DETERMINANTS AND IMPACT OF EARLY VASCULAR AGING IN CHILDREN AND ADOLESCENTS

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DETERMINANTS AND IMPACT OF EARLY VASCULAR AGING IN CHILDREN AND ADOLESCENTS

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EDITORIAL
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Editorial: Determinants and Impact of Early Vascular Aging in Children and Adolescents

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Keywords: adolescents, arterial stiffness, blood pressure, cardiovascular health, children, early vascular aging, preventative cardiology

Editorial on the Research Topic

Determinants and Impact of Early Vascular Aging in Children and Adolescents

Cardiovascular health is essential for longevity and limited morbidity with increasing age. However, exposure to several risk factors from as early as preconception (genetic or epigenetic predispositions, lifestyle and behavioral risk factors, and early life programming) may probe accelerated vascular deterioration, also known as early vascular aging (EVA). The central hallmark of EVA is arterial stiffness, although several other markers have been proposed to identify individuals at increasing risk of accelerated biological aging. The collection of articles published under this Research Topic addresses the need for more scientific evidence to refine the current concepts of EVA within the scope of pediatrics.

Early life development or early life programming is a critical period in which a fetus' biological trajectory is determined for cardiovascular disease susceptibility. In a prospective birth cohort study by Wang et al., maternal gestational weight gain, as one of the early life programming risk factors, was measured before pregnancy, in the first trimester ($\leq$12 weeks), and before delivery. The aim of their study was to determine whether excessive maternal gestational weight gain, as an adverse intrauterine environment, could contribute to alterations in left ventricular geometry and function in offspring. Excessive maternal gestational weight gain in the second and third trimesters was associated with interventricular septum thickening, a risk factor for left ventricular hypertrophy in the offspring.

While several intrauterine and maternal risk factors contribute to the EVA trajectories of neonates, there are also non-modifiable factors that predisposes individuals to higher EVA risk, e.g., ethnicity/race, gender/sex, as well as sociocultural and socioeconomic factors. A group of researchers from the Eastern Cape Province in South Africa developed a review of vascular dysfunction and its determinants, with a focus on children of African ancestry (Matjuda et al.). This review is timely, since limited evidence on African normative values exists for either biomarkers measured clinically or in a laboratory and rely on guidelines and reference values from other countries. In children and adolescents, obesity and hypertension remain the main contributors to the development of vascular dysfunction and accelerated vascular aging.

A bidirectional relationship between the large and small arteries (cross-talk) has been described in adults (1), whereby changes in the macrovasculature exacerbates abnormalities in the

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microvasculature and vice versa. In a study by Breet et al., large artery stiffness was associated with retinal arterial narrowing and venular widening in children, suggestive of cross-talk between the macro- and microvasculature. This study also identified ethnic differences between black and white children, highlighting the need for further investigation into the impact of ethnicity on the macro-microvasculature interactions. In addition to the crosstalk between large and small arteries, as well as the contribution of obesity to EVA, high-risk youth are subjected to reduced diastolic function. Madson et al. illustrated that high arterial stiffness may lead to diastolic dysfunction in youth, and highlighted the importance of prevention and treatment to curb the onset of premature heart failure with preserved ejection fraction in adulthood.

Three studies examined the impact of lifestyle (namely physical activity and/or sedentary behavior) on various markers of vascular aging. In a cross-sectional analysis of 1,324 primary school aged children (7.2 $\pm$ 0.4 years), Kochli et al. examined the impact of fitness and obesity on central hemodynamics. This study found that a one-unit increase in body mass index was independently associated with higher central blood pressure and borderline independent higher central pulse pressure, but lower augmentation of the reflected pulse wave. A one-unit increase in shuttle run (stages) was associated with lower central blood pressure, but not after adjustment for confounders, and higher amounts of vigorous activity (min/day) was associated with lower augmentation index. Another study showed that higher amounts of exercise in adolescence is associated with lower cardiovascular risk $\sim$3 years later, however there was no association with carotid IMT (Königstein et al.). Higher volumes of exercise at both baseline and follow up was associated with borderline lower carotid stiffness. Böhm et al., showed in 94 children with a mean age of 12.2 years that the amount of time spent sedentary per day was the strongest predictor of low arterial compliance, but there was no association with endothelial function. Taken together, it is evident from these studies and the existing literature that modifiable lifestyle factors contribute to EVA in childhood.

Type 1 diabetes mellitus (T1DM) is an important cardiovascular risk factor. Although clinically overt cardiovascular complications are extremely rare in children and adolescents with T1DM, many studies have shown that the subclinical features of macro- and microvascular injury are present in diabetic children. Šuláková et al. assessed carotid-femoral pulse wave velocity (cfPWV) in children with T1DM and compared them with an age and sex matched group of healthy, normotensive children. This study showed that children with T1DM, despite similar blood pressure values in ambulatory blood pressure measurements, had higher cfPWV values compared to healthy children. Moreover, the biological age of the arteries estimated by cfPWV in children with T1DM was 5 years higher than in the control group.

A rare disease, tuberous sclerosis, is associated with constitutional activation of the mammalian target of rapamycin (mTOR) pathway. Although experimental studies indicate that mTOR activation causes a disruption of the structure of arteries, there are no studies to assess the structure and elastic properties of arteries in children with tuberous sclerosis. Skrzypczyk et al. showed that children with tuberous sclerosis had higher blood pressure, cfPWV, and carotid intima-media thickness values compared to the age and sex matched group of healthy children. In addition, they found that central blood pressure correlated with renal cysts size.

