Endothelial Function in Children and Adolescents Is Mainly Influenced by Age, Sex and Physical Activity

— An Analysis of Reactive Hyperemic Peripheral Artery Tonometry —

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Background: As adolescents rarely experience cardiovascular events, surrogate markers of atherosclerosis are useful to justify and monitor effects of primary prevention and therapy of risk factors. Endothelial function assessed by reactive hyperemic peripheral arterial tonometry (RH-PAT) resulting in a reactive hyperemic index (RHI) is a noninvasive method with limited data for use in children and adolescents.

Methods and Results: We performed a total of 931 RHI measurements in 445 high-school students, aged 10–17 years, over a time period of 5 years. Students were randomized by class to 60 min physical exercise (PE) at school daily (intervention group), or 2 units of 45-min PE weekly (control group). To characterize the factors influencing the RHI, anthropometry, cardiopulmonary exercise testing, blood cholesterol and quality of life were assessed and used to build mixed linear models. Main influential factors were age, with an increase of RHI from 1.53 ± 0.42 in the youngest to 1.96 ± 0.59 in the oldest students, sex, with higher values in girls, and physical activity. This increase adjusted by age and sex was estimated as 0.11 [0.08, 0.14] per year. RHI was higher in the intervention group by 0.09 [–0.05, 0.23] in comparison with the control group.

Conclusions: If RH-PAT is used in research or as a clinical tool in adolescents, the shown age- and sex-dependence of RHI have to be taken in account.

Key Words: Adolescents; Endothelial function; Physical exercise; Primary prevention; Reactive hyperemic index

Atherosclerosis starts early in life if the currently known cardiovascular risk factors are present. Postmortem studies have demonstrated the presence of atherosclerotic lesions from as early as the first decade of life.¹ As children rarely experience cardiovascular events, surrogate markers of cardiovascular disease are needed to provide additional risk stratification, and to justify and monitor effects of prevention and therapy of risk factors.^{2,3} A standard evaluation of endothelial function uses brachial artery reactivity measurements such as flow-mediated dilatation (FMD);^{4,5} however, this technique requires much experience and is operator dependent.⁶ Assessment of endothelial (dys-)function using reactive hyperemic peripheral arterial tonometry (RH-PAT) has shown excellent correlation with measures of coronary and peripheral endothelial dysfunction in adults.^{7,8} It provides clinicians with a reliable and reproducible index (the reactive hyper-

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emic index (RHI)) of endothelial function in a 15-min test.⁹

In adults, an RHI <1.35 indicates impaired endothelial function.⁷ Data providing normal values for RHI in children and adolescents are limited. However, the method is used frequently for study purposes, usually to compare endothelial function in children with cardiovascular risk factors against healthy subjects.^{2,3} Additionally, interventional studies investigating the effect of physical exercise (PE) on RHI in adolescents are lacking.

Thus, using RH-PAT in a large cohort of healthy school children, we aimed to explore the factors influencing the RHI under particular consideration of PE at school.

Received October 2, 2016; revised manuscript received December 27, 2016; accepted January 5, 2017; released online February 11, 2017 Time for primary review: 18 days

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Methods

Study Design

Children and adolescents were recruited within the “Leipzig School Project” (clinical trial registration: <http://www.clinicaltrials.gov>, Identifier: NCT00176371). The study protocol and procedures were approved by the Ethics Review Board at the University of Leipzig. The study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. All participants were younger than 18 years at the beginning of the study, so they gave verbal assent and parents gave written informed consent to participate in the study protocol.

A detailed study design was published previously.^{10,11} In brief, the “Leipzig School Project” was a class-wise cluster randomized intervention study designed to investigate the influence of a 60-min PE session daily at school on cardiovascular risk factors in the intervention group in comparison with the otherwise standard of 2 PE units of 45 min weekly in the control group. At participating schools, 5th and 6th graders were randomized by class to the intervention or control group. A third study group was a pure observational group in a sports school and consisted of sports students (high-level group) who frequently participated in competitive sporting events, thus representing a maximum of physical fitness attainable under reasonable conditions in school-age children. After baseline assessment, we saw all groups yearly for control measurements.

This subanalysis focusses on the measurement of endothelial function using RH-PAT for assessment of RHI.

