 (7)	66 (9)	-
VO_{2peak} (ml/kg/min)*	40.8 (9.6)	44.2 (3.9)[#]	45.0 (7.4)
HR_{peak} (bpm)	187 (12)	-	187 (10)
HRR_{60} (bpm)	34 (10)	-	32 (10)
Physical activity (METs/week)	39.3 (21.3)	49.5 (23.6)	-

*Mean (SD). CoA= Coarctation of the Aorta. D=days. Y=years. SBP=systolic blood pressure. DBP=diastolic blood pressure. VO_{2peak} = peak oxygen uptake. HR_{peak}=maximal heart rate at peak exercise. HRR_{60}=heart rate recovery 1 minute into recovery. MET=metabolic equivalent task. *significant difference between patients and healthy control groups ($p<0.05$). [#]estimated using prediction equation[237].*

Table 2 Ambulatory measurement of HR, PEP, RSA and CO

	Activity	CoA patients	Healthy controls (1)
Heart rate (bpm)	Sleep	67 (8)	65 (9)
	Quiet sitting	79 (9)	80 (13)
	Active sitting	83 (9)	85 (11)
	Moderate PA	105 (12)	110 (15)
	Heavy PA	124 (16)	128 (16)
RSA (ms)	Sleep	113 (52)	119 (57)
	Quiet sitting	93 (56)	93 (46)
	Active sitting	88 (30)	81 (36)
	Moderate PA	42 (21)	35 (14)
	Heavy PA	29 (14)	24 (11)
PEP (ms)	Sleep	95 (20)	98 (14)
	Quiet sitting	93 (19)	92 (13)
	Active sitting	95 (19)	95 (15)
	Moderate PA	82 (17)	76 (14)
	Heavy PA	72 (11)	66 (8)
CO (L/min)	Sleep	3.2 (1.0)	3.7 (1.0)
	Quiet sitting	3.5 (1.1)	4.0 (1.2)
	Active sitting	3.5 (1.0)	4.2 (1.3)
	Moderate PA *	4.0 (1.3)	4.9 (1.6)
	Heavy PA *	4.0 (1.2)	4.8 (1.7)

*Mean (SD). PA=physical activity. HR=heart rate. RSA=respiratory sinus arrhythmia. PEP=pre ejection period. CO=cardiac output. *significant difference between groups (p<0.01).*

Results of the mixed linear modelling included a main effect of ambulatory activity on all four measures. For HR, no main effect of group was found ($F(1,94)=3.27$, $p=0.07$) but there was a group*activity interaction effect ($F(4,88)=3.16$, $p=0.02$). HR was higher in healthy

controls during periods of moderate or heavy physical activity. For PEP, no main effect of group (F(1,76)=0.34), p=0.56) but an interaction effect of group*activity (F(4,83)=4.03, p=0.005) was found. Sympathetic activity, as reflected in shorter PEP, was higher in healthy controls during periods of moderate or heavy physical activity. RSA showed neither a group effect (F(1,93)=1.48, p=0.23) nor an interaction of group*activity (F(4,97)=1.39), p=0.24). Lastly, CO showed a main effect of group (F(1,96)=7.08, p=0.009) which was due to lower CO in the CoA patients throughout the entire ambulatory recording period.

Table 3 cardiac function parameters

	CoA patients	Heathy controls (1)
Longitudinal strain (%)	16.4 (2)	17.0 (3)
LV TDI S' (cm/s)	8.8 (3.1) *****	10.9 (3.1)
LV TDI E' (cm/s)	15.2 (4.7) *****	19.3 (3.9)
Septum TDI S' (cm/s)	6.9 (1.0)	7.2 (1.0)
Septum TDI E' (cm/s)	12.7 (2.4) *****	14.1 (2.1)
RV TDI S' (cm/s)	12.9 (2.2)	12.4 (1.9)
RV TDI E' (cm/s)	16.0 (3.4)	14.8 (3.3)
PWV proximal aorta (m/s)	4.8 (1.3)	-
PWV distal aorta (m/s)	3.9 (0.7)	-
LV mass (g)	85 (27)	-

Mean (SD). S=systolic motion. E=early filling. TDI=Tissue Doppler Imaging. LV=left ventricle. RV=right ventricle. PWV=Pulse wave velocity *******significant difference between groups after Bonferroni correction for multiple comparisons (p<0.005).*

Cardiac function was significantly different between the two groups (Pillai's Trace, V=0.340, F(10,71)=3.66, p=0.001). Univariate tests show a significantly lower peak E wave velocity in the left ventricular wall and the intra ventricular septum in patients (*table 3*). VO_{2peak} was lower (p=0.010) in patients compared to controls (*table 1*). Exercise heart rate dynamics was also different between groups (Pillai's Trace, V=0.007, F(2,159)=0.553, p=0.576). Univariate test results revealed no difference in maximal heart rate nor heart rate recovery (*table 1*). The correlation between basal RSA during sleep and PWV in the proximal aorta was significant (r=0.47; p=0.012) as were the correlation between basal PEP during sleep and LV longitudinal strain (r=-0.45; p=0.012), between basal PEP and LV mass (r=0.55;

p=0.002) and between basal PEP and peak S' wave in the intraventricular septum (r=-0.45; p=0.010). Rerunning the analysis without the three patients on medication did not alter the pattern of results.

Table 4 correlation between cardiac ANS and cardiac function in the CoA group

	PEP (sleep)	RSA (sleep)
Longitudinal strain	-0.45 (95%CI: -.39;-.05) *	0.12 (95%CI:-.13;.24)
LV TDI S'	-0.31 (95%CI: -.67;.05)	0.04 (95%CI: -.33;.42)
LV TDI E'	-0.25 (95%CI: -.62;.12)	0.10 (95%CI: -.29;.48)
Septum TDI S'	-0.45 (95%CI: -.78;-.11) *	0.23 (95%CI: -.13;.59)
Septum TDI E'	-0.23 (95%CI: -.60;.15)	0.06 (95%CI: -.32;.44)
RV TDI S'	-0.04 (95%CI: -.42;.34)	-0.14 (95%CI: -.52;.24)
RV TDI E'	-0.01 (95%CI: -.38;.37)	0.04 (95%CI: -.34;.41)
CO (sleep)	-0.09 (95%CI: -.47;.29)	-0.03 (95%CI: -.41;.35)
SBP	0.20 (95%CI:- 17.;.53)	0.08 (95%CI: -.29;.43)
DBP	0.10 (95%CI: -.26;.45)	0.13 (95%CI:- 23.;.48)
LV mass	0.55 (95%CI: .23;.86) *	0.13 (95%CI: -.24;.50)
PWV PA	-0.04 (95%CI: -.45;.37)	0.47 (95%CI: .11;.83) *
PWV DA	-0.01 (95%CI: -.41;.40)	-0.02 (95%CI: -.42;.38)

*S=systolic motion. E=early filling. TDI=Tissue Doppler Imaging. LV=left ventricle. RV=right ventricle. SBP=systolic blood pressure. DBP=diastolic blood pressure. PWV=pulse wave velocity. PA=Proximal aorta. DA=Distal aorta. *significant correlation (p<0.05).*

DISCUSSION

The aim of this study was to extensively evaluate children after CoA repair, including ANS activity, cardiac function and exercise capacity and to explore the relationship between cardiac ANS and cardiac function. The main findings of this study were that cardiac ANS was not different between the CoA patients and the healthy controls but ambulatory CO during physical activity, exercise capacity and LV function were decreased in patients compared to controls.

The current study does not find differences in ambulatory cardiac ANS regulation between healthy controls and patients after CoA repair measured using ambulatory impedance cardiography. This is in accordance with Kenny et al. who studied cardiac ANS activity in CoA patients by measuring (15 minute supine) heart rate- and blood pressure variability and baroreceptor sensitivity[94;95]. In contrast, Beekman et al. and Millar et al. who report decreased heart rate variability and baroreceptor function post-repair[96;252]. Before

intervention, the decreased blood pressure in the lower part of the body –including the kidneys- may activate the renin-angiotensin system to increase systemic blood pressure. Also, increased pressure proximal to the coarctation, decreased elastic wall properties[102] and secondary flow patterns[103] in the aortic arch may alter baroreceptor function. After intervention, ANS may be altered by damaging of the ANS nerves traveling along the aorta. Although not significantly, in the current study PEP was higher in patients compared to controls in most ambulatory activity conditions. PEP –the time delay between the electrical depolarization and the onset of left ventricular ejection- is a measure of contractility. Contractility is influenced by the SNS and not the PNS and therefore PEP provides a 'pure' measure of SNS activity, in contrast to heart rate which reflects the activity of both ANS branches. Lower myocardial contractility results in a longer time delay between electrical depolarization and blood outflow as it will take the left ventricle longer to build up enough force to overcome arterial pressure and open the aortic valve. A longer PEP thus reflects lower contractility. However, PEP is dependent on afterload. When the heart has to pump against a high afterload, it will also take more time to build up enough force to open the aortic valve. Hence, afterload may influence PEP independent of cardiac inotropic drive [47;253]. Therefore a difference in PEP between two groups could be the result of difference in contractility due to SNS activity, or a difference in afterload.