Given the growing prevalence of cardiovascular risk factors in childhood (2, 3) and the likely impact this will have on EVA, focus should shift to primordial prevention to promote cardiovascular health from early life. For the time being, more work is required to identify the determinants and consequences of EVA in children and adolescents and the most effective and sustainable lifestyle prescription to counteract EVA in early life and to promote longevity.

AUTHOR CONTRIBUTIONS

RK, ML, and RC gave substantial contributions to the conception and design of the editorial, drafting the work, revising it critically for important intellectual content, provided approval for publication of the content, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors contributed to the article and approved the submitted version.

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Association of Maternal Gestational Weight Gain With Left Ventricle Geometry and Function in Offspring at 4 Years of Age: A Prospective Birth Cohort Study

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Background: Maternal gestational weight gain (GWG) may be associated with cardiovascular diseases in the offspring from childhood to adulthood. We aimed to investigate the association between maternal GWG and the left ventricle (LV) geometry and function in the offspring, and explore the influence of the intrauterine environment on early childhood cardiac change.

Methods: Data of 981 mother-offspring pairs from the Shanghai Birth Cohort was used. Maternal pre-pregnancy weight and height, weight in the first trimester (≤12 weeks), and before delivery were measured. The echocardiography, blood pressure, and anthropometry assessment were evaluated in the offspring at 4 years of age.

Results: Interventricular septal thickness during diastole had a significantly positive correlation with total GWG [β = 0.009, (0.001, 0.017)]. In the second and third trimesters, LV mass index [β = 0.149, (0.015,0.282)], interventricular septal thickness in systole [β = 0.027, (0.011,0.043)], and in diastole [β = 0.014, (0.005,0.023)] were positively associated with GWG. The risks of eccentric [OR = 1.115, (1.232, 1.010)] and concentric hypertrophy [OR = 1.133, (1.259,1.018)] increased with the elevation of maternal GWG.

Conclusions: This study suggested that the excessive maternal GWG was associated with the thickening of the interventricular septum in the offspring, especially during the second and third trimesters. Excessive GWG in the second and third trimesters was a risk factor for LV eccentric and concentric hypertrophy in the offspring.

Keywords: gestational weight gain, left ventricle geometry, left ventricle hypertrophy, birth cohort, cardiovascular risk

INTRODUCTION

Left ventricle (LV) geometry and function are important factors that influence cardiac remodeling, and are also determinants of cardiovascular events in adulthood (1, 2). LV geometry may increase morbidity and mortality even in asymptomatic conditions, which could occur before the onset of overt hypertension and heart failure (3–6).

The LV geometry and functional changes are often evaluated in adults and adolescents. Morphologic changes in the LV could progress due to cumulative exposures from an early age, which could impair LV function ultimately in later life (3). However, studies on early LV geometry and functional changes in children are limited.

Apart from early influences during childhood, the LV geometry and function could also be affected by various maternal factors, such as obesity, gestational hypertension, and diabetes (7–9). Gestational weight gain (GWG) could reflect the health condition of both the fetus and mother during pregnancy (10, 11). It may play an important role in the development of cardiovascular diseases originating from the intrauterine environment (11–13). Increased GWG and maternal obesity may increase the risk of hypertension (14, 15), ventricular myocardial hypertrophy (16, 17), myocardial fibrosis (18), and congenital heart defects (19), which have been demonstrated in previous adult and animal studies. However, the impact of GWG on early LV geometry and function in young offspring remains unclear.

Based on the Shanghai Birth Cohort (SBC), we aimed to investigate the association of maternal GWG with offspring LV geometry and function at 4 years of age to explore the influence of the intrauterine environment on early childhood cardiac geometry and function.

MATERIALS AND METHODS

Participants

The SBC is an ongoing prospective cohort study conducted in six collaborating hospitals in Shanghai, China. Volunteer couples were recruited during preconception care or in early pregnancy from 2013 to 2016, and the mother-fetus pairs were followed from preconception or early pregnancy to the end of the gestation. A detailed description of the cohort has been provided elsewhere (20). In our study, only women who had a singleton live birth, with recorded weight and height during pre-pregnancy, and recorded weight at pre-delivery were included. Miscarriage, stillbirth, multiple pregnancies, lost to follow-up, and women without available medical records were excluded. For offspring, children with congenital heart disease, lost to follow-up, uncooperative, and without available records were excluded. Finally, 981 mother-offspring pairs were included in the analysis. Ethical approval was granted by the Ethical Committee of Xinhua Hospital affiliated to Shanghai Jiao Tong University School of Medicine (Protocol no. XHEC-C-2013-001-2). All parents or guardians of participants signed the written informed consent before enrollment.

Measurement of Maternal Factors

Information on demographic and sociodemographic characteristics (e.g., maternal age, race, education level), reproductive characteristics (e.g., parity, gestational week, delivery mode, birth weight and length), lifestyle factors (e.g., passive smoking or alcohol drinking status during pregnancy), and history of gestational hypertension or diabetes were collected through structured questionnaires and extraction of the inpatient history of the pregnant women from medical records. Pre-pregnancy weight and height, weight in the first trimester ($\leq$12 weeks), and weight before delivery were measured at each clinical visit. Maternal pre-pregnancy body mass index (BMI), total GWG, GWG in the first ($\leq$12 weeks) or second and third trimesters (>12 weeks) were calculated. Based on the World Health Organization (WHO) criteria, the pre-pregnancy BMI was categorized as underweight ($<$ 18.5 kg/m^2), normal weight (18.5–24.9 kg/m^2), overweight (25.0–29.9 kg/m^2), and obese ($\geq$ 30.0 kg/m^2) (21).