Study Subjects

In 7 schools in the area of Leipzig and Chemnitz, Saxony, Germany, 18 classes with 491 students in grades 5 or 6 were invited to participate in this open-ended, controlled, randomized, school-based exercise program. The program started in 2005, inclusion time was until 2008, and last follow-ups were completed in 2013.

To answer our questions concerning RHI in children, a

set of all pupils having at least 1 RHI measurement ($n=445$) was used. For logistic reasons, it was not possible to perform RH-PAT in all students at each yearly visit, but usually biannually, so the yearly analyses contain different students. The study flowchart is shown in **Figure 1** and the baseline characteristics of the participating students are given in **Table 1**.

Measurements

Procedures were identical in the intervention, control and high-level groups and consisted of RH-PAT, anthropometric data, body composition, questionnaire, blood sample, and cardiopulmonary treadmill exercise test (CPX).

RH-PAT

RH-PAT was performed using the EndoPAT® device (Itamar Medical Ltd., Caesarea, Israel). Measurements were performed in thermo-neutral quiet surroundings with students positioned supine, following an overnight fast. Fingertip probes were placed on both index fingers and pulse wave amplitudes (PWA) were recorded. After 5 min of baseline measurement arterial flow in the non-dominant arm was occluded using a blood pressure (BP) cuff inflated to 30 mmHg above systolic pressure. After 5 min of occlusion, the cuff was rapidly deflated to allow for reactive hyperemia. PWA were recorded for a further 5 min after the cuff was deflated. An integrated software program calculated the RHI as the ratio of the average PWA measured over 60 s starting 1 min after cuff deflation to the average PWA measured at baseline. This ratio is normalized to the concurrent signal from the contralateral finger to correct for changes in systemic vascular tone. Lower RHIs are reflective of greater endothelial dysfunction and risk for atherosclerosis. Details of RH-PAT have been published elsewhere.^{12,13}

As mentioned before, it was not possible to perform RHI measurements in all students at each yearly visit for the following reasons: follow-up was performed all day, but RHI measurements only in the morning to ensure

Table 1. Characteristics of the Schoolchildren Aged 10–17 Years at Each Time Point of RHI Measurement

