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

Harteveld, L.M.

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Chapter

3

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

Lisette M. Harteveld

Ineke Nederend

Arend D.J. ten Harkel

Nienke M. Schutte

Susanne R. de Rooij

Tanja G.M. Vrijkotte

Helena Oldenhof

Arne Popma

Lucres M.C. Jansen

Jill Suurland

Hanna Swaab

Eco J.C. de Geus

FemNAT-CD collaborators*

* Consortium of authors who collaborated in the FemNAT-CD study

Abstract

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

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

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

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

What is New?

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

What Are the Clinical Implications?

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

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Introduction

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

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

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

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

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

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

Methods

Study Populations

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

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

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

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

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

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

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

Age Grouping

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

Data Collection

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

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

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

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

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

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

ANS Adjustment

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

Statistical Analysis

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

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

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

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Results

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

Table 1. Characteristics Per Study Cohort

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

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

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

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Developmental Trajectories in Mean PEP and HRV

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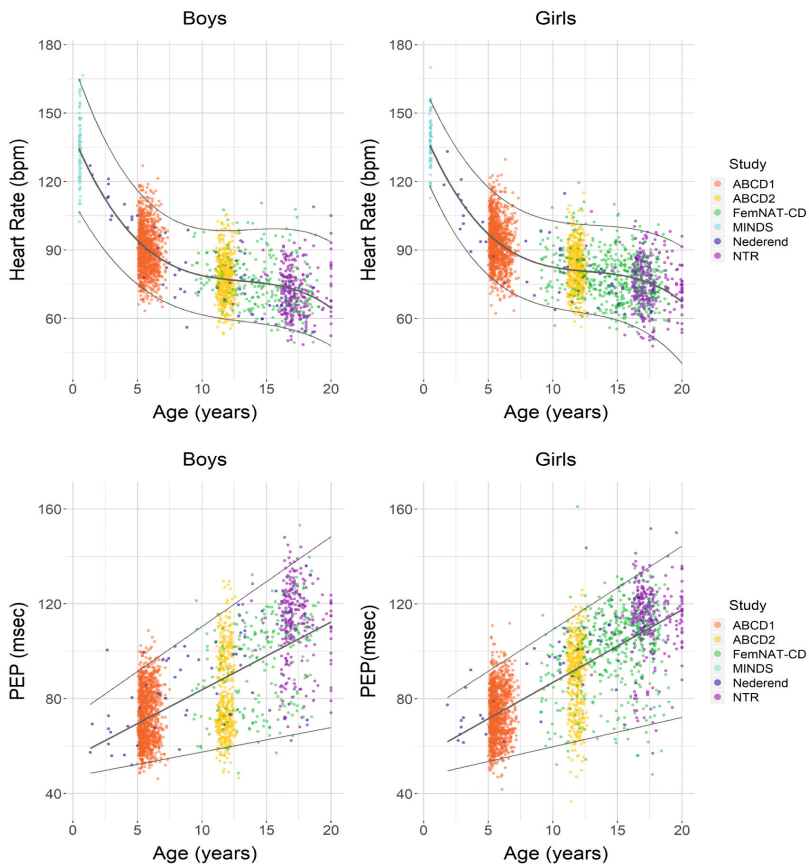
Figure 1. Scatterplots of age-related heart rate and pre-ejection period for boys and girls with cubic linear smoothing of median.