Measurement of Offspring Factors

The height and weight of the offspring were measured according to the standard protocol. BMI was calculated in the 4-year-old children. Blood pressure (BP) and heart rate (HR) of the 4-year-old children were assessed by one trained staff while they were supine using the OMRAN HBP-1300 automatic BP device (Omron Healthcare, Guangzhou, China) on the left arm at heart level and with the appropriate cuff size for arm circumference. Three measurements were taken at 5-min intervals. The mean of the measurements was used in all analyses.

Transthoracic echocardiography examinations were performed for the children according to the American and European guidelines (22) by trained operators using the Philip EPIQ7C (Philips Healthcare, Andover, USA) ultrasound that uses the X5-1 (1-5MHz) or S8-3 (8-3MHz) matrix-array transducers (Philips Healthcare, Andover, USA). Measurements of the left ventricle (LV) dimensions were acquired from two-dimensional (2D)-guided M-mode echocardiograms, including the thickness of LV interventricular septum (IVSs and IVSd), posterior wall (LVPWs and LVPWd), and the internal diameter (LVIDs and LVIDd) of the LV during systole and diastole. LV ejection fraction (LVEF) and fractional shortening (FS) were calculated to evaluate the systolic function of LV.

Relative wall thickness (RWT) was calculated by the sum of the thickness of the LV posterior wall in diastole (LVPWd) and interventricular septal thickness in diastole (IVSd), then divided by the internal LV diameter in diastole (LVDd) (23). LV mass (LVM) was calculated using the Devereux formula (24) and the LVM index (LVMI) was calculated using the formula: $\text{LVMI} = \text{LVM}/\text{Height}^{2.7}$ (25).

Pulse wave Doppler was used to measure the mitral early diastolic flow velocity (E), late diastolic flow velocity (A) to calculate the E/A ratio. The Doppler time intervals, including the ejection time (ET), isovolumic contraction time (ICT), and isovolumic relaxation time (IRT), were obtained at the mitral inflow and LV outflow tracts to calculate the Tei index, with the formula: Tei index = (ICT + IRT)/ET. These indices were used to assess the diastolic function of the LV.

For 2D speckle-tracking analysis, 2D images of 3–5 cardiac cycles were collected and analyzed with the commercial Qlab version 10.5 software (Philips Healthcare, Andover, USA)] at a frame rate of ≥ 60/s. Peak longitudinal strain was measured in the apical four, two, and three-chamber and global peak longitudinal strain (GLS) was calculated using the strain value of each segment in LV.

The LV geometry patterns were defined using the LVMI and RWT according to Ganau and colleagues' descriptions (5). There were no recommended LVMI and RWT cutoff points (26) for 4-year-old healthy children in China. We used the sex-specific 95th percentiles of LVMI and RWT derived from the cohort as cutoff points. LVMI = 33.24 g/m$^{2.7}$, RWT = 0.27 in girls and LVMI = 33.76 g/m$^{2.7}$, RWT = 0.27 in boys represented the sex-specific 95th percentiles in the cohort. Four groups were constructed for LV geometry patterns: (1) LVMI < 33.24 g/m$^{2.7}$ for girls and < 33.76 g/m$^{2.7}$ for boys, and RWT < 0.27 was classified as normal left ventricular geometry; (2) normal LVMI with increased RWT (>0.27) was classified as concentric remodeling; (3) increased LVMI (girls ≥33.24 g/m$^{2.7}$, boys ≥ 33.76 g/m$^{2.7}$), and normal RWT (<0.27) was defined as eccentric hypertrophy; and (4) increase in both variables was identified as concentric hypertrophy.

All the examinations were performed by a single experienced operator. Both the sonographers and the observers were blinded to the participants' details.

Statistical Analyses

Linear regression models were used to investigate the associations between maternal GWG and offspring LV geometry and function changes. Five sets of models were constructed: the basic model was adjusted for none of the maternal factors or offspring factors. Model 1 was adjusted for maternal factors, including age at delivery, race, educational level, alcoholic drink intake history, exposure to passive smoke, pre-pregnancy BMI, gestational diabetes mellitus (GDM), and gestational hypertension or pre-eclampsia. Model 2 was adjusted additionally for gestational age, sex of offspring, delivery mode, and parity. Model 3 was adjusted additionally for BMI at 4 years of age. Model 4 was adjusted additionally for BP at 4 years of age. The GWG was divided into three groups according to the gestational trimesters as the total, first trimester, and second and third trimesters.

To further eliminate the effect of pre-pregnancy BMI on the LV geometry and function change in offspring, the study population was stratified into three groups (underweight, normal weight, and overweight or obese) according to the WHO criteria (21). Linear regression models were also constructed.

To test the risk of LV geometry pattern changes caused by an increase in GWG, including eccentric hypertrophy, concentric hypertrophy, and remodeling, multiple logistic regression analysis was performed in different groups, and odds ratio (OR) was calculated.

Statistical analysis was carried out using the SPSS 19.0 software program (IBM Corp., Armonk, NY, USA). All tests were two-sided with a significance level of 0.05.