Characteristic	Baseline	1st follow-up	2nd follow-up	3rd follow-up	4th follow-up	5th follow-up
n	154	271	146	187	112	61
I/C/HL group, n (%)	84 (55)/56 (36)/14 (9)	152 (56)/90 (33)/29 (11)	61 (42)/57 (39)/28 (19)	107 (57)/70 (37)/10 (5)	52 (46)/41 (36)/19 (17)	33 (54)/27 (44)/1 (2)
Female in I/C/HL, n (%)	37 (44)/20 (36)/6 (43)	75 (49)/51 (57)/12 (41)	28 (46)/21 (37)/7 (25)	46 (43)/39 (56)/5 (50)	26 (50)/18 (44)/4 (21)	15 (45)/17 (63)/0 (0)
RHI	1.53±0.42	1.49±0.45	1.58±0.50	1.71±0.49	1.97±0.66	1.96±0.59
RHI <1.35, n (%)	72 (46.8)	153 (56.6)	61 (41.8)	53 (28.3)	17 (15.2)	9 (14.8)
Age (years)	11.66±0.93	12.44±0.67	13.17±0.77	14.38±0.68	15.23±0.76	16.47±0.46
Height (cm)	151.24±8.99	156.34±8.11	161.82±8.98	166.89±8.72	170.61±8.73	172.59±10.62
Body weight (kg)	43.79±10.18	45.93±9.62	51.20±11.13	57.05±9.68	61.65±11.99	63.83±9.91
BMI (kg/m ²)	18.93±3.02	18.66±2.9	19.38±2.95	20.42±2.71	21.04±3.07	21.37±2.26
BMI percentile	55.76±28.54	47.44±28.93	49.73±28.39	53.91±25.73	54.26±28.65	53.66±24.69
BMI >90th percentile, n (%)	45 (10.5)	41 (9.6)	40 (10.3)	40 (11.4)	24 (9.4)	7 (9.0)
BMI-SDS	0.20±0.94	−0.10±1.02	0.01±0.95	0.13±0.86	0.15±0.96	0.12±0.77
Heart rate at rest (1/min)	79±10.82	96±14	83±19	72±10	72±12	70±16
SBP (mmHg)	99±10	102±11	108±12	99±9	107±11	97±8
DBP (mmHg)	63±7	70±8	69±10	62±8	62±8	60±3
Fat-free mass (% body weight)	79.28±6.99	80.55±7.03	81.20±6.87	78.99±6.99	78.92±8.32	78.58±7.91
Muscle mass (% body weight)	48.21±5.45	48.96±5.78	50.46±5.82	50.18±5.94	50.96±6.82	51.56±6.52
Heart rate at maximum (1/min)	204±10	204±10	203±10	199±7	201±8	197±6
VO _{2peak} (mL/min/kg)	50.86±9.68	48.21±8.72	53.69±8.84	48.78±8.58	53.86±10.02	47.31±9.28
Maximum power (W)	132.78±35.71	155.89±36.58	181.08±46.72	187.61±51.84	209.19±63.35	172.50±53.15
VE peak (L/min)	78.25±17.93	80.28±17.16	96.94±25.37	95.10±22.86	118.03±31.08	105.35±26.96
Time to max. effort (s)	1,010.96±91.90	1,083.97±95.84	1,101.88±113.14	1,077.66±101.87	1,099.31±109.62	1,097.24±108.98
Motor quotient	95.65±16.94	112.77±13.85	108.55±16.61	116.62±11.50	123.00±7.21	121.00±5.66
Cholesterol (mmol/L)	4.21±0.69	4.17±0.64	4.14±0.67	3.88±0.66	3.93±0.77	3.79±0.80
HDL (mmol/L)	1.41±0.33	1.44±0.33	1.48±0.33	1.41±0.32	1.36±0.32	1.42±0.29
LDL (mmol/L)	2.21±0.57	2.18±0.56	2.23±0.59	2.19±0.56	2.08±0.58	2.00±0.59
TG (mmol/L)	1.09±0.57	1.08±0.66	1.07±0.55	0.98±0.50	0.99±0.52	1.00±0.54
Leisure activity index	3.40±0.67	3.73±0.57	3.58±0.57	3.59±0.58	3.58±0.66	3.67±0.54
Sports index	3.18±0.59	3.35±0.61	3.31±0.71	3.19±0.69	3.33±0.71	3.25±0.66
Life quality index	3.49±0.50	3.96±0.48	3.85±0.46	3.78±0.47	3.86±0.51	3.80±0.48

This substudy focused on RHI measurements. The yearly measurements contain different students, as RHI measurements were not performed yearly for each student. Data are mean±standard deviation unless otherwise indicated. BMI, body mass index; C, Control group; DBP, diastolic blood pressure; HDL, high-density lipoprotein; HL, High-level group; I, Intervention group; LDL, low-density lipoprotein; MET, metabolic equivalent of task; RHI, reactive hyperemic index; SBP, systolic blood pressure; TG, triglycerides; VE, expiratory volume; VO_{2peak}, peak oxygen uptake at spirometry (cardiopulmonary exercise).

overnight fast; in the first 2 study years the technique was not available at our study center.

Anthropometry, Body Composition, Questionnaire and Blood Samples

Height, body weight and BP were measured with standard medical devices. The body mass index (BMI) was calculated as the quotient of weight in kg divided by the square of height in meters. Age- and sex-adjusted BMI percentiles and standard deviation score (BMI-SDS) were determined by using age- and sex-adjusted curves for children and adolescents.¹⁴

Students were asked to attend the RH-PAT and blood tests after fasting. We assessed blood count, glucose, low-density lipoprotein-cholesterol, high-density lipoprotein-cholesterol, and triglycerides. Body composition [fat-free mass (FFM) and muscle mass (MM)] was assessed by

bioimpedance (Akern, Italy).

All participants had to complete a questionnaire containing 13 items that evaluated quality of life, and physical activity in general and in leisure time. From these items 2 indices, activity index in general, and leisure index for leisure physical activity, were calculated. To assess family history all parents were asked at baseline to complete a questionnaire on cardiovascular risk factors, including obesity (height and weight were requested), diabetes, arterial hypertension, smoking, hyperlipidemia, and coronary artery disease. We investigated whether the known cardiovascular family risk factors were associated with RHI in children: (1) if one or both parents had at least one risk factor; and (2) if the total number of parental risk factors in mothers, fathers or both parents showed an association with RHI in children.