Hypertension is a well-known and common complication in CoA patients [104]. Indeed, resting blood pressure was higher in patients compared to controls (see *table 1*). The difference in blood pressure between the groups (i.e. afterload being higher in the CoA group) will probably influence the length of the PEP in that it prolongs PEP independent of SNS activity. In the patient group, a negative relationship was found between basal PEP during sleep and longitudinal left ventricular strain, and between basal PEP and septal peak systolic velocity (S'). A positive relationship was found between basal PEP and LV mass. Thus, patients with a high basal PEP (i.e. a low SNS activity or a high afterload) showed lower longitudinal strain, lower septal peak systolic velocity and higher LV mass. These associations were not found in the healthy control group. Since increased afterload on the heart may in time cause deterioration of LV function and LV hypertrophy, PEP arguably reflects afterload rather than SNS activity in CoA patients.

The fourth association found in the current study was a positive relationship between pulse wave velocity (a measure of artery stiffness) in the proximal aorta and basal RSA (a measure of PNS activity). The only study investigating the relationship between PWV and an index of ANS found a negative relationship between baroreceptor sensitivity and aortic PWV[95]. The authors argue hypertension becomes manifest when the ANS fails to

compensate adequately in these patients. Although plausible, more research is necessary on this topic.

In accordance with the findings from the ambulatory cardiac ANS measurements, exercise heart rate dynamics (peak heart rate and heart rate recovery one minute after exercise cessation) did not show a difference between patients and controls in the current study. Exercise capacity was different in the two groups studied. Patients showed a lower VO_{2peak} while weekly physical activity was not different between the groups. Pediatric studies show different results compared to the current study. Norozi et al. [239] describe normal exercise capacity measured by workload in pediatric CoA patients, however no oxygen consumption was measured. Balderston et al. [254] found no difference in VO_{2peak} between children after CoA repair and controls. It should be noted that the difference in exercise capacity found in the current study means that as a group, these patients have a lower exercise capacity but individual patients may have a normal or even above normal VO_{2peak}; in the current cohort, 8 of 32 patients have a VO_{2peak} higher than 100% of the predicted value [237]. The decreased exercise capacity might be explained by the decreased CO, as seen in the physically active periods during ambulatory assessment.

Klitsie et al.[248] investigated LV function before, shortly after and 1 year after correction of CoA. Before surgery, LV function was significantly impaired compared to healthy age matched controls. This improved after surgery, however LV function remained significantly lower compared to controls. The current study adds to that by showing a decreased LV diastolic function (peak velocity of the E' wave) after longer term (12.3±3.5y) follow up. The increased afterload caused by the high blood pressure and the stiffness of the proximal aorta in these patients are likely to explain LV hypertrophy and decreased LV diastolic function. A limitation of this study is that we did not employ 24-hour blood pressure measurements. Therefore, the blood pressure might be overestimated.

In conclusion, despite no differences in cardiac ANS activity between children after successful CoA repair and healthy peers, ambulatory cardiac output, left ventricular function and exercise tolerance were significantly decreased in these young patients. These results underline the importance of life long follow up in CoA patients, even after successful repair. Also, although cardiac ANS is not abnormal in childhood, it might still play a role in the long term complications at older age and this should be addressed in future studies.

Chapter 8

Summary and discussion

SUMMARY

The first aim of this thesis was to investigate the feasibility and validity of ambulatory recording of cardiac ANS activity and cardiac output using the ECG and ICG in healthy children and in children with a repaired CHD. Assuming valid recording of these signals, a second aim was to examine whether altered cardiac SNS and PNS activity is found in pediatric CHD patients.

For this thesis, a database was build containing ambulatory cardiac ANS measurements in children with a CHD and healthy children. For these measurements, we used the VU-AMS system that was developed at the Vrije Universiteit Amsterdam more than two decades ago and has been under constant development and improvement ever since. Echocardiograms, exercise tests and magnetic resonance imaging in these children were completed at the LUMC. Additionally, exercise testing data was obtained from two additional cohorts: healthy school children in Rotterdam and adolescent twins and their siblings of the Netherlands Twin Register. In this section, the findings from this thesis will be summarized.

In chapter 3, an overview of the literature on cardiac ANS control in children with a congenital heart defect is described. Congenital heart disease is the most common congenital defect, affecting about 1 in 100 new-borns. Fortunately, thanks to the techniques that are available today, survival of these patients is good and during childhood, most patients do well. However later in life, cardiac problems often reappear and the mechanism behind these long term sequelae is unclear. Altered cardiac ANS is found in various cardiac patient groups and is associated with an increased risk of cardiac events and sudden cardiac death. In these patients, the altered ANS typically is characterized by an increased sympathetic and a decreased parasympathetic activity. The SNS in particular has shown to play a key role in the progression of heart failure. It is plausible that altered cardiac ANS also plays a significant role in the long term sequelae of patients with a CHD but large scale studies relating ANS control to clinical outcome are lacking. When studying differences in ANS control in congenital patients, it is important to segregate groups based on the type of CHD and whether they have had an intervention. The pathophysiology and type of intervention needed for every CHD is different and this is expected to affect ANS control differently.

From the studies that are available it can be tentatively concluded that ANS function seems to be altered both before and after intervention in children with a CHD. However, studies linking cardiac autonomic control and clinical outcomes are warranted in order to gain more insight into the potentially causative role of the ANS in the etiology of long term outcomes in CHD and the potential benefits of intervention targeting the ANS. Gold standards for measuring cardiac ANS function include measurement of

norepinephrine regional spillover, microneurography, microdialysis and pharmacological blockade. Unfortunately, these methods are also the most invasive methods. Non-invasive methods include for example measurement of baroreceptor sensitivity, skin conductance, exercise heart rate dynamics, heart rate variability and impedance cardiography. The latter two are of particular interest for they are able to discriminate between sympathetic and parasympathetic activity. Also, heart rate variability and thorax impedance are amenable to longer term ambulatory measurement in real life settings, which arguably have the highest clinical relevance.

In chapter 4, the validity of impedance cardiography in pediatric populations is investigated. Classically, three points of interest points of interest are derived from the ICG. The B-point represents the opening of the aortic valve and thus the start of left ventricular outflow. The C-point coincides with peak flow in the aorta and the X-point corresponds to the moment of closing of the aortic valve and marks the end of left ventricular outflow. The B-point marks the end of the pre-ejection period (PEP); a measure of sympathetic activity. The PEP is defined as the time between the start of ventricular depolarization (onset of the Q wave in the electrocardiogram) and the start of left ventricular outflow (B-point in the ICG). For the estimation of stroke volume from ICG, all 3 points (B-, C- and X) are needed for the equation because stroke volume estimation includes left ventricular outflow time (i.e. the time between opening –B-point- and closing –X-point- of the aortic valve) and the amplitude of the C-point. Unfortunately, as with many modalities for physiological measurement, the scoring of the different points is often complex.

In the research described in chapter 4 of this thesis, thoracic ICG and transthoracic echocardiography are measured simultaneously in 128 healthy volunteers and in 66 patients with a CHD. After studying the agreement between ICG and transthoracic echocardiograms of multiple potential candidates for the B- C and X points, we provide fixed rules for optimal scoring. Using the optimal scoring, the agreement between ICG and echocardiography is moderate for the PEP (healthy volunteers: ICC: 0.57, 95%CI: 0.27-0.76. Patients: ICC: 0.50, 95%CI: 0.22-0.67) and good for left ventricular ejection time (healthy volunteers: ICC: 0.69, 95%CI: 0.27-0.86. Patients: ICC: 0.59, 95%CI: 0.10-0.80).

The original development of ICG based stroke volume estimation was done using band electrodes. Currently, spot electrodes are used more frequently as it greatly decreases the obtrusiveness and measurement burden on the subjects. However, this has repercussions for the stroke volume estimation as it relies on baseline thorax impedance, which is much lower when using spot electrodes compared to band electrodes. As a result, absolute stroke volume is overestimated. In the research described in chapter 4, stroke volume estimation is optimized by correcting the formula for the lower thoracic impedance. After adjustment of the equation for stroke volume the ICC improved from

0.26 to 0.72 in healthy subjects and from 0.13 to 0.37 in patients. Reliable SV assessment using ICG remained more difficult in patients compared to healthy controls. This was not surprising since SV assessment in patients also proved more difficult when using TTE as shown by the lower intra class correlations between the various methods (biplane, VTI, 3D) in patients compared to controls. We conclude that SV assessment from ICG is non-inferior to other modalities that are available for SV assessment and therefore is usable in pediatric populations. However, when employing this method in cardiac patients caution is warranted as reliability is less compared to healthy persons.