RESULTS

Basic Characteristics

The baseline characteristics of the study participants are presented in **Supplementary Tables 1**, **2**. The mean total GWG was 14.4 ± 5.2 kg. On average, mothers gained a weight of 2.5 ± 3.2 kg in the first trimester and 11.9 ± 4.4 kg in the second and third trimesters. Most pregnant women enrolled had normal BMI (73.2%) before pregnancy. There were 14.1% who were underweight and 12.7% who were overweight or obese. In the offspring, the majority were male (52.8% male vs. 47.2% female). The average results of the LV structure and function data in the offspring were all within the reference range.

Maternal GWG and Offspring LV Geometry and Function

In the basic model (**Supplementary Table 3**), almost all the indicators of LV internal cavity and wall thickness had a positive correlation with total GWG. However, there was no significant association between GWG with the LV systolic and diastolic function indices at 4 years of age. After adjusting for other maternal or offspring factors (**Tables 1**–**3**) which could influence the LV geometry and function in children, only the IVSd [β = 0.009, (0.001, 0.017)] had a significant positive correlation with the total GWG. There was no significant correlation between the LV global function indices and total GWG found in any models.

The gestational time was divided into the total, first trimester (≤12 weeks), and second and third trimesters (>12 weeks). In the basic model, LVMI [β = 0.147, (0.027, 0.266)], LVDd [β = 0.051, (0.011, 0.091)], IVSd [β = 0.033, (0.017, 0.047)], and IVSs [β = 0.014, (0.006, 0.022)] had a positive association with the second and third trimester GWG. GWG in the first trimester had no significant correlation with any structural and functional indices. After adjusting for maternal and offspring factors, LVMI [β = 0.149, (0.015, 0.282)], IVSs [β = 0.027, (0.011, 0.043)], and IVSd [β = 0.014, (0.005, 0.023)] continued to be positively associated with GWG in all models during the second and third trimesters. The RWT and the LV function indices had no significant correlation with GWG in any trimester.

Offspring LV Geometry and Function in Different Pre-pregnancy BMI Groups

GWG was strongly related to pre-pregnancy BMI (27). Taking the pre-pregnancy BMI into consideration, we divided the maternal-offspring pairs into three groups (underweight, normal weight, and overweight or obese) to eliminate the influence of pre-pregnancy BMI (**Table 4**). For underweight women, LVPWs was directly associated with the total [β = 0.046, (0.001, 0.091)] and the first trimester GWG [β = 0.068, (0.001, 0.135)]; LVPWd was positively associated with the total [β = 0.035, (0.007, 0.062)] and the second and third trimester GWG [β = 0.035, (0.002, 0.067)]; IVSs was associated with the total GWG [β = 0.049, (0.002, 0.095)]. For normal-weight women, IVSd were positively correlated with the total [β = 0.012, (0.003, 0.022)], and the second and third GWG [β = 0.015, (0.004, 0.026)]; IVSs had a positive correlation with the second and third trimester GWG [β = 0.020, (0.001, 0.039)]. But in overweight and obese women,

TABLE 1 | Association between maternal total GWG and offspring LV geometry and function.

	Total GWG			
	Model 1	**Model 2**	**Model 3**	**Model 4**
LV Structure				
LVMI	0.14(−0.006,0.214)	0.106(−0.007,0.218)	0.097 (−0.018,0.213)	0.096(−0.023,0.215)
LVPWs	0.018(0.004,0.031)	**0.019(0.005,0.032)**	0.008(−0.006,0.021)	0.010(−0.003,0.024)
LVPWd	0.012(−0.001,0.025)	0.012(−0.001,0.025)	0.005(−0.008,0.018)	0.002(−0.011,0.016)
LVDs	**0.045(0.017,0.074)**	**0.040(0.011,0.069)**	0.003(−0.025,0.030)	0.001(−0.027,0.029)
LVDd	**0.058(0.022,0.095)**	**0.051(0.014,0.088)**	−0.006(−0.040,0.028)	−0.006(−0.040,0.028)
IVSs	**0.022(0.009,0.037)**	**0.022(0.008,0.036)**	0.011(−0.003,0.025)	0.013(−0.001,0.027)
IVSd	**0.010(0.003,0.018)**	**0.011(0.004,0.019)**	**0.009(0.001,0.017)**	**0.009(0.001,0.017)**
RWT	0.001(−0.001,0.001)	0.001(−0.001,0.001)	0.001(−0.001,0.001)	0.001(−0.001,0.001)
LV Function				
E/a	−0.001(−0.005,0.004)	−0.002(−0.006,0.003)	−0.002(−0.006,0.003)	−0.002(−0.007,0.02)
Tei Index	0.001(−0.001,0.001)	0.001(−0.001,0.001)	0.001(−0.001,0.002)	0.001(−0.001,0.002)
EF	0.058(−0.052,0.169)	0.073(−0.038,0.183)	0.079(−0.034,0.193)	0.082(−0.037,0.201)
AP2 strain	−0.014(−0.068,0.040)	−0.014(−0.070,0.041)	0.001(−0.055,0.005)	0.002(−0.054,0.058)
AP3 strain	−0.052(−0.115,0.011)	−0.049(−0.113,0.015)	−0.031(−0.094,0.033)	−0.030(−0.095,0.036)
AP4 strain	−0.052(−0.115,0.011)	−0.001(−0.053,0.052)	0.013(−0.039,0.066)	0.013(−0.041,0.067)
GLS	0.013(−0.041,0.067)	−0.021(−0.067,0.023)	−0.006(−0.051,0.038)	−0.005(−0.051,0.040)

The bold values mean the mean difference is significant (P > 0.05).