Figure 2. Sex (A) and age (B) dependency of reactive hyperemia index (RHI) in children and adolescents.

CPX

To assess fitness, participants underwent a graded treadmill exercise test (Woodway USA, Inc., WI, USA) with spirometry until exhaustion, according to a modified Bruce protocol for children, starting at 1.7 mph and 0 degrees. Oxygen uptake ($\dot{V}O_2$) was recorded using a portable spirometry system (K4b2, Cosmed Srl., Italy). Workload was increased every 3 min. At the end of each level the subjects had to indicate their personal perception of exhaustion according to the Borg rating on a scale from 1 to 10 (1=no exhaustion, 10=maximal exhaustion), and cardiopulmonary indicators were recorded [BP, heart rate (HR), $\dot{V}O_2$, expiratory volume ($\dot{V}E$), respiratory exchange ratio (RER, ratio of carbon dioxide production to $\dot{V}O_2$)]. Requirements to ensure that subjects reached their maximum effort with this protocol included at least 2 of the following: maximal HR >200 beats/min, RER >1.1, a plateau in oxygen consumption, and subjective Borg rating

Figure 3. Estimated percentiles of reactive hyperemia index (RHI) in (A) girls and (B) boys.

of 10. $\dot{V}O_2$ was calculated in relation to body weight and is given in mL/min/kg.

All students and parents were given the test results of anthropometry and CPX with a rating on health status and performance.

Endpoints

The primary endpoint in this substudy was the RHI. Variables analyzed as secondary endpoints were parameters of CPX [$\dot{V}O_{2\text{peak}}$, HR at rest and at maximum, maximum running duration (Tmax) and peak power], anthropometric values and body composition.

Statistical Analysis

Data preparation and descriptive statistics were performed by IBM SPSS Statistics version 23, and R (packages moments, gamlss, ggplot2, lme4, Hmisc) for advanced statistics.

The study cohorts were characterized by standard descriptive statistics. Because missing status of RHI values does not depend on the RHI values themselves, the assumption “missing completely at random” is plausible, which means that complete case analyses give unbiased results.

We compared the RHI of normal and overweight/obese children per year by paired t-test. Most analyses were

	RHI		Age		$\dot{V}O_{2peak}$	
	Boys	Girls	Boys	Girls	Boys	Girls
Baseline						
Mean	1.48	1.59	11.6	11.6	46.9	41.9
95% CI	1.41, 1.56	1.47, 1.71	11.5, 11.7	11.5, 11.8	45.5, 48.3	40.7, 43.1
1st follow-up						
Mean	1.43	1.56	12.3	12.4	51.6	44.6
95% CI	1.35, 1.50	1.48, 1.63	12.3, 12.4	12.3, 12.4	50.4, 52.7	43.4, 45.8
2nd follow-up						
Mean	1.51	1.69	13.4	13.4	53.4	43.9
95% CI	1.42, 1.61	1.54, 1.84	13.3, 13.4	13.3, 13.6	52.2, 54.6	42.7, 45.2
3rd follow-up						
Mean	1.66	1.77	14.3	14.4	55.1	43.8
95% CI	1.57, 1.75	1.66, 1.88	14.2, 14.4	14.3, 14.5	53.9, 56.4	42.7, 45.0
4th follow-up						
Mean	1.88	2.09	15.3	15.2	55.7	42.3
95% CI	1.71, 2.04	1.90, 2.27	15.1, 15.4	15.0, 15.4	54.2, 57.2	40.9, 43.7
5th follow-up						
Mean	1.89	2.02	16.5	16.3	58.2	41.5
95% CI	1.71, 2.06	1.79, 2.25	16.3, 16.7	16.1, 16.5	55.9, 60.4	39.9, 43.1

CI, confidence interval. Other abbreviations as in Table 1.

performed applying linear mixed-effects models (LMM).¹⁵ This tool is able to adjust for age and sex, and to model correlation structures of repeated measurements and clustered data. Additionally, LMM are able to deal with missing values. We considered models with group and sex as fixed factors, age, BMI and $\dot{V}O_{2peak}$ as continuous covariates, and subject and class as factors with random intercepts. We checked for possible interactions, but removed the interaction terms if no significant improvement was seen. Finally, we applied LMM to look for variables possibly multiply associated with the RHI. To do so, we started with a full model (24 variables) and reduced step by step to get a sparse model with significant variables. Fixed effects were estimated, and 95% confidence intervals were calculated by profile likelihood.