ABCD indicates the Amsterdam Born Children and their Development study; FemNAT-CD, the Neurobiology and Treatment of Adolescent Female Conduct Disorder study; MINDS, the Mother-Infant Neurodevelopment Study; and NTR, Netherlands Twin Register; and PEP, pre-ejection period.
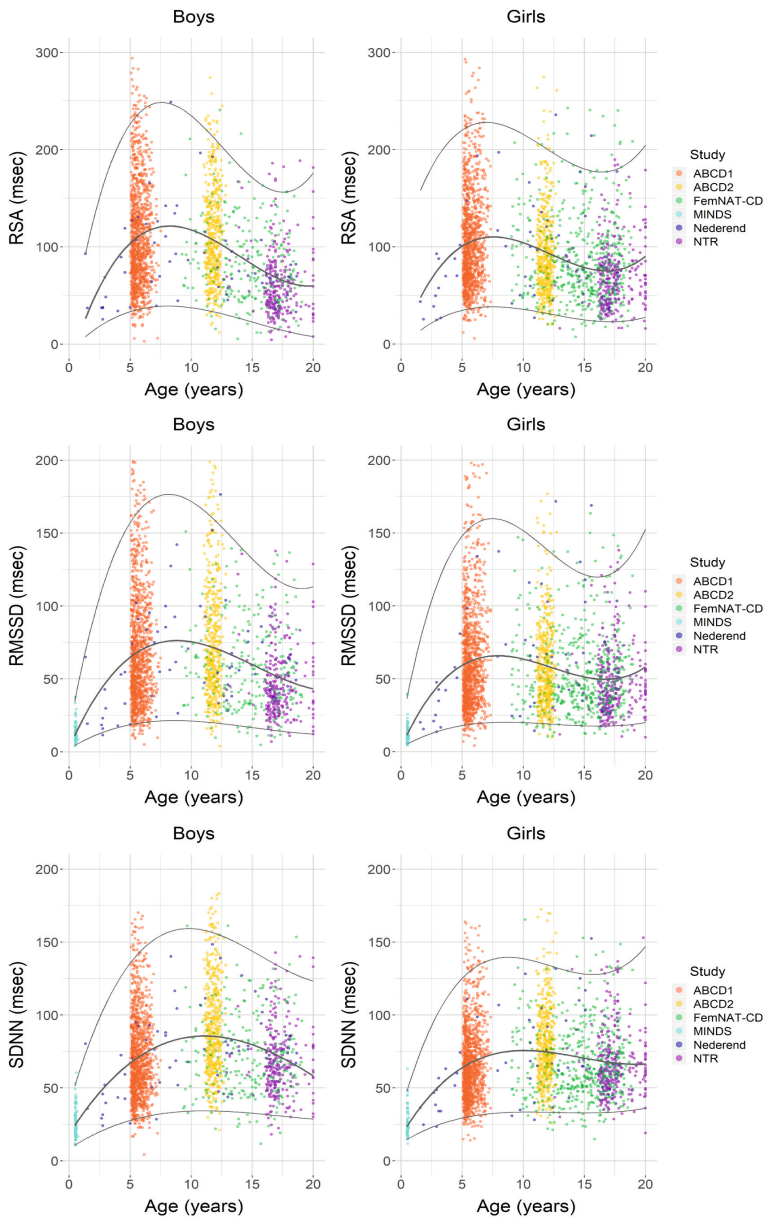
Figure 2. Scatterplots of age-related heart rate variability with Cubic smoothing of median.