Model 1: adjusted for maternal factors, including age, race, educational level, drinking, passive smoking, pre-pregnancy BMI, GDM and gestational hypertension.

Model 2: adjusted additionally for gestational age, sex of offspring, delivery mode, parity.

Model 3: adjusted additionally for BMI at 4 years of age.

Model 4: adjusted additionally for BP at 4 years of age.

AP2 strain, peak longitudinal strain measured on apical two chambers; AP3 strain, peak longitudinal strain measured on apical three chambers; AP4 strain, peak longitudinal strain measured on apical four chambers, BMI, body mass index; EF, ejection fraction; GDM, gestational diabetes mellitus; GLS, global peak longitudinal strain; GWG, gestational weight gain; IVS, ventricle interventricular septal; IVSs, ventricle interventricular septal in systole; IVSd, ventricle interventricular septal in diastole; LVH, left ventricle hypertrophy; LVMI, LV mass index; LVPWd, LV posterior wall in diastole; LVPWs, LV posterior wall in systole: LVDd, LV diameter in diastole; LVDs, LV diameter in systole; RWT, relative wall thickness.

only IVSs were correlated with the second and third trimester GWG. There was no significant association of LV function indices with GWG in any of the groups.

Risk of Left Ventricle Hypertrophy in Offspring at 4 Years of Age

The risk of four types of LV geometry change patterns in offspring with maternal GWG in different trimesters is presented in **Figure 1**. In the second and third trimesters, the risk of eccentric [OR = 1.115, (1.232, 1.010)] and concentric hypertrophy [OR = 1.133, (1.259, 1.018)] increased with an elevation of maternal GWG after adjusting for the maternal and offspring factors. Eccentric, concentric hypertrophy, and remodeling were three types of LVH. It indicated that excessive GWG in the second and third trimesters was an independent risk factor for LVH in the offspring.

DISCUSSION

In this prospective cohort study, we found that greater GWG was associated with LV morphologic changes in the offspring as early as 4 years of age, especially the thickening of IVS. Greater GWG during the second and third trimesters was an independent risk factor for LV eccentric and concentric hypertrophy in the offspring.

Cardiac structural changes usually occur before global functional alteration, which is often evaluated during adulthood and adolescence. However, morphologic changes in the LV could progress due to accumulated exposures during childhood, which could impair the LV function ultimately in later life (3). In our study, regional cardiac structural changes occurred as early as 4 years of age during follow-up, whereas there were no changes in the global function. This result provided evidence for early screening of the cardiovascular structure changes in young children, which may be useful for preventing the generation and progression of cardiovascular disease in adulthood.

LV wall thickening is the manifestation of myocardial hypertrophy (28). The thickness of the IVS is one of the reliable markers for evaluating the adverse outcomes in cardiovascular diseases, including coronary artery disease (29), atrial fibrillation (30), and valve replacement (31). In patients with hypertension, the thickening of IVS has been observed before LVMI and functional changes (32); conversely, isolated hypertrophy of the IVS with normal LVM and function has been demonstrated to increase the risk of the development of hypertension in the future (33–35). The thickness of IVS is a multifactorial index influenced by the growth and BP of children. In our study, the

TABLE 2 | Association between maternal GWG and offspring LV geometry and function in the first trimester.

	First trimester GWG			
	Model 1	**Model 2**	**Model 3**	**Model 4**
LV Structure				
LVMI	−0.027(−0.200,0.146)	−0.012(−0.186,0.161)	−0.028(−0.203,0.146)	−0.039(−0.220,0.141)
LVPWs	0.0189(−0.002,0.040)	0.020(−0.002,0.041)	0.010(−0.011,0.031)	0.011(−0.010,0.033)
LVPWd	**0.014(0.001,0.027)**	**0.014(0.001,0.027)**	0.009(−0.004,0.022)	0.009(−0.005,0.023)
LVDs	0.018(−0.027,0.063)	0.024(−0.021,0.069)	−0.009(−0.050,0.033)	−0.009(−0.051,0.034)
LVDd	0.001(−0.056,0.059)	0.011(−0.046,0.069)	−0.039(−0.090,0.011)	−0.042(−0.03,0.010)
IVSs	−0.002(−0.024,0.020)	−0.001(−0.022,0.021)	−0.011(−0.032,0.010)	−0.012(−0.033,0.009)
IVSd	0.001(−0.011,0.012)	0.001(−0.011,0.012)	−0.002(−0.014,0.010)	**0.003(0.001,0.015)**
RWT	0.001(−0.001,0.001)	0.001(−0.001,0.001)	0.001(−0.001,0.001)	0.001(−0.001,0.001)
LV Function				
E/a	−0.006(−0.142,0.130)	−0.006(−0.144,0.132)	0.006(−0.132,0.144)	0.002(−0.137,0.141)
Tei Index	−0.001(−0.007,0.007)	0.001(−0.007,0.007)	−0.001(−0.007,0.007)	−0.001(−0.007,0.006)
EF	0.001(−0.001,0.001)	0.001(−0.001,0.002)	0.001(−0.001,0.002)	0.001(−0.001,0.002)
AP2 strain	−0.013(−0.096,0.069)	−0.005(−0.088,0.079)	0.009(−0.074,0.092)	0.009(−0.074,0.093)
AP3 strain	−0.022(−0.117,0.074)	−0.018(−0.115,0.079)	0.001(−0.096,0.096)	−0.012(−0.110,0.085)
AP4 strain	0.048(−0.030,0.126)	0.047(−0.032,0.126)	0.060(−0.018,0.138)	0.067(−0.013,0.147)
GLS	0.004(−0.063,0.071)	0.008(−0.060,0.075)	0.023(−0.044,0.089)	0.021(−0.047,0.089)

The bold values mean the mean difference is significant (P > 0.05).