To create percentile plots, a set of independent values had to be created. Thus, exactly 1 RHI per student was randomly chosen. Percentiles were calculated by the R package gamlss and percentile curves were plotted.¹⁶ Positive skewness and kurtosis >4 restricted the number of possible functions. The aim was to fit relatively smooth curves with low total deviation, good agreement between observed and fitted percentiles, and without artefacts.

Significance level for two-sided tests was generally 5%.

Results

Study Population

Baseline and follow-up parameters are given in Table 1. The analyses containing all 931 RHI were comparable with the calculations when only the longitudinal data of the 130 students with 3 RHI measurements over the study time were used. Therefore, the following results include all 931 measured RHI.

RHI, Age and Sex

RHI increased significantly with increasing age (Table 1,

Figures 2,3). The mean RHI of girls increased from 1.59 [1.47, 1.71] in the youngest cohort at baseline to 1.69 [1.54, 1.84] after 2, and 2.09 [1.90, 2.27] after 4 years; the average RHI of boys increased from 1.48 [1.41, 1.56] to 1.51 [1.42, 1.61] and 1.88 [1.71, 2.04] after 2 and 4 years, respectively. Most students had higher RHI in the follow-up in comparison with baseline. This increase adjusted by age and sex was estimated as 0.11 [0.084, 0.14] per year and was significant. The RHI for boys and girls by year are given in Table 2.

At all time-points the RHI was higher in girls than in boys (Figures 2,3). The mean difference was 0.11 [−0.002, 0.23], which became significant after adjustment for BMI percentile or intervention group (Table 3B,C).

Based on our data we calculated reference values for boys and girls (Table S1, Figure 3).

RHI and Intervention or Sports Performance

Regular physical activity had an effect on RHI. Students in the intervention group had RHI that were higher than those of the control group by 0.09 [−0.052, 0.23], adjusted for age and sex. The annual difference between the high-level and control groups was 0.14 [−0.05, 0.33] (Table 3).

On the other hand, a dependency of $\dot{V}O_{2peak}$ after adjustment for age and sex was hardly seen at all (0.0023 per 10 mL/min/kg [−0.022, 0.068]).

RHI and BMI

We observed a weak association of BMI percentiles and RHI (Table 3): an increase of BMI percentile by 10 was associated with an increase in RHI by 0.012 [0.000, 0.024]. However, a difference of mean RHI between children with normal BMI and those who were overweight/obese could not be proven.

Variables Possibly Associated With RHI

We looked for a possible association between 24 variables

Table 3. Linear Mixed Models for Association With RHI

	Coefficient	95% CI
A) Adjustment for sex and age		
Constant	1.33	1.23, 1.43
Age (years)	0.111	0.084, 0.137
Girl	0.114	−0.002, 0.230
B) Possible influence of intervention		
Constant	1.26	1.14, 1.39
Age (years)	0.111	0.090, 0.131
Girl	0.117	0.046, 0.189
Intervention		
I group	0.090	−0.052, 0.233
HL group	0.141	−0.050, 0.332
C) Dependence from BMI percentile		
Constant	1.27	1.17, 1.38
Age (years)	0.108	0.087, 0.129
Girl	0.112	0.042, 0.183
BMI percentile (per 10)	0.012	0.000, 0.024
D) Possible association of RHI with $\dot{V}O_{2peak}$		
Constant	1.21	0.95, 1.47
Age (years)	0.109	0.088, 0.130
Girl	0.142	0.061, 0.225
$\dot{V}O_{2peak}$ (per 10 ml/min/kg)	0.0023	−0.022, 0.068
E) With RHI-associated variables (baseline and follow-up)		
Constant	1.84	1.59, 2.11
Age	0.103	0.082, 0.125
Girl	0.124	0.051, 0.196
BMI percentile (per 10)	0.017	0.005, 0.030
DBP at rest (per 10)	−0.090	−0.126, −0.055