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

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

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

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

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

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

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

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

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

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

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

Adjustment for Heart Rate

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

Developmental Changes in the Variance of SNS and PNS Parameters

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

Temporal Stability from Age 6 to Age 11.5 Years

In the subset of 784 children from the ABCD study measured twice across a 5.5-year period, temporal stability computed as the Pearson correlation coefficient for boys and girls is shown in Table 5. Moderate temporal stability was found for PEP and good stability for HR, RSA, RMSSD and SDNN (Pearson 0.26<r<0.58; ICC 0.25<r<0.57). Log transformation of HRV variables barely changed these correlations (Pearson and ICC 0.42<r<0.53) despite significant longitudinal changes in mean values with age, all of which were compatible with the cross-sectional comparisons of the relevant age groups. Furthermore, adjustment for HP, which adds temporal instability in the HP itself, barely influenced the stability of the ANS parameters (Pearson, 0.28 <r< 0.53; ICC, 0.27<r<0.54).
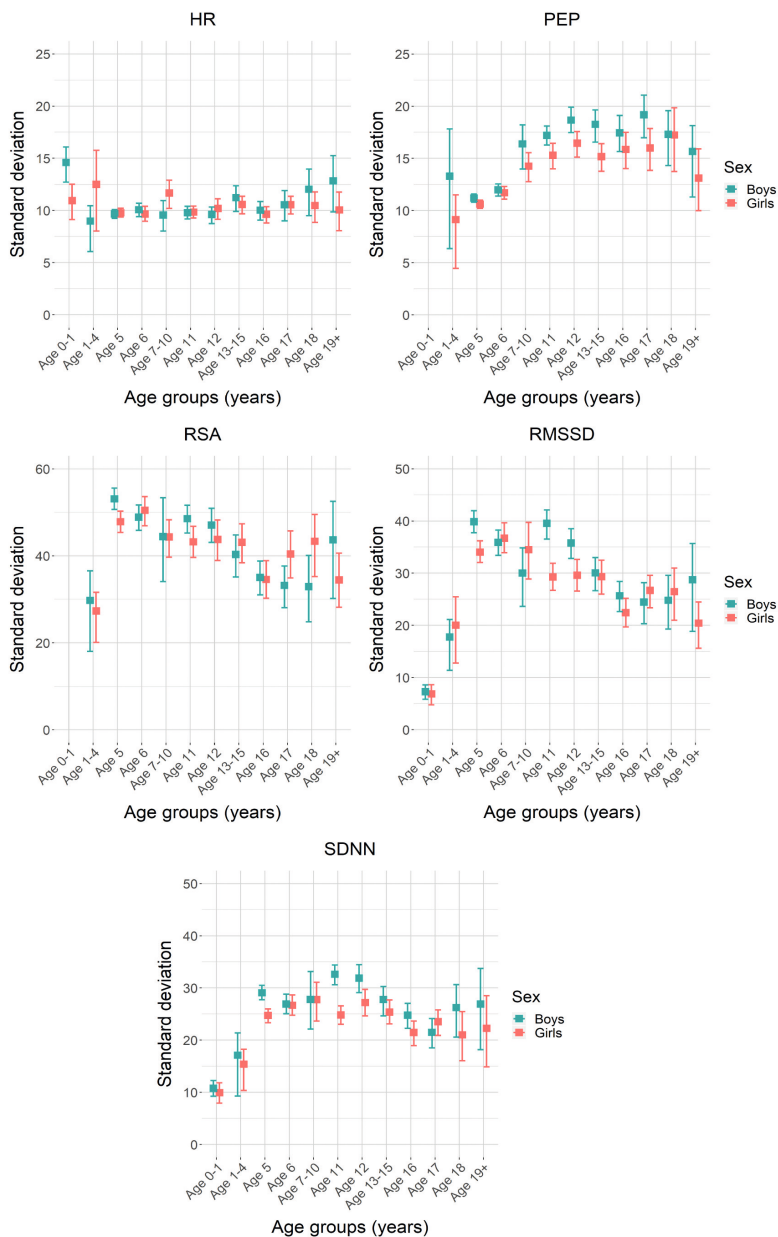
Figure 3. Histograms of SDs per age group for heart rate, pre-ejection period, respiratory sinus arrhythmia, root mean square of successive differences between normal sinus beats and SD of the inter-beat interval of normal sinus beats;

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

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

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

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

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

† P value <0.001.

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Discussion

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

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

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

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

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

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

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

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

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

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

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

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

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

Conclusion

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

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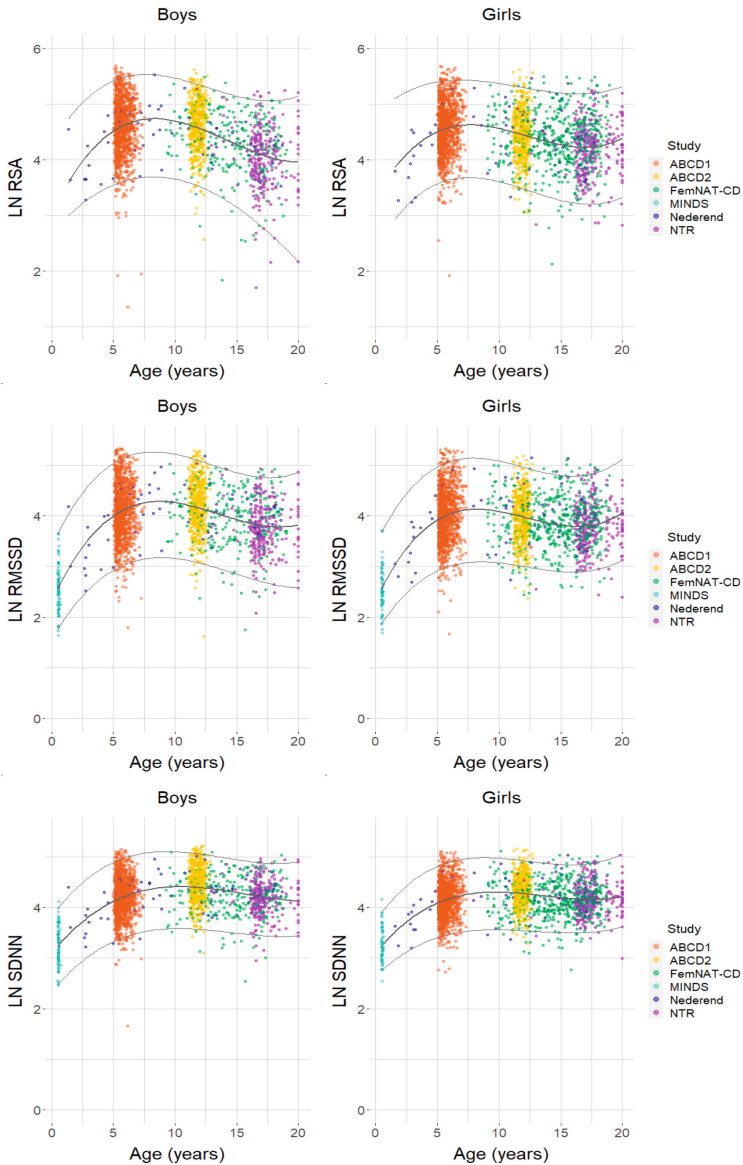
Supplemental Material