Model 1: adjusted for maternal factors, including age, race, educational level, drinking, passive smoking, pre-pregnancy BMI, GDM and gestational hypertension.

Model 2: adjusted additionally for gestational age, sex of offspring, delivery mode, parity.

Model 3: adjusted additionally for BMI at 4 years of age.

Model 4: adjusted additionally for BP at 4 years of age.

AP2 strain, peak longitudinal strain measured on apical two chambers; AP3 strain, peak longitudinal strain measured on apical three chambers; AP4 strain, peak longitudinal strain measured on apical four chambers, BMI, body mass index; EF, ejection fraction; GDM, gestational diabetes mellitus; GLS, global peak longitudinal strain; GWG, gestational weight gain; IVS, ventricle interventricular septal; IVSs, ventricle interventricular septal in systole; IVSd, ventricle interventricular septal in diastole; LVH, left ventricle hypertrophy; LVMI, LV mass index; LVPWd, LV posterior wall in diastole; LVPWs, LV posterior wall in systole: LVDd, LV diameter in diastole; LVDs, LV diameter in systole; RWT, relative wall thickness.

thickness of the IVS was found to be significantly associated with greater GWG, independent of offspring's BMI, BP, and other factors.

We focused on the LV geometry and functional patterns in a birth cohort comprising randomly selected children. These LV structural changes, including thickening of the IVS, may be physiologic during early childhood since the pathological changes usually occur in adolescents (26, 36) and adults (9), or in high-risk groups of children, such as those with obesity (37), obstructive sleep apnea (38), or hypertension (39). The transition to pathologic remodeling could be heralded by progressive ventricular dilatation, distortion of the shape of the cavity, and disruption of the normal cardiac geometry and function (40). The LV geometry patterns described the four different LV remodeling features. Eccentric hypertrophy, concentric hypertrophy, and cardiac remodeling were classified as different types and states of LVH. LVH indicated a pathological hyperdynamic state, which may alter LV structure and function that may predispose to the development of heart failure or other adverse cardiovascular prognosis (41). The pressure overload pattern of concentric hypertrophy was associated with high systolic blood pressure and high peripheral resistance. Eccentric LV hypertrophy was associated with normal peripheral resistance, but high cardiac indexes consistent with excess circulating blood volume. Concentric remodeling was characterized by high peripheral resistance, low cardiac index, and increased arterial stiffness (5, 40, 42). In Cuspidi's meta-analysis (43), the risk of LVH was 4.2-fold greater in obese than in non-obese participants and eccentric hypertrophy was the most common type of LVH in obesity. Consistently, we found that the maternal GWG during the second and third trimesters was an independent risk factor for hyperdynamic status in children at 4 years of age. It indicated that the maternal intrauterine environment may have a long-term influence on their offspring's hemodynamic status. However, this hypothesis needs further validation in future studies with larger sample sizes.

Greater GWG was associated with many adverse maternal and offspring outcomes from birth to adulthood. Greater maternal pre-pregnancy weight and GWG were associated with higher systolic blood pressure (36), adverse accumulation of lipid and inflammatory profiles (9, 44), increased risk of congenital heart disease (19, 45), myocardial hypertrophy (16), hypertension (15), and premature death in later life in the offspring (46, 47). The GWG in the second and third trimesters accounted for a majority of the total GWG and played an important role in offspring growth, adiposity, and metabolic state (48). It was a key period for organ development, including the heart, muscles, bones, and liver (48–50). In our study, greater GWG during the second and third trimesters was significantly associated with thickening of the LV wall and was a risk factor for LVH in the offspring, independent of

TABLE 3 | Association between maternal GWG and offspring LV geometry and function in the second and third trimesters.

	Second and third trimesters GWG			
	Model 1	**Model 2**	**Model 3**	**Model 4**
LV Structure				
LVMI	**0.154(0.027,0.280)**	**0.155(0.026,0.284)**	**0.146(0.017,0.276)**	**0.149(0.015,0.282)**
LVPWs	0.009(−0.007,0.025)	0.009(−0.007,0.025)	0.003(−0.013,0.018)	0.006(−0.010,0.022)
LVPWd	0.005(−0.005,0.015)	0.006(−0.004,0.015)	0.002(−0.007,0.012)	0.003(−0.007,0.013)
LVDs	**0.043(0.010,0.076)**	**0.037(0.003,0.070)**	0.015(−0.016,0.046)	0.010(−0.022,0.041)
LVDd	**0.063(0.021,0.105)**	**0.054(0.011,0.096)**	0.021(−0.017,0.059)	0.018(−0.020,0.056)
IVSs	**0.031(0.015,0.046)**	**0.031(0.015,0.047)**	**0.024(0.008,0.039)**	**0.027(0.011,0.043)**
IVSd	**0.013(0.004,0.021)**	**0.015(0.006,0.023)**	**0.013(0.005,0.022)**	**0.014(0.005,0.023)**
RWT	0.001(−0.001,0.001)	0.001(−0.001,0.001)	0.001(−0.001,0.001)	0.001(−0.001,0.001)
LV Function				
E/a	0.001(−0.004,0.006)	0.001(−0.004,0.006)	0.001(−0.004,0.007)	0.001(−0.005,0.006)
Tei Index	0.001(−0.001,0.002)	0.001(−0.001,0.002)	0.001(−0.001,0.002)	0.001(−0.001,0.002)
EF	0.010(−0.091,0.112)	0.009(−0.096,0.114)	0.019(−0.086,0.124)	0.030(−0.077,0.136)
AP2 strain	−0.011(−0.072,0.051)	−0.016(−0.080,0.048)	−0.005(−0.068,0.058)	−0.003(−0.067,0.051)
AP3 strain	−0.055(−0.126,0.016)	−0.054(−0.128,0.020)	−0.040(−0.113,0.033)	−0.031(−0.107,0.044)
AP4 strain	−0.017(−0.075,0.041)	−0.028(−0.088,0.032)	−0.018(−0.078,0.042)	−0.023(−0.084,0.039)
GLS	−0.028(−0.078,0.021)	−0.033(−0.085,0.018)	−0.022(−0.072,0.029)	−0.020(−0.072,0.032)