Chapter 5 of this thesis focused on exercise heart rate recovery (HRR) and vagal rebound. In the first minute after exercise cessation, the decrease in heart rate is mainly due to increased parasympathetic activity. A lower HRR is associated with an increased risk of (cardiac) mortality. Although the predictive power of HRR is well established, the origin of the individual differences in HRR is not yet clear. A poorer cardiac vagal control leading to a slower vagal rebound after exercise seems paramount in explaining these predictive effects. In chapter 5, the bivariate heritability of heart rate recovery and vagal rebound after exercise were studied in a cohort of adolescent twins and their siblings. Twin studies enable us to decompose total variance in genetic, common environmental and unique environmental components. Heritability (the relative contribution of genetic influences to the total variance) of heart rate recovery and vagal rebound in the 1st minute after exercise cessation was 60% and 23% respectively, meaning that 60% of the individual differences in heart rate recovery can be explained by genetic factors. The heritability of long term HRR and vagal rebound, 3 minutes after exercise cessation, were estimated at 65% and 3%. The heritability of resting heart rate (68%), resting parasympathetic control (58%) and voluntary exercise behavior (80%) were also estimated and were consistent with what was found in earlier studies. Exercise behavior was correlated to resting heart rate and long term HRR only. In keeping with the hypothesis that HRR is related to cardiac parasympathetic control, the phenotypic correlations hinted towards the existence of an 'general cardiac vagal factor' (including resting heart rate, resting RSA and the vagal rebound effects after exercise) and a more specific 'cardiac vagal exercise recovery' factor (including immediate HRR and vagal rebound and long-term HRR and vagal rebound). The existence of these factors was confirmed by multivariate genetic modelling, showing two separate genetic factors underlying the general and exercise recovery vagal factors respectively.

In chapter 6 and 7, we investigated differences in ambulatory cardiac ANS activity, exercise heart rate recovery, exercise capacity and cardiac function between a cohort of children after repair of their CHD and a group of healthy peers. The second aim of these studies was to investigate the relationship between basal cardiac ANS activity and cardiac function. In this thesis we studied a group of patients after CoA repair (chapter 6) and a

group of patients after VSD repair (chapter 7). Cardiac ANS was measured for 24 hours by the use of ambulatory ICG (VU-AMS). Both studies showed no difference in ambulatory cardiac ANS control between patients and controls. Exercise heart rate recovery yielded the same findings with no differences found in HRR between patients and controls.

Another resemblance between the two studies was that the patient groups showed a lower exercise capacity (VO_{2peak} (mL/kg/min)), while weekly physical activity of the patients was not significantly lower compared to the age- and sex matched healthy control group. In CoA patients, VO_{2peak} was 40.8±9.6 versus 45.0±7.4 in matched controls. In VSD patients it was: 39.0±5.4 vs in matched controls 44.3±7.2. Cardiac function was also impaired in the CHD patients, with impairment showing a clear dissimilarity between CoA and VSD patients. This underscores the difference in pathophysiology and the need to study well-defined homogeneous patient groups (chapter 1). In VSD patients, right ventricular function was diminished compared to controls. Potentially, exposure of the heart (especially the ventrally situated RV) during sternotomy has irreversible effects on the myocardium. Prognosis after VSD repair is generally believed to be excellent but in this study we show that using advanced techniques, subtle differences in cardiac performance are detected 10 years after repair. Basal cardiac ANS activity was not related to cardiac function in this group. In CoA patients, left ventricular function was decreased. Most likely this can be explained by increased afterload caused by high blood pressure –the most common complication in this patient group- and the increased stiffness of the proximal aorta. Furthermore in CoA patients, basal PEP was negatively related to ventricular function (LV longitudinal strain and septal peak systolic velocity (S')) and positively to LV mass and basal RSA was positively related to PWV in the proximal aorta. These associations were not found in the healthy control group.

GENERAL DISCUSSION

In this section, the findings of this thesis will be evaluated in the light of current knowledge and directions for future research will be provided.

Validity of impedance cardiography measurement

Systolic time intervals

Various groups including our own have noted the ambiguity in scoring of the B-, C- and X-point [47;146-148]. This is particularly so for the B-point, which is needed for PEP calculation as

PEP is defined as the time between the start of ventricular depolarization (onset of the Q wave in the electrocardiogram) and the start of left ventricular outflow (B-point in the ICG). Not surprisingly, efforts have been made to estimate PEP from other, easier detectable points in the signals. For example, the group of Meijer et al. [255] introduced the initial systolic time interval which they defined as the time delay between the –easy to score- R-peak and the C-point. Such an alternative is attractive because it enables replacement of manual inspection by detection by computer algorithms. Unfortunately, the initial systolic time interval could not adequately replace PEP [146]. However, the time interval might be helpful in monitoring fluid responsiveness –a daily struggle on the intensive care unit- in patients on the intensive care [256]. Lozano et al. [147] suggest a polynomial function for the estimation of the R-B interval ($RB=1.233RZ-0.0032RZ^2-31.59$ where RZ is the time between the B- and the C-point in the ICG) based on the relationship between the timing of the opening of the aortic valve and peak aortic blood flow in the cardiac cycle. When tested in our sample, it underperforms when compared to the optimal scoring method proposed in chapter 4 (in controls: ICC=0.44, 95%CI: 0.1-0.7 compared to ICC= 0.50 95%CI: 0.2-0.7 using our method and in patients: ICC=0.41, 95%CI: 0.2-0.6 compared to ICC=0.48, 95%CI: 0.3-0.7 using our method). However, in signals with no detectable B point due to noise or morphology, this may provide a good alternative to having to put the B-point to missing. Other B-point scoring guidelines that have been put forward include the use of the point on the dZ/dt limb at 15% of the dZ/dt_{max} value and the point of dZ/dt zero crossing. Both options are discouraged [47]. When tested in chapter 4 the point of DZ/dt crossing indeed proved to show very bad agreement to the actual moment of aortic valve opening.

The guidelines proposed in chapter 4 should aid researchers in scoring the (ambiguous) ICGs. To further aid ICG scoring for researchers working on pediatric data, an overview of physiologically plausible PEP and LVET value ranges in children taken the observed HR is displayed in *table 1*. By the use of a diary filled in by the participant, 24 hour recordings were divided into fixed periods, coded for activity. Ensemble averaged ICG and ECG over these periods were then classified according to the heart rate in that period (40-60 bpm, 60-80 bpm etc. see *table 1*), in which the average PEP and LVET were calculated. The ranges displayed in *table 1* are based on the mean±2SD of ambulatory recordings of 118 healthy children between 1 and 18 years, aggregated per heart rate range bin.

Caution has previously been voiced in the use of the PEP as an index of SNS activity in comparisons of groups or conditions with notably different cardiac preload or afterload [160;257]. Specific caution seems to be warranted in the application of ICG-derived systolic time intervals in cardiac pediatric patients groups as they may systematically differ in afterload and preload from healthy controls. For example, we find reduced ventricular

function and increased LV mass to be associated with prolonged PEP in CoA patients. This seems rather confusing at first sight. Longer PEP typically signals lower cardiac sympathetic activity, which we expect to protect LV function. However, PEP is also determined by afterload, and the afterload effect on PEP occurs independent of SNS activity. As mentioned earlier, hypertension is a very common complication in CoA patients. Indeed, CoA patients showed a higher blood pressure compared to their healthy peers (SBP 120±13 vs 113±12). Hypertension causes an increased afterload on the heart which in time may cause decrease of LV function and an increase of LV mass. The same afterload can also paradoxically prolong PEP even if cardiac sympathetic activity is unchanged. Therefore, longer PEP arguably reflects higher afterload rather than decreased SNS control in CoA patients.

