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)

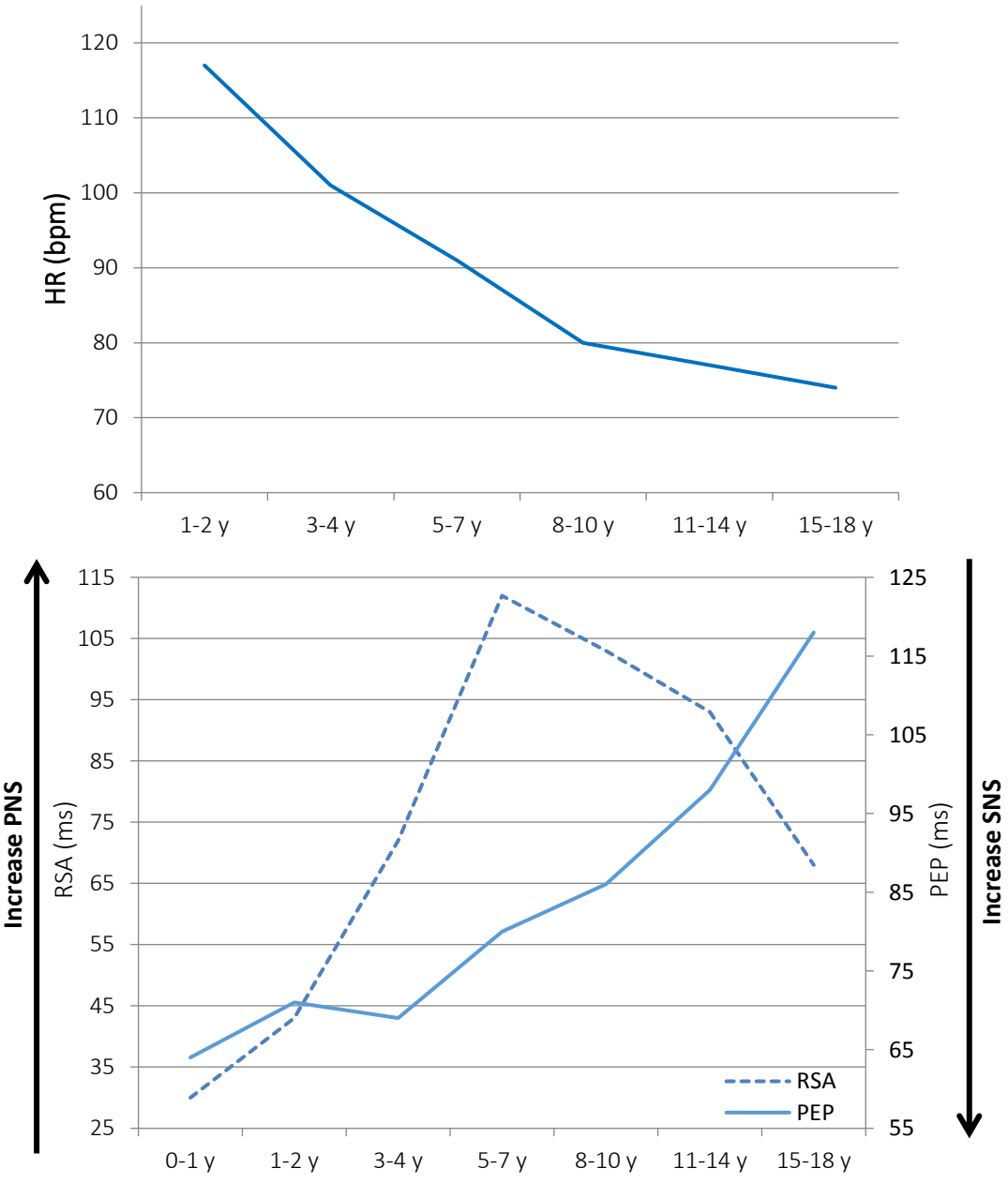

Figure 1 Maturation of HR, PEP and RSA (weighted average means from table 2)

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.