The bold values mean the mean difference is significant (P > 0.05).

Model 1: adjusted for maternal factors, including age, race, educational level, drinking, passive smoking, pre-pregnancy BMI, GDM, and gestational hypertension.

Model 2: adjusted additionally for gestational age, sex of offspring, delivery mode, parity.

Model 3: adjusted additionally for BMI at 4 years of age.

Model 4: adjusted additionally for BP at 4 years of age.

AP2 strain, peak longitudinal strain measured on apical two chambers; AP3 strain, peak longitudinal strain measured on apical three chambers; AP4 strain, peak longitudinal strain measured on apical four chambers; BMI, body mass index; EF, ejection fraction; GDM, gestational diabetes mellitus; GLS, global peak longitudinal strain; GWG, gestational weight gain; IVS, ventricle interventricular septal; IVSs, ventricle interventricular septal in systole; IVSd, ventricle interventricular septal in diastole; LVH, left ventricle hypertrophy; LVMI, LV mass index; LVPWd, LV posterior wall in diastole; LVPWs, LV posterior wall in systole; LVDd, LV diameter in diastole; LVDs, LV diameter in systole; RWT, relative wall thickness.

the influence from offspring growth or BP. Therefore, judicious monitoring of GWG during the second and third trimesters should be recommended, which may help promote the health of both the mother and her child.

There are several possible explanations for the mechanisms responsible for a greater GWG increasing the risk of LVH. Greater maternal GWG is an abnormal metabolic state, which modifies the intrauterine environment, thereby influencing placental function and fetal programming of the cardiovascular system, which, in turn, affects fetal heart development. A mother with excessive weight gain or obesity has a suboptimal uterine nutritional climate, which may disrupt the inflammatory and hormone metabolic homeostasis of the mother, including insulin resistance, increased level of leptin, lipid, and pro-inflammatory cytokines. These metabolic disorders may change the placental hemodynamics causing placental vascular insufficiency, increased lipogenesis, infarction, hypoxia, and inflammatory activation (12). These may cause an abnormal accumulation of glycogen and glucose uptake in the myocytes, thus, increasing the load on the fetal heart, ultimately resulting in myocyte hypertrophy (28, 51). Furthermore, the abnormal intrauterine environment may influence the epigenetic modifications of genes associated with cardiovascular function and development (13). The effect of epigenetics is inborn and lifelong. It may have a cumulative effect on the vascular and myocyte function in the offspring from the fetal stage to adulthood. These may increase the risk of the early development of hypertension, ventricular hypertrophy, atherosclerosis, and premature cardiac failure, consequently influencing the prognosis of cardiovascular health in later life (12).

Early screening of LV geometry and function by echocardiography at 4 years of age without the administration of any sedative agents was feasible. Thus, this age may be a suitable time for early screening. Furthermore, early screening of LV structural changes should be recommended in the offspring of mothers who had excessive weight gain during pregnancy, and emphasize the need for additional monitoring and weight management during the second and third trimesters.

Strengths and Limitations

To our knowledge, this study was the largest prospective birth cohort study that assessed LV geometry and function using detailed echocardiography during early childhood in China. Furthermore, previous research on the association between maternal and offspring factors associated with LV geometry usually focused on maternal obesity (46) and were performed in adolescents, adults, or high-risk groups of offspring with obesity (43), abnormal blood pressure (52), or other metabolic syndromes (26). This study provided the first evidence of

TABLE 4 | Association between maternal GWG and LV geometry and function in offspring divided by pre-pregnant BMI.