Table 1 Physiologically plausible ranges for PEP and LVET in children, for various heart rates

Heart rate range	Plausible physiological range for PEP (ms)	Plausible physiological range for LVET (ms)
HR 40 - 60	70 -141	277 -375
HR 60 - 80	63 -125	254 -354
HR 80 - 100	55 -118	217 -318
HR 100 - 120	46 -104	188 -290
HR 120 - 140	44 - 85	155 -272
HR 140 - 160	44 - 77	130 -249
HR 160 - 180	38 - 77	121 -217
HR 180 - 200	43 - 58	113 -185

Stroke volume

Much effort has been put into the non-invasive measurement of CO. Interest on this area comes from different areas including medical research, elite sport research, and astronautics. Nowadays, several methods, both invasive and non-invasive are available. Thermodilution is such an invasive technique that enables measurement of CO by injecting a liquid of known amount and temperature into the superior vena cava or right atrium [258]. CO can be calculated as it is inversely related to the fall in temperature, which is measured by the same catheter at the pulmonary artery. The dye dilution method for measurement of CO includes injecting a known quantity of dye in the circulatory system and withdrawing blood at a distal site in order to create a concentration curve from which CO can be calculated [259]. CO can also be estimated by the Fick method [260] which calculates CO by dividing oxygen consumption by the arteriovenous oxygen difference. The aforementioned methods are not able to measure beat-to-beat changes in SV. Also, these methods are invasive since a catheter is needed. Later, the Fick theory was used to form the indirect Fick method, where CO is estimated from breath gas instead of blood gas, making the method non-invasive [261]. Cardiac magnetic resonance imaging (MRI) enables CO measurement in a non-invasive way. No catheter or blood draw is needed, however especially in children or claustrophobic persons it can be still quite intrusive. The most frequently used method to obtain SV from MRI is by drawing the endocardial contours at end-systole and end-diastole in all slices of the left ventricle[262]. Movement artefacts can seriously hamper image quality and thereby reliability of SV assessment. Another non-invasive method available for SV estimation is TTE. Using the biplane method [263] SV can be calculated from 2 dimensional TTE. In the long axis apical 2- and 4 chamber view, epicardial contours have to be drawn at end-diastole and end-systole. Drawing endocardial contours is not always unambiguous and introduces a source of inaccuracy. Doppler ultrasound can be used to estimate SV by multiplying the aortic cross sectional area with the velocity time integral from the pulse wave Doppler signal [264]. A disadvantage of this technique is that the signal is sensitive to the angle of insonation, introducing variation in SV measurement. Of the non-invasive methods available, ICG is the least intrusive way to measure SV.

ICG is the only method currently available to measure CO in a naturalistic setting for prolonged periods of time. The ICG does not require much attentive cooperation of the participant as for example TTE or MRI do, which can be especially challenging in children. Studies evaluating the validity of SV measurement using ICG in children with CHD show predominantly good results [125;135;136;152;265;266] except during and directly after surgery for their CHD [141;142]. Three of those studies reported lower agreement between ICG and the reference method in patients with intraventricular shunts [135;265;266]. However, in all three

studies the Fick method was employed as a reference method which may be less reliable in persons with a shunt [267]. Studies evaluating validity in healthy children are scarce. Pianosi et al.[268] evaluated SV measurement by the use of ICG during an incremental exercise test in healthy children by comparing it to CO using the indirect Fick method at two different stages of exercise and found good agreement. Later, the same group studies ICG again in a bigger cohort of healthy children and concluded that CO behaved according to expectation as it increased linearly with oxygen uptake [175]. In the current thesis, agreement between ICG-derived SV and TTE-derived SV was less in cardiac patients compared to controls, this was also noted in a review by Raaijmakers et al. [113]. This might bias the comparison of CO between groups. However, inspection of the Bland-Altman plots revealed that the lower ICC was not due to an offset bias but due to larger measurement error in both directions, i.e. ICG in the patient groups produced both larger errors of overestimation and underestimation. The difference found in ambulatory CO in chapters 6 and 7 could not be simply attributed to a systematic underestimation of SV in patients.

For the estimation of SV by the use of impedance cardiography, several equations have been proposed [269;270]. Virtually all equations proposed for the estimation of ICG-derived stroke volume are descendants of the Kubicek equation [10]. A possible exception to that rule is PhysioFlow, that uses a proprietary algorithm of which the only detail exposed is that they do not employ basal thorax impedance (Z0) in the equation [143;159]. The equation as proposed in chapter 4 of this thesis is a descendant of the Kubicek equation but also removed basal thorax impedance from the equation. Basal thorax impedance is sensitive to electrode placement and body shape. By removing it from the equation, SV estimation became more valid.

Respiratory frequency and Respiratory Sinus Arrhythmia

Respiratory frequency can be measured by the use of thoracic impedance. Thoracic impedance is constantly varying around the basal thoracic impedance value of the person. This variation is due to two main reasons. High frequency changes (in the heart frequency range) reflect beat-to-beat variation in blood pumped out by the heart. Impedance will drop during systole because of the increased blood volume in the aorta and the alignment of erythrocytes which together cause a momentary decrease in impedance. These high frequency changes in thoracic impedance (dZ), when integrated over time, constitute the ICG (dZ/dt) and are used to extract systolic time intervals and SV. The high-frequency changes in thoracic impedance are superposed on a much slower change in dZ in the respiratory frequency range. This lower-frequency variability in dZ has a much larger amplitude and reflects the effects of breathing. With appropriate band pass filtering for

both the high frequency heart signal and much lower frequency upper body movement (at least when they are not aligned with breathing) a clear respiration signal can be extracted from the variability in dZ coupled to breathing.

By combining the respiration signal from the impedance with the inter beat interval time series from the ECG, RSA can be computed by the peak-valley method [15]. The shortest inter beat interval during inhalation is subtracted from the longest inter beat interval during exhalation in order to get an index of RSA. The physiology behind this respiration-coupled variation in heart rate originates in the brain [45;46]. Connections exist between the nuclei that control the respiratory generator in the pre-Bötzinger and Bötzinger complexes, and the parasympathetic and sympathetic neurons. As a result, the firing of motor neurons in the nerves ambiguous and the sympathetic nuclei is phasically inhibited (during inspiration) and exited (during exhalation). This coupling to the respiration thus affects both the parasympathetic and the sympathetic branch and they both directly innervate the sinoatrial node. However, hyperpolarization of the sinoatrial node as a result of parasympathetic outflow occurs within hundreds of milliseconds while sympathetic outflow does this only on the scale of seconds. Therefore sympathetic outflow barely alters RSA. When parasympathetic outflow is high, the effect of the phasic inhibition and excitation will be most pronounced, resulting in a higher RSA compared to a situation when there is less parasympathetic activity. The absolute value of RSA thus provides us with a measure of PNS effects on the heart [46].

Validity of peak-valley RSA as a measure of cardiac PNS effects has been shown by Grossman et al. [271;272] in a series of studies using muscarinergic blockade. In this thesis we supply an alternative indication of the validity of peak-valley RSA as a good indicator of cardiac vagal activity. In the research described in chapter 5, we measured RSA in the first minute after maximal exercise and saw an increase from almost zero at maximal exercise intensity to 40ms (average RSA of the first minute after exercise cessation). This is in keeping with textbook knowledge that the PNS is completely suppressed during exercise but rapidly returns shortly after exercise, and that HRR is induced mainly by an increased parasympathetic control while sympathetic control remains practically unchanged in the first minute [186]. Also, in the ambulatory measurements, as expected we saw a stepwise decrease in RSA with increasing physical activity (chapters 6&7). The advantage of peak-valley RSA over spectral analysis is that it enables measurement of vagal control over periods of time as short as 30 seconds while spectral analysis, longer periods (>100 seconds) are necessary for analysis. Breathing frequency is a potential confounder that can influence RSA independent of vagal activity [257;273] and several researchers have expressed their concerns about this issue [45;155]. Also, respiration depth can influence RSA. However,

normally breathing frequency and depth are tightly coupled and variation in breathing frequency will account for the bulk of variation in RSA. For this reason, impedance cardiography derived RSA has a clear advantage over other measures of HRV in the respiration frequency band such as RMSSD (root mean square of successive differences); it enables correction for respiration rate. In this thesis, RSA corrected for respiration frequency was employed to measure PNS activity by adding the average respiration rate per experimental/ambulatory condition as a covariate to the analyses of RSA (chapter 6 and 7).

Feasibility of ambulatory impedance cardiography measurement

Ambulatory measurement has the clear advantage of improved ecological validity compared to measurements done in the laboratory or doctor's office. This has been demonstrated clearly in studies on emotional stress where exposure to laboratory manipulations evoke a physiological stress response that does not transfer well to stress responses in real life situations [274;275]. It is very likely that this also applies to cardiac ANS reactivity. Apart from having a higher construct validity, the expectation is also that measurement of physiologic parameters in a naturalistic setting provide a better prediction of morbidity and mortality risk in the future. Such an added value of ambulatory measurements is well demonstrated by blood pressure measurements; the prognostic power of ambulatory measurement is much stronger than in the 'office blood pressure' [276]. Thanks to ambulatory measurements, the unjust prescription of blood pressure medication has been successfully confined.

Ambulatory ICG measurement has been used frequently in adult studies [16;117;277]. The current thesis now for the first time showed that it is also feasible in children aged 1-18 years, although in children below 3 years of age drop-out rate was high. From the 194 subjects included in this study, 184 completed the 24-hour measurement. From the 10 children that did not complete the measurement, 9 were under the age of 3 and did not participate to the ambulatory part of the study but all completed the short ICG measurement simultaneous with the echocardiography for the validation study. Additionally, data from two ambulatory measurements were lost due to technical problems. Eventually we successfully realized 182 complete ambulatory measurements.