Intervention group had 1 h daily of physical exercise in school in contrast to the control group with 2×45-min units per week. High-level group contains athletes with regular intensive training sessions up to 20 h weekly. Abbreviations as in Tables 1,2.

and RHI. Potential candidates were, for example, age, sex, year of measurement, BMI, FFM and MM, family history, HR, systolic and diastolic BP at rest, sports and leisure time index, as well as $\dot{V}O_{2peak}$, maximal running time, and maximal power. We found a model with age, sex, BMI percentile and diastolic BP at rest. The correlation of the first 3 factors has already described. Additionally, an increase in diastolic BP by 10 mmHg was associated with a decrease in RHI by -0.09 [-0.13 , -0.05]. Family history was assessed at baseline by self-reported questionnaire, but parental information was only available from approximately 55% of the families (most of them did not fill out the questionnaires or the data were incomplete). With the limited and possibly biased data, associations between cardiovascular risk factors in the parents and the RHI in adolescents could not be proved. Additionally, the calculated “effect” of parental risk factors was small compared with the effects of age and sex. The highest association was found if risk factors were present in both parents, with reduction in RHI by -0.073 [-0.191 ; 0.045]. Maternal risk factors, especially smoking, had more influence than paternal ones on reducing the RHI, but also without reaching significance, and with a wide confidence interval including 0.

Discussion

The purpose of this study was to assess the factors

influencing the RHI in healthy children and adolescents with a particular focus on the effect of PE at school.

First, a fundamental comment: there was a very wide intra- and interindividual variation in RHI (**Figure 2B**). We found some associations, but are far from knowing the causal relationships.

Interestingly, we found an interaction between RHI, age and sex. Additionally, we measured a nonsignificantly higher RHI in our intervention group with daily PE lessons and even higher in the high-level group. In contrast, RHI was not directly dependent on fitness measured by $\dot{V}O_{2peak}$. Other influential factors were a weak association of BMI percentile and RHI, and an inverse association between diastolic BP and RHI.

Use of RH-PAT in Children and Adolescents

We used the EndoPAT® device to assess endothelial function, which focuses on small resistance vessels to evaluate endothelium-dependent flow-mediated dilation. This dynamic testing method enables evaluation of the endothelial cell response to mechanical stress to test for the presence of endothelial dysfunction as a systemic disorder.

As with other studies, we agree that RH-PAT is a promising noninvasive technique to evaluate endothelial function and was well tolerated by all the children. None of the RH-PAT-tests had to be terminated early, and all measurements were analyzable. Further advantage of the measurement of

RHI using EndoPat® is that it is simple to use and needs less training than FMD.¹³

Factors Associated With RHI: Clinical Aspects

Endothelial dysfunction is present in children with risk factors for atherosclerosis, such as hypercholesterolemia, obesity, smoking or diabetes, before anatomical evidence of plaque formation in the arteries studied.¹⁷ As endothelial dysfunction is believed to be a pan-systemic disease associated with numerous disease states and shown to respond well to treatment even in childhood, lifestyle modification may augment RHI. The higher RHI values that we found in the intervention group and high-level group may also account for this. As we have shown a positive effect of daily PE lessons on $\dot{V}O_{2\text{peak}}$ in the first 2 years of our study,^{11,18} but less after 4 years of intervention,¹⁰ it could be argued that the positive effect of PE on endothelial function lasts longer than on cardiovascular performance. This underlines the necessity of lifelong regular physical activity to prevent cardiovascular diseases.

Another important and interesting finding of our study was the sex difference for the RHI in all age groups. Associations between pubertal development and RHI, and between age, sex, and RHI in children and adolescents have been reported in smaller cohorts.^{19–21} A possible effect of sex hormones could explain this difference even in our youngest study group because of the earlier puberty of girls, as estrogens might protect the different vascular wall layers against early changes. Additionally, it has to be kept in mind that this cohort of adolescents was mainly healthy. Thus, the traditional cardiovascular risk factors will have minimal effect on vascular features.