	Total GWG			First trimester GWG			Second and third trimesters GWG		
	Underweight	**Normal weight**	**Overweight and obese**	**Underweight**	**Normal weight**	**Overweight and obese**	**Underweight**	**Normal weight**	**Overweight and obese**
LV Structure									
LVMI	0.173 (−0.243,0.588)	0.137 (−0.004,0.279)	−0.037 (−0.305,0.230)	−0.150 (−0.770,0.469)	0.073 (−0.152,0.297)	−0.264 (−0.632,0.105)	0.321 (−0.155,0.797)	0.144 (−0.016,0.305)	0.168 (−0.180,0.517)
LVPWs	**0.046 (0.001,0.091)**	0.008 (−0.009,0.025)	−0.003 (−0.029,0.023)	**0.068 (0.001,0.135)**	0.010 (−0.017,0.038)	−0.001 (−0.037,0.036)	0.020 (−0.033,0.074)	0.005 (−0.015,0.024)	−0.004 (−0.038,0.030)
LVPWd	**0.035 (0.007,0.062)**	0.007 (_0.003,0.019)	−0.007 (−0.025,0.011)	0.018 (−0.024,0.061)	0.013 (−0.004,0.030)	−0.003 (−0.028,0.022)	**0.035 (0.002,0.067)**	0.004 (−0.009,0.016)	−0.009 (−0.033,0.014)
LVDs	−0.025 (−0.127,0.078)	0.015 (−0.017,0.048)	−0.032 (−0.099,0.036)	0.045 (−0.107,0.197)	0.006 (−0.045,0.058)	−0.080 (−0.173,0.012)	−0.059 (−0.177,0.059)	0.016 (−0.021,0.053)	0.017 (−0.072,0.105)
LVDd	−0.009 (−0.146,0.128)	−0.008 (−0.047,0.032)	−0.013 (−0.096,0.070)	0.042 (−0.161,0.245)	−0.034 (−0.096,0.028)	−0.096 (−0.210,0.018)	−0.037 (−0.195,0.121)	0.010 (−0.035,0.054)	0.062 (−0.046,0.171)
IVSs	**0.049 (0.002,0.095)**	0.009 (−0.007,0.026)	0.012 (−0.019,0.043)	0.025 (−0.045,0.095)	−0.011 (−0.038,0.015)	−0.027 (−0.071,0.016)	0.048 (−0.006,0.102)	**0.020 (0.001,0.039)**	**0.045 (0.005,0.085)**
IVSd	0.011 (−0.019,0.040)	**0.012 (0.003,0.022)**	0.006 (−0.011,0.022)	−0.016 (−0.060,0.027)	0.002 (−0.013,0.017)	−0.001 (−0.024,0.022)	0.024 (−0.010,0.057)	**0.015 (0.004,0.026)**	0.010 (−0.011,0.032)
RWT	0.001 (−0.001,0.003)	**0.001 (0.000,0.002)**	−0.001 (−0.001,0.001)	−0.001 (−0.003,0.002)	0.001 (−0.001,0.001)	0.001 (−0.001,0.002)	0.002 (−0.001,0.004)	0.001 (−0.001,0.001)	−0.001 (−0.002,0.001)
LV Function									
E/a	0.002 (−0.016,0.201)	−0.001 (−0.007,0.005)	−0.003 (−0.014,0.007)	−0.013 (−0.037,0.010)	0.008 (−0.008,0.009)	−0.001 (−0.014,0.013)	0.012 (−0.008,0.031)	−0.001 (−0.007,0.006)	−0.005 (−0.019,0.008)
EF	0.177 (−0.174,0.528)	0.041 (−0.070,0.151)	−0.137 (−0.370,0.096)	0.209 (−0.097,0.515)	−0.039 (−0.225,0.147)	−0.171 (−0.479,0.136)	−0.090 (−0.437,0.256)	0.073 (−0.055,0.200)	−0.061 (−0.358,0.236)
Tei Index	0.001 (−0.003,0.004)	0.001 (−0.001,0.002)	−0.001 (−0.002,0.002)	0.001 (−0.004,0.006)	0.001 (−0.002,0.002)	0.001 (−0.003,0.004)	0.001 (−0.004,0.004)	0.001 (−0.001,0.002)	−0.001 (−0.003,0.003)
AP2 strain	−0.015 (−0.234,0.205)	−0.007 (−0.074,0.060)	0.040 (−0.114,0.194)	−0.108 (−0.299,0.082)	−0.019 (−0.131,0.094)	0.136 (−0.063,0.335)	0.121 (−0.091,0.334)	0.001 (−0.077,0.078)	−0.060 (−0.253,0.133)
AP3 strain	0.150 (−0.104,0.404)	−0.072 (−0.151,0.007)	0.086 (−0.087,0.259)	0.019 (−0.207,0.244)	−0.084 (−0.217,0.050)	0.118 (−0.110,0.345)	0.121 (−0.129,0.371)	−0.057 (−0.149,0.035)	0.029 (−0.190,0.249)
AP4 strain	0.004 (−0.219,0.223)	0.020 (−0.043,0.084)	0.025 (−0.132,0.183)	0.063 (−0.133,0.258)	0.085 (−0.021,0.191)	0.108 (−0.098,0.314)	−0.074 (−0.292,0.144)	−0.014 (−0.087,0.060)	−0.058 (−0.255,0.140)
GLS	0.042 (−0.128,0.212)	−0.020 (−0.074,0.035)	0.049 (−0.082,0.179)	−0.012 (−0.162,0.138)	−0.005 (−0.096,0.087)	0.119 (−0.049,0.288)	0.055 (−0.111,0.222)	−0.024 (−0.087,0.039)	−0.032 (−0.196,0.133)

The bold values mean the mean difference is significant (P > 0.05).

Linear regression was adjusted for maternal and offspring factors, including maternal age, race, educational level, drinking, passive smoking, pre-pregnancy BMI, GDM and gestational hypertension, gestational age, sex of offspring, delivery mode, parity, BMI, and BP of offspring at 4 years of age.

AP2 strain, peak longitudinal strain measured on apical two chambers; AP3 