Limitations of ambulatory impedance cardiography measurement

Despite its obvious boost to ecological validity, there are also specific disadvantages of ambulatory measurement. First, there is no control over the activities of the participant as ambulatory assessment is unstructured by nature. This is problematic because the ANS is a major source of cardiovascular homeostasis and therefore very sensitive to changes in posture and physical activity level. Even simply going from sitting to standing evokes substantial increases in SNS and decreases in PNS activity. Physical activity is a further powerful determinant of ANS activity. As a consequence, careful registration of body posture and physical activity is required, as the interpretation of group differences in ambulatory recordings, e.g. patients versus controls, is meaningless if differences in daily activities are not taken into account. In the ambulatory ANS measurements described in the current thesis, a paper diary was used in combination with build-in accelerometer of the VU-AMS device that helped to demarcate the 24-hour measurement in periods based on posture and physical activity. This procedure, although standard in the field, has two clear set-backs. Manual labelling of daily activities is very time consuming and there is a large burden on the participant. A completely automated approach based on motion sensors, GPS and possibly beacon signals from the environment, paired to intelligent algorithms able to detect the body position and type of activity (e.g. biking, walking, stair climbing) automatically would greatly reduce burden on participants and researcher labour. This would enable much bigger study samples. Also, it would increase reproducibility because in the current situation, labelling the data for activity is done manually by the researcher and differences in labelling are expected between researchers. Alternatively, a completely different approach to parse ambulatory data could be employed; instead of clustering data based on reported activities, data could be clustered on heart rate [278].

Second, scoring of ambulatory data is time-consuming and this is specifically true for impedance cardiographic data. Despite improved filtering, ensemble averaging [144], and improved automated detection algorithms, visual interactive correction remains the current practice for ICG. Results in chapter 4 of this thesis are promising in this regard. Smarter detection algorithms may at some point obviate the need for laborious visual ICG scoring.

Normative values and maturation for cardiac ANS activity in children

Examining cardiac ANS control in children with a CHD is difficult for two main reasons. First, age- and sex-specific normative values for cardiac ANS measures in healthy children are

lacking from the literature. Secondly, related to the first issue is the lack of knowledge on the patterns of maturation of both branches of cardiac ANS across childhood. A few large studies reported mean values in healthy children but only in a narrow age range [54;279-282]. A sex effect on cardiac ANS values in children was noted but not consistently across all studies. All studies finding a difference, reported a higher HRV in boys compared to girls. Van Dijk [54], described this sex difference in resting values of PEP and RSA in children 5-7 years old, Jarrin et al. [279], described it in children 10 years of age and Faulkner [283] found significant differences between boys and girls in a cohort of 13-19 year olds. Michels et al. [280] in their cohort of 5-10 year old children found a higher HRV in boys only at the age of 5-6 years old. In contrast to the above, Seppala et al. [281] did not find any gender differences in their group of children between 6-8 years old. Finally, Umetani et al. studied HRV in persons from 10-99 years old and also found gender differences, but not over the entire lifespan. From 50 years of age onwards, the gender difference in HRV disappeared [284].

Table 2 presents the means and standard deviations of HR, PEP and RSA while sitting quietly for our 128 healthy volunteers, divided into 6 age groups. No significant sex or age-by-sex differences were found in this cohort, but note that cell sizes are very modest. *Table 2* also displays means and standard deviations from cohorts from the Mother-Infant Neurodevelopment Study (MINDS) [285], the Amsterdam Born Children and their Development (ABCD) study [54], twins from the Netherlands Twin Register (NTR) study described in chapter 5 and the TRacking Adolescents' Individual Lives Survey (TRAILS) study [282]. These studies used very comparable PEP and RSA measures as the study in 128 children presented in this thesis. Three of them (TRAILS is the exception) in fact used the same ambulatory ICG/ECG recording with the VU-AMS system for data collection. The MINDS study is a longitudinal study that aims to investigate factors of influence on emotional and behavioural problems in children [286]. ABCD study also is a longitudinal study, with the goal to investigate factors in early life (both pre- and postnatal) that cause health later in life (www.abcd-studie.nl). TRAILS study investigates the social and physical development of adolescents [287]). The last column in *table 2* displays the weighted means and standard deviations for every age group (last column). The MINDS, ABCD and TRAILS cohorts are much larger compared to our cohort and thus the weighted average is largely influenced by their means. However, the data from the ABCD, NTR and TRAILS cohorts are in striking accordance with the values found in our cohort. Therefore, our data alone and the weighted average from all studies together show the same trend.

Maturation of the cardiac ANS has been mainly investigated in preterm versus term neonates. Most studies report significantly lower HRV in preterm compared to term neonates [288;289], suggesting an important maturation in the last weeks of gestation.

However the preterm neonates do seem to catch up by the age of 2-3 years old [290]. A clear and steep increase in HRV in the breathing frequency range [291] and an increase in the amount of myelinated fibers of the vagus nerve [292] already in the first months of life suggest that cardiac PNS activity starts to increase at very young age. Previous studies of the change in RSA across different age groups suggested that PNS activity continues to increase rapidly in early childhood and levels off at late childhood, reaching its peak in adolescent age [49;293]. Our data is consistent with such a maturational pattern as can be deduced from inspection of *table 2* and *Figure 1*. The lower panel of *figure 1* shows a graphical representation of the maturation of PEP and RSA based on the weighted means from *table 2*. RSA increases very rapidly in early childhood and thereafter levels off. Data from the MINDS study further reinforces that for RSA in their infants <1 year old is lower than the youngest age group (1-2 year olds) in our sample. For the maturation of SNS however, the time course and direction of maturation is still largely unknown. Our data suggests that PEP shows little change up to the age of 5 and then starts to increase (meaning a decrease in SNS activity). The almost linear heart rate decline with age (*figure 1* upper panel) seems to be mainly due to increased PNS activity in early childhood while in late childhood the HR decline seems to be mainly mediated by a decreased SNS activity, suggesting a differential maturation of PNS and SNS.

Table 2 HR, PEP and RSA, per age category

	Age	*N*	Mean (SD) sitting	*N*	MINDS Mean (SD)	*N*	ABCD Mean (SD)	*N*	NTR Mean (SD)	*N*	TRAILS Mean (SD)	Weighted average Mean (SD)
HR (bpm)	0-1 y	-	-									-
	1-2 y	*13*	117 (9)									117 (9)
	3-4 y	*12*	101 (6)									101 (6)
	5-7 y	*16*	87 (10)			*2624*	91 (10)					91 (10)
	8-10 y	*10*	80 (14)									80 (14)
	11-14y	*43*	77 (11)									77 (11)
	15-18 y	*23*	70 (10)					*455*	74 (11)			74 (11)
PEP (ms)	0-1 y	-	-	*101*	64 (6)							64 (6)
	1-2 y	*11*	71 (11)									71 (11)
	3-4 y	*12*	69 (12)									69 (12)
	5-7	*15*	84 (13)			*2624*	80(12)					80 (12)
	8-10 y	*10*	86 (14)									86 (14)
	11-14y	*41*	98 (12)									98 (12)
	15-18 y	*22*	104 (19)					*455*	113 (16)	*555*	123 (9)	118 (12)
RSA (ms)	0-1 y	-	-	*101*	30 (2)							30 (2)
	1-2 y	*12*	43 (20)									43 (20)
	3-4 y	*12*	72 (27)									72 (27)
	5-7 y	*16*	113 (43)			*2624*	112(53)					112 (53)
	8-10 y	*10*	103 (60)									103 (60)
	11-14y	*41*	93 (44)									93 (44)
	15-18 y	*23*	84 (47)					*455*	67 (37)			68 (37)

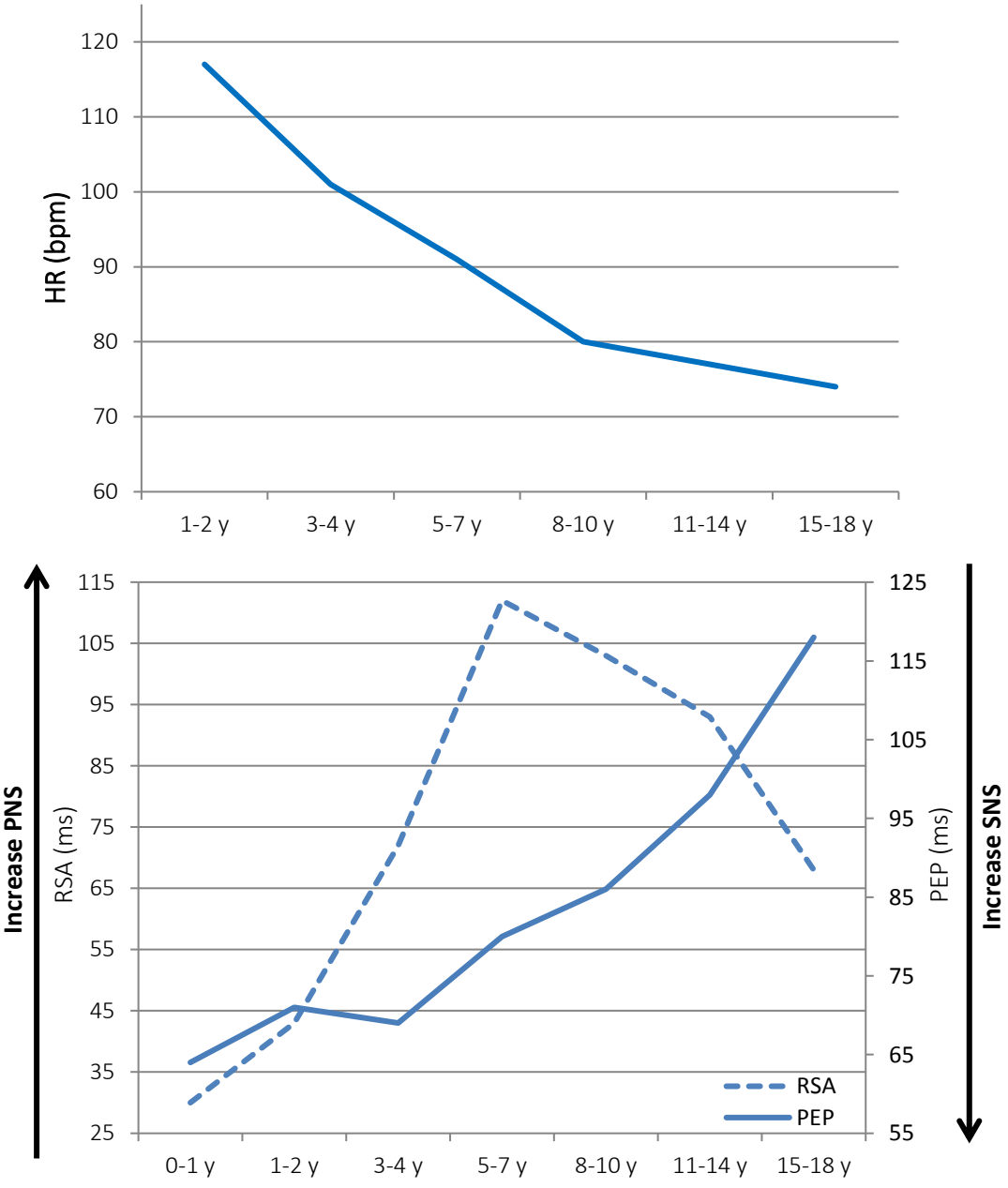

Figure 1 Maturation of HR, PEP and RSA (weighted average means from table 2)

Clinical implications

Most children do well after repair of their CHD, and they might believe that they are 'cured' for life. However, evidence is starting to emerge that repair may not be synonymous to 'cure' of cardiovascular abnormalities, even in relatively simple defects as a VSD or CoA. This thesis presents encouraging evidence that in childhood, cardiac ANS is unaffected in children after CoA or VSD repair. However, it is still possible that altered ANS activity plays a role in the development of long term problems in CHD patients and studies available do seem to point in the direction of altered ANS activity in these patients [164;294;295]. Therefore, it is important for future studies to investigate this further in older CHD patients in order to unravel the exact role of the cardiac ANS in disease progression and eventually to prevent or reverse this process.

In spite of intact cardiac ANS activity, we find a clear reduction in exercise capacity in our CHD patients, coupled to diminished ventricular function and cardiac output. Exercise capacity is related to the risk of hospitalization and sudden cardiac death [188;296]. Furthermore, a good exercise capacity is related to general well-being and an improved quality of life. Therefore, regular evaluation of (changes in) exercise capacity using a cardiopulmonary exercise testing is advisable.

It was also shown in this thesis that cardiac function at rest was impaired in patients compared to controls: in VSD patients the right ventricle showed decreased function (both systolic and diastolic, as measured by TDI) and in CoA patients decreased function was shown of the left ventricle (both systolic and diastolic, as measured by TDI). During daily activities, ambulatory CO was systematically lower in patients compared to controls. It is important to regularly monitor these patients, in order to intervene as early as possible when necessary. The reduction in exercise capacity and the decreased cardiac output and ventricular function might all benefit from an increase in regular physical activity. In contrast to other studies [297;298], no significant differences in physical activity level were found between VSD/CoA patients and controls in our studies (chapter 6&7). A decreased physical activity level in patients may be due to residual cardiac problems but also due to psychosocial factors as for example fear or parental overprotection. This overprotection is uncalled for since physical activity has been proven safe in children with a CHD where it improves exercise capacity just as it does in unaffected children [299;300]. Moreover, especially in children, non-pharmacologic measures like exercise are preferred over pharmacological interventions. Below we review the literature suggesting that regular exercise may induce positive effects on exercise capacity, ventricular function and cardiac output in children.

Exercise as an intervention on cardiac ANS

Both resting heart rate [179-181] and HRR [187-194;301] are well-established prognostic factors for mortality. A lower resting heart rate and a higher HRR decrease mortality risk, and this may explain part of the beneficial effects of regular exercise on mortality risk. Both faster HRR and lower HR partly reflect an increase in cardiac parasympathetic control, which by itself has also proven cardioprotective through increased electrical stability of the heart [61-64]. Exercise training studies show that exercising causally lowers resting heart rate and this is also seen in children [302]. Cross-sectional studies show a higher PNS control in exercisers compared to non-exercisers [303;304] and a positive correlation between physical fitness and PNS control is found [203;305] although not in all studies [306;307]. An association has also been reported between exercise behavior and HRR in both patients and healthy persons [226-228;308]. A causal exercise induced increase in PNS control has been put forward to explain these associations [203;205;230]. Training studies that set out to support this hypothesis have been somewhat equivocal [306;309],in part because they may not have been long enough to induce a PNS effect. Even so, the resting bradycardia in exercisers is largely due to a decreased intrinsic heart rate so the decrease in vagal control may play a relatively minor role in exercise bradycardia [230;310]. In contrast the increased HRR found in regular exercisers compared to non-exercisers may be mediated largely by the increase in PNS control [311].

The clear prognostic value of HRR and the fact that it is an easy to measure variable makes it an attractive clinical parameter. However, most studies are in adults and little is known about HRR and its relationship with exercise behavior and cardiovascular event or mortality risk in children. The only pediatric population in which HRR was studied as a possible predictor is in children after heart transplant and HRR showed to be a significant predictor of mortality [312;313]. Ohuchi et al. [314] studied a group of children and adults (age ranging from 8 to 36) after CHD repair and found a decreased HRR compared to controls. Also, they found a negative relationship between resting PNS activity and HRR. Their study supports the idea that HRR predicts cardiac events because of the close relationship between HRR and cardiac ANS. Singh et al.[315] studied the determinants of HRR one minute after maximal exercise in healthy 9-18 year old children. Age, gender, BMI and resting heart rate were significant predictors that together explained 39% of the variance in their study.

Nagai et al. [316] studied the effect of physical exercise on cardiac ANS control in school children. They compared children with the lowest HRV (in the frequency domain; total power) to sex, weight, height and age matched controls. After a 12 month exercise

intervention of 20 minutes of moderate intensity exercise, HRV had increased (total-, low- and high frequency power) the group with initial low HRV while in the control group, only low frequency power had increased. While they did not measure HRR, an effect may be expected. The study of Nagai et al. shows similarity to a study in adults by Duarte and colleagues [224] who assigned their participants to groups based on their resting high frequency HRV. After a 12 week training program of 40 minutes of moderate intensity exercise 3 times a week, they found increased vagal control and HRR in the group with initially low HRV while in the group with high HRV, only HRR showed an increase. This is in agreement with the hypothesis of the existence of different physiologic factors for resting heart rate and HRR [223] as confirmed by our analysis described in chapter 5.

An important question remains whether an improvement of HRR after an exercise intervention actually affects mortality risk. Jolly et al. [311] studied 1347 adult patients that were referred for their standard 12 week cardiac rehabilitation programme. An exercise test was performed before and after the program. They found that patients with an abnormal HRR (defined as <12bpm decrease in the first minute after exercise cessation) at baseline but normalized after the exercise intervention had a similar mortality risk to patients who had a normal HRR at baseline. Patients with an abnormal HRR at both exercise tests had the highest mortality risk. The results of this study suggest that increasing HRR (e.g. by an exercise intervention) indeed decreases mortality risk. Singh et al. [317] suggest that these exercise intervention benefits can be fully reaped by CHD patients. They studied the effect of a 12 week exercise program (twice a week one hour) on HRR in children with various CHD and found a significant increase in HRR.