As with other studies, we observed high variability in the RHI values.²² Haller et al described a wide range of RHI in their cohort of children with type 1 diabetes (age 14.6 ± 2.7 years): RHI was 1.63 ± 0.5 when compared with children without diabetes (mean RHI 1.95 ± 0.3 , $P < 0.01$).³ Our values in the 14-year-olds were higher than in the diabetes group but lower than in the healthy group. As other studies have found comparable data (e.g., male diabetic subjects vs. controls (1.60 ± 0.32 vs. 1.92 ± 2.5),² we point out the necessity of considering the age and sex of the test persons. Our age range was small (< 0.9 years) because of class-wise testing. High standard deviations of age in other studies may have a strong effect on RHI. Therefore a lot of unobserved associations (e.g., social, genetic, or adolescent) have to be taken in account as additional influences on the results. In terms of children and adolescents, family history may also be important for the individual's RHI. However, associations between cardiovascular risk factors in the parents and the RHI in children could not be proven in our study, in contrast to the “Cardiovascular risk in Young Finns study”, where parental smoking status in childhood had an influence on FMD in young adults.²³ Because those subjects were much older than our cohort (24–39 years), a direct comparison cannot be made. An investigation of our population 10–20 years later could answer the question whether the small, but not significant, “effects” of parental risk factors in adolescents lead to more endothelial dysfunction in young adulthood. Furthermore, the low rate of response to the questionnaires may have biased our data.

Our findings may also be relevant for the planning of further studies, because the large variability implies the need for larger study sizes to detect effects when using RH-PAT for evaluation of endothelial function.²²

RHI and Other Noninvasive Parameters of Endothelial Function: Are They Comparable?

Noninvasive measurements for risk stratification include, for example, FMD, intima-media thickness (IMT), carotid artery compliance (CAC), and RH-PAT. In adults, RH-PAT has been shown to correlate closely with FMD and IMT, as well as with direct measures of coronary arterial function.^{7,13} From adult studies we know that endothelial function deteriorates with increasing age in otherwise healthy subjects.²⁴ This raises the question of why do we see an increase in RHI during adolescence, which seems paradoxical, despite the presence of atherosclerotic lesions in the first decade of life.¹

The data on the relationships between age, sex, and indices of vascular damage are limited and partly controversial in adolescence as in adulthood. Age-dependency with an increase of RHI in adolescence has been found previously.¹⁹ Recently, Hopkins et al²⁵ determined the relationship between FMD, age, and sex in 978 healthy children and adolescents aged 6–18 years. They observed that FMD depended on brachial artery diameter. However, they found an overall sex and age difference in FMD, mainly driven by changes in FMD from 16 to 18 years between males and females, with the decrease in FMD apparent in postpubertal males, but not in females. Comparable to our RHI data, they reported high variability in FMD. Furthermore, they reported unsteady FMD values throughout the years. It is difficult to explain why FMD at the age of 8 years was higher than at 6 or 10 years, and at 10 years higher than at 7, 14 and 15 years.²⁵ However, those authors concluded that age- and sex-specific FMD data are not needed in pediatric populations. In contrast, our data show that age and sex have to be taken into account when analyzing RH-PAT findings in children and adolescents. An important strength of our study is that we provide longitudinal study data not only for RHI in 10- to 17-year-old subjects, but also for 130 students with 3 RHI measurements at different ages, showing comparable age- and sex-related differences of RHI.

In the “Cardiovascular risk in Young Finns” study follow-up,²⁶ measurements of FMD, IMT and CAC were performed in healthy young adults aged 24–39 years. An age-dependency of IMT and CAC was found, but no interaction between age and FMD. Additionally, the sex differences in these parameters were mostly explained by differences in risk factors and vessel size; however, FMD was initially higher in women, but after adjustment for baseline brachial artery diameter, men had significantly higher values.²⁶ We reiterate that our study population was much younger, which also means that fewer cardiovascular risk factors were present in our subjects. Moreover, depending on the study subjects and study size, other studies dealing with endothelial function show variable results with and without sex and age differences in carotid or femoral IMT.^{27–30} Another strength of our analysis is that, in addition to sex and age, we included many other potential covariates in the LMM, though age and sex remained the most relevant variables with regard to RHI.

Even if RH-PAT is technically easier to perform than FMD, influences on measurement such as stress in the morning, anxiety, clandestine breakfast, passive smoking at home and other factors may have more subtle influences in children and adolescents than in adults, especially on small vessel reactivity.