Figure S1. Scatterplot of age-related natural logarithm of RSA, RMSSD and SDNN with Cubic smoothing of median.
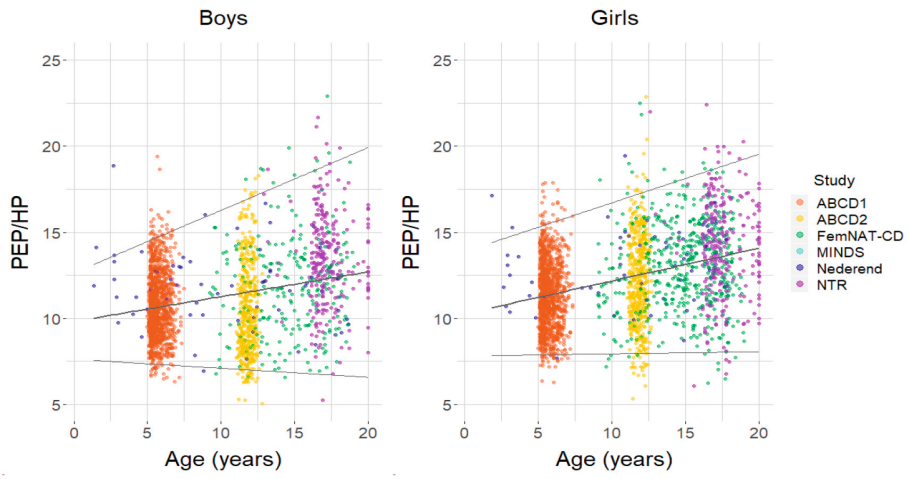
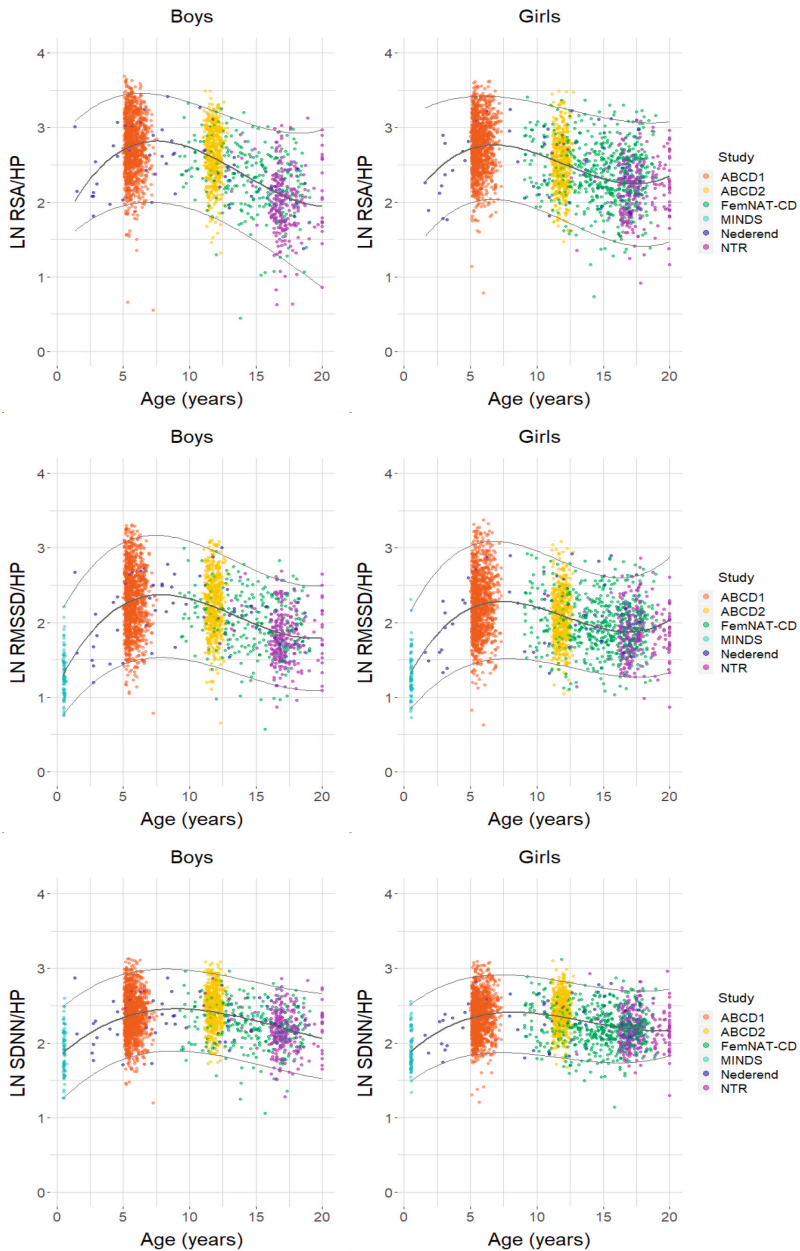**Figure S2.** Scatterplot of age related for heart period (HP) corrected PEP with Quadratic smoothing of median.

Figure S3. Scatterplot of age related for heart period (HP) corrected natural logarithm of RSA, RMSSD and SDNN with Cubic smoothing of median.
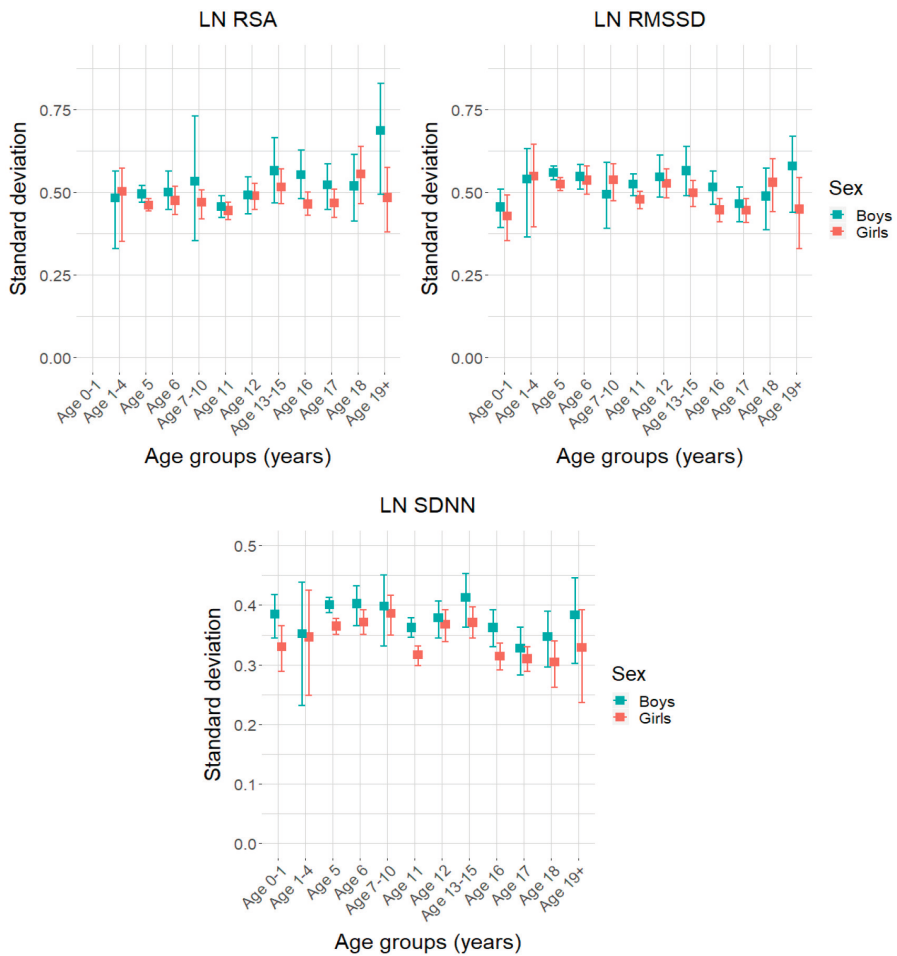
Figure S4. Histograms of standard deviations per age group for natural logarithm of RSA, RMSSD and SDNN.

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