Exercise as an intervention on ventricular function and CO

As described, exercise can cause a decrease in resting heart rate. Since CO is the product of heart rate and SV, resting SV will increase in order to compensate for the decreased heart rate. Indeed in healthy trained children, an increased SV at rest is found [302;318]. The origin of increased exercise capacity in response to an exercise intervention is not fully understood but may -at least in part- be the result of improved SV response to exercise in healthy adult persons [319], children [320] and in patients [321]. This is not uncontested. Wagner [322] argues that in adults the increased VO_{2peak} after training is mainly due to an enhanced diffusion of oxygen rather that an increased CO. Maximal heart rate may be either unchanged or slightly decreased in adult exercisers [323] and the same is seen in trained children [318], which pleads for an increased SV during exercise. Morphological adaptions to training, explaining

the increased SV in children may include an increased end-diastolic left ventricular dimension [302;324]. The effects of increased exercise on ventricular function and SV in patients with a CHD is largely unknown [300]. In a small study of 4 adult patients with a Fontan circulation, Cordina et al. [321] found that cardiac filling, SV and CO increased after an 11 week exercise intervention. Sklansky et al. [325] did not find changes in LV end-diastolic dimension nor in LV wall thickness after their 8-week training in 11 children after Tetralogy of Fallot repair. More recently, Duppen et al. [326] extensively studied cardiac remodelling after a 12 week exercise training program. They did not find significant changes in cardiac function measured by TTE and MRI. However, as with ANS effects, it is unknown what the minimum length of an intervention must be in order to evoke an effect. Moreover, all studies tested exercise effects on cardiac function in static resting conditions in the clinic. The patient cohorts described in chapter 6 and 7 of this thesis showed a decreased exercise capacity which might be explained by the decrease in ambulatory CO –which were seen especially in the physically active periods during the day-, also described in these chapters. Possibly, increased exercise in patients after CHD repair would increase their capacity to engage in daily physical activities, through an increase of their SV.

Future directions

There is a scarcity of research on pediatric cardiac ANS activity and I hope to have inspired more studies like the ones presented in this thesis. For these future studies the key lies in expanding sample sizes and studying homogeneous groups of patients, ideally in terms of type of congenital heart disease, type and timing of correction and presence of residual abnormalities as those are expected to be of influence on ANS control. Longitudinal studies on this topic are especially needed in order to test the existence and the direction of a relationship between cardiac ANS function and long term sequelae in adults with a congenital heart disease. Studies evaluating cardiac ANS activity in CHD patients are advised to include ambulatory impedance cardiography. Ideally they do so in an intervention design aimed at detecting the efficacy of regular exercise programs to increase exercise capacity, pre- and post-exercise PNS activity, cardiac output and ventricular function in this unique patient population.

Dutch summary

(Nederlandse samenvatting)

Van alle aangeboren afwijkingen komt een aangeboren hartafwijking het meest voor. Ongeveer 1 op 100 baby's wordt geboren met een hartafwijking. Dankzij de (chirurgische) technieken die tegenwoordig beschikbaar zijn, kunnen gelukkig veel kinderen toch volwassen worden. Het vergaat deze kinderen over het algemeen goed in de kindertijd, maar helaas is ook bekend dat, als deze patiënten volwassen zijn, zij vaak weer hartproblemen krijgen. Het is onduidelijk wie hier het meeste risico op hebben, wanneer de problemen precies ontstaan en wat het exacte mechanisme is achter het terugkeren van de problemen. Mogelijk speelt het autonome zenuwstelsel hierin een rol.

Het autonome zenuwstelsel beschermt ons lichaam tegen veranderingen van de interne en de externe omgeving (bv fysieke inspanning, een bloeding) door onze lichaamsfuncties te coördineren. Het autonome zenuwstelsel bestaat uit twee verschillende takken; het sympathische zenuwstelsel en het parasympathische zenuwstelsel. De sympathische tak is verantwoordelijk voor de zogenaamde 'vecht of vlucht' reactie; het maakt het lichaam klaar om in actie te komen. De reactie bestaat o.a. uit het verhogen van de hartslag, ademfrequentie, de knijpkracht van het hart en de bloeddruk. De parasympathische tak zorgt juist voor het tegenovergestelde; de 'rust en verteer' reactie. Activatie van deze tak zorgt o.a. voor een verlaging van de hartslag en activatie van het verteringsstelsel. Het sympathische en het parasympathische systeem zijn bijna altijd tegelijk actief. De hartfrequentie is het resultaat van die balans; wanneer je in rust bent is de parasympathicus het meest actief en wanneer je sport is de sympathicus het meest actief. Het is uit eerdere studies bekend dat het autonome zenuwstelsel uit balans is bij verschillende groepen (hart)patiënten en dat dit geassocieerd is met hartproblemen en plotse hartdood in de toekomst. Ook is bekend dat overactiviteit van het sympathische zenuwstelsel een belangrijke rol speelt bij het verergeren van hartfalen. Door schade tijdens de hersteloperatie of als gevolg van compensatiemechanismen zou de werking van het autonome zenuwstelsel bij patiënten met een gerepareerde aangeboren hartafwijking kunnen veranderen. Een veranderde activiteit van het autonome zenuwstelsel zou daarom ook een rol kunnen spelen bij het (weer) ontstaan van hartproblemen op volwassen leeftijd

bij deze patiënten. Helaas zijn er weinig grote studies gedaan naar de activiteit van het autonome zenuwstelsel en de relatie met de functie van het hart. In hoofdstuk 3 geven we een overzicht van studies naar de activiteit van het autonome zenuwstelsel bij kinderen met een aangeboren hartafwijking. De beschikbare studies zijn veelal klein of bestuderen kinderen met verschillende hartafwijkingen in één groep. Ook werd vaak slechts korte tijd de activiteit van het autonome zenuwstelsel gemeten en keek men niet naar beide takken van het autonome zenuwstelsel. Hierdoor is het nog niet precies duidelijk of autonome zenuwstelselactiviteit bij kinderen met een hartafwijking verschilt van dat van gezonde kinderen.

Er zijn verschillende manieren om de activiteit van het autonome zenuwstelsel te meten. Helaas zijn de methoden die dit het meest precies kunnen, ook het meest invasief. Impedantiecardiografie maakt het mogelijk om met slechts 7 stickers op het bovenlichaam de activiteit van de beide takken van het autonome zenuwstelsel te meten. Een tweede voordeel van deze techniek is dat het voor een langere periode (bv 24 uur lang) en in de thuissituatie gebruikt kan worden, wat wellicht de hoogste klinische relevantie heeft. Door continu de thoracale impedantie (weerstand) te meten kan de ademfrequentie worden gemeten alsmede de mechanische activiteit van het hart. In de onderzoeken beschreven in dit proefschrift heb ik gebruik gemaakt van deze techniek. Hierbij gebruiken we respiratoire sinusaritmie (RSA, het verschil tussen de langste hartperiode tijdens uitademing en de kortste hartperiode tijdens inademing) als maat voor parasympathische activiteit en de pre ejectie periode (PEP, de tijd tussen de start van de elektrische depolarisatie van de hartspier en de start van de uitstroom van bloed uit het hart naar de aorta) als maat voor de activiteit van het sympathische zenuwstelsel.

In hoofdstuk 4 onderzochten we de validiteit van impedantiecardiografie. Uit het impedantiecardiogram kunnen drie punten worden gehaald: 1. het B-punt (correspondeert met het moment van openen van de aortaklep en het begin van uitstroomfase van bloed de aorta in) 2. het C-punt (correspondeert met piekstroom in de aorta) en 3. het X-punt (correspondeert met het sluiten van de aortaklep en het einde van de uitstroomfase van bloed). Helaas is het scoren van deze punten niet altijd makkelijk; er zijn vaak meer potentiële B- C- en X-punten. Daarom hebben we het impedantie cardiogram en een echocardiogram simultaan gemeten om te onderzoeken welk punt gekozen moet worden wanneer er meerdere potentiele punten zijn. Vervolgens keken we hoe goed de punten overeenkwamen in de twee verschillende meetmethoden als we de optimale scoring toepasten. Na het toepassen van de nieuwe scoringsmethode bleken de punten gemiddeld tot goed overeen te komen. Ook kan met behulp van het impedantiecardiogram

slagvolume (de hoeveelheid bloed die per hartslag het hart verlaat) worden berekend. Hiervoor zijn alle drie de punten (B-, C- en X) nodig. Na het optimaliseren van de scoring van het B- C- en X-punt hebben we de methode om slagvolume te meten verbeterd door de formule te corrigeren voor het gebruik van huidelectroden. De oude formule is namelijk gebaseerd op gebruik van bandelectroden, welke een hogere weerstand hebben dan de huidelectroden die we tegenwoordig gebruiken. De intraclass correlatie tussen slagvolume gemeten met impedantiecardiografie en slagvolume gemeten met echocardiografie, verbeterde van 0.26 met de oude formule naar 0.72 met de gecorrigeerde formule.

In hoofdstuk 5 onderzochten we hartslagherstel na inspanning. In de eerste minuut na (maximale) inspanning zorgt de re-activatie van het parasympathische zenuwstelsel voor een hartslagdaling. Uit voorgaande studies is bekend dat mensen bij wie de hartslag in de eerste minuut na inspanning niet voldoende daalt, een groter risico hebben op overlijden. Het lijkt er sterk op dat een verminderde functie van het parasympathische systeem een rol speelt bij het verklaren van het verband. Het is niet bekend wat de oorzaak is van individuele verschillen in hartslagherstel. Met behulp van tweelingen kan worden uitgezocht of de individuele verschillen worden veroorzaakt door genetische factoren (erfelijkheid), factoren uit de omgeving, of factoren die uniek zijn voor het individu. Als één-eiige tweelingen (100% genetisch identiek) meer op elkaar lijken dan twee-eiige (delen gemiddeld 50% van hun genetisch materiaal) tweelingen, is dat indicatief voor een rol van genen. Uit deze studie, waarvoor 491 tweelingen tussen 12 en 25 jaar uit het Nederlands Tweelingen Register een maximale inspanningstest ondergingen, bleek dat de erfelijkheid van hartslagherstel in de eerste minuut na inspanning 60% is. Dit betekent dat 60% van de verschillen die bestaan tussen mensen, toe te schrijven is aan het feit dat mensen verschillende genen hebben. Ook hebben we gekeken naar de langere termijn hartslagherstel, 3 minuten na inspanning. De erfelijkheid daarvan is 65%. Ook hebben we de re-activatie van het parasympathische zenuwstelsel (respiratoire sinus aritmie) op 1 en 3 minuten na inspanning gemeten. De erfelijkheid hiervan was respectievelijk 23% en 3%. De erfelijkheid van rust hartfrequentie (68%), parasympathische activiteit in rust (58%) en sportgedrag (80%) gemeten in deze studie, kwamen zeer goed overeen met de bestaande literatuur. Een tweede doel van het onderzoek beschreven in dit hoofdstuk was om de hypothese te toetsen dat er twee genetische factoren bestaan; een voor basale parasympathische activiteit en een voor de parasympathische re-activatie na inspanning. Het bestaan van deze twee factoren hebben we getoetst met behulp van een multivariaat model. Dit model bevestigde de hypothese van het bestaan van een erfelijke ‘basale cardiale parasympathische’ factor (met rust hartfrequentie, parasympathische activiteit in rust, hartslagherstel) en een erfelijke ‘cardiale parasympathische re-activatie’ factor (met

hartslagherstel en parasympathische re-activatie). Ook vonden we dat een genetische factor zowel sportgedrag als rust hartfrequentie en hartslagherstel beïnvloed. Wanneer de erfelijkheid van een fenotype hoog is, betekent dat niet dat het fenotype niet kan worden veranderd met een interventie. De parasympathische activiteit in rust als wel de re-activatie na inspanning kunnen bijvoorbeeld door middel van regelmatige intensieve inspanning worden verbeterd.

In hoofdstuk 6 en 7 onderzochten we of er verschil is in autonome zenuwstelselactiviteit tussen kinderen die zijn geopereerd aan hun aangeboren hartafwijking en gezonde leeftijdsgenootjes. Dit onderzochten we bij kinderen geboren met een ventrikel septum defect (een gat tussen de hartkamers, hoofdstuk 6) en bij kinderen geboren met een coarctatie (een plaatselijke vernauwing in de grote lichaamsslagader, hoofdstuk 7). Daarnaast onderzochten we of er bij de patiënten een relatie bestaat tussen hartfunctie en autonome functie tijdens een normale schooldag en de daaropvolgende nacht. Hiervoor hebben we met behulp van impedantiecardiografie de autonome activiteit en met behulp van echocardiografie de hartfunctie van de kinderen in kaart gebracht. We vonden geen verschil in de 24-uurs activiteit van het cardiale autonome zenuwstelsel tussen patiënten en gezonde kinderen. Bij patiënten met een ventrikel septum defect vonden we een verminderde functie van de rechterkamer, wat potentieel een resultaat kan zijn van het gebruik van de hart-longmachine. In deze groep vonden we geen relatie tussen cardiale functie en autonome activiteit. Bij patiënten met een coarctatie vonden we een verminderde linkerkamerfunctie wat het resultaat zou kunnen zijn van een verhoogde bloeddruk wat veel voorkomt in deze patiënten groep. In deze groep vonden we een negatieve relatie tussen PEP tijdens slaap en hartfunctie en een positieve relatie tussen PEP tijdens slaap en linker ventrikelmassa. Ook vonden we een positieve relatie tussen de RSA tijdens slaap en de vaatstijfheid van het proximale deel van de aorta. Patiënten met een langere PEP (lage sympathicus activiteit) hadden dus een slechtere ventrikelfunctie en hogere ventrikelmassa terwijl het tegenovergestelde zou worden verwacht. PEP is echter gevoelig voor pre- en afterload en omdat een verhoogde afterload op den duur kan leiden tot verslechterde linker ventrikelfunctie en verhoogde spiermassa zou dit mogelijke een verklaring kunnen zijn voor de gevonden verbanden.

Hoewel het geruststellend is dat we geen verschillen vonden tussen de patiënten en hun leeftijdsgenootjes wat betreft autonome zenuwstelselactiviteit, sluit dit niet uit dat autonome zenuwstelseldysfunctie op latere leeftijd nog een rol gaat spelen in de pathofysiologie van de problemen op latere leeftijd. Dit zal verder onderzocht moeten worden, idealiter in longitudinale studies in grote groepen patiënten homogeen wat betreft soort hartafwijking omdat de soort afwijking en de benodigde ingre(e)p(en) effect zullen

hebben op de mate van eventuele autonome dysfunctie. Verder ondersteunen de resultaten uit dit proefschrift het groeiende bewijs voor het feit dat ook patiënten met een simpele 'gerepareerde' aangeboren hartafwijking altijd subtiele verschillen kunnen blijven vertonen. De verlaagde ventrikelfunctie die kort na operatie gevonden wordt bij deze patiënten, blijft aanwezig in de latere kinderleeftijd. Ook bestaat er een subtiel maar significant verschil in inspanningscapaciteit tussen patiënten en gezonde leeftijdsgenootjes. Hierom is het raadzaam om deze patiënten ook in de volwassen leeftijd te blijven volgen.

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Abbreviations

Ach	acetylcholine
ANS	autonomic nervous system
ASD	atrial septal defect
ASO	arterial switch operation
BDG	bidirectional Glenn shunt
BP	blood pressure
BPV	blood pressure variability
BRS	baroreceptor sensitivity
CHD	congenital heart disease
CO	cardiac output
CoA	coarctation of the aorta
CPL	complex cyanotic heart disease
DFA	detrended fluctuation analysis
DZ	dizygotic
dZ/dt	first derivative of thoracic impedance
dZ/dt_{max}	amplitude of dZ/dt
E	epinephrine
EB	exercise behavior
ECG	electrocardiogram
HR	heart rate
HRR	heart rate recovery
HRR_{180}	heart rate recovery 3 minutes after exercise
HRR_{60}	heart rate recovery 1 minute after exercise
HRV	heart rate variability
IBI	inter beat interval
ICC	intraclass correlation
ICG	impedance cardiography
LBNT	low body negative pressure test
LRS	left to right shunt
LV	left ventricle
LVET	left ventricular ejection time
MET	metabolic equivalent task
MIGB	metaiodobenzylguanidine
MRI	magnetic resonance imaging
MZ	monozygotic
NE	norepinephrine
NN-interval	normal-to-normal interbeat interval
NYHA	New York Heart Assosiation
PA	physical activity
PEP	pre ejection period
PNS	parasympathetic nervous system
PST	pulmonary stenosis
PWV	pulse wave velocity
Qp/Qs	pulmonary to systemic flow ratio
RB	time between R peak and B-point
RMSSD	root mean square of successive differences
RSA	respiratory sinus arrhythmia
RSA_{300}	respiratory sinus arrythmia in minute 2-5 after exercise
RSA60	respiratory sinus arrythmia in the first minute after exercise
RV	right ventricle
RX	time between R peak and X-point
SD	standard deviation
SNS	sympathetic nervous system
SV	stroke volume
TGA	transposition of the great arteries
TOF	tetralogy of Fallot
TTE	transthoracic echocardiography
VA	ventricular arrhythmia
VO_{2peak}	maximal oxygen uptake
VSD	ventricle septal defect
VTI	velocity time integral
VU-AMS	Vrije Universiteit ambulatory monitoring system
Z	thoracic impedance
Z_0	baseline impedance

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