Molecular Mechanisms

For many years, paracrine control of vessels' function has been attributed exclusively to the endothelium.³¹ Endothelium-dependent dilation is influenced by various molecules. Nitric oxide (NO) is the major endothelium-derived relaxing factor; others are prostacyclin and endothelial-derived hyperpolarizing factor (EDHF).³² Although NO affects the macro- and microcirculation, the importance of EDHF increases as the vessel's diameter decreases.³³ Therefore, EDHF plays a prominent role in the resistance vessels of the microcirculation.³⁴ Because of EDHF, microvascular endothelial cells hyperpolarize upon agonist stimulation more effectively than do macrovascular cells.³⁵ In addition to the vessel's size and endothelial function, the vasoactive role of adventitial cells and perivascular adipose tissue is now a topic of research.³¹ Different thickness of vessel layers in resistance and conduit vessels, interactions of various concentrations of vasoconstrictive and vasodilative molecules, and interactions between the endothelium, perivascular tissue and smooth muscle cells, may account for some failure of RHI- and FMD-correlation, as they may reflect different aspects of vascular damage. Although different methods are used to noninvasively assess endothelial function, they may not be interchangeable.³⁶ RHI and FMD do not measure the same vascular responses to shear stress induced by 5-min occlusion of the brachial artery. RH-PAT reflects the vasodilator function in the microvasculature of the fingers, whereas FMD considers the vasodilator function in large conduit vessels. Moreover, for both techniques the interaction between vessel wall-derived vasodilator and vasoconstrictor factors has to be discussed. We also considered whether or not high-flow-mediated constriction prior to vasodilation³⁷ may be found more in the finger tips than in the brachial artery, and thus may also have an influence on the relation of FMD and RHI, especially in young study cohorts. Therefore, the correlation between the different vasoactive substances and FMD and RHI remains to be established.

We considered the same concerns raised by Kelly et al:¹⁹ that is, the age-dependency of RHI may be a measurement error related to fingertip probes, which have one size for all persons. Some studies therefore favor FMD. However, in a former study we found a close correlation between the RHI in children and the endothelial NO synthase (eNOS)-Ser¹¹⁷⁷ phosphorylation ($r=0.66$) induced by high-density lipoprotein-cholesterol (as it is capable of modifying the production of NO) at the stimulatory site, and the eNOS-Thr⁴⁹⁵ phosphorylation ($r=-0.60$) at the inhibitory site.³⁸ Therefore, larger studies are needed to clarify the usefulness of the tests, the influence of growth-related changes to vessel function, the relation between both, and the correlation with concentrations of vasoactive molecules.

Study Limitations

This substudy was, in essence, purely explorative. Yearly measurements of RHI in all participants might have added additional information, but were not possible for several reasons (availability of device, high costs of probes, and subjects tested in the afternoon without RHI).

Because of the hormonal influence on endothelial function in postpubertal girls, tests on endothelial function should be performed in the third week of the menstrual cycle, as the test results of endothelial function are the highest at that time. This was not always feasible. However, it can be supposed that doing so would have increased the

measured values in postpubertal girls, and therefore led to a larger difference between boys and girls.

Conclusions

In adolescence RHI increases with age. Girls have higher values than boys. Daily PE at school may improve endothelial function measured by RH-PAT. If RH-PAT is used in research or as a clinical tool, the age- and sex-dependency and wide variation in results have to be taken in account.

Acknowledgments

We thank the children and their parents, the teachers and principals of Annenmittelschule Chemnitz, Evangelisches Schulzentrum Leipzig, Johannes-Kepler-Gymnasium Chemnitz, Mittelschule und Gymnasium Brandis, Sportgymnasien Chemnitz und Leipzig for their participation. We also thank David Petroff for assistance with the manuscript.

Funding Sources

The study was supported by an unrestricted grant from Novartis, the Deutsche Forschungsgemeinschaft (KO 3512/1-1), and the "Roland Ernst Stiftung".

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Supplementary Files

Supplementary File 1

Table S1. Percentiles of RHI by age in half-yearly decrements in 10–17 year-old schoolchildren

Please find supplementary file(s);
<http://dx.doi.org/10.1253/circj.CJ-16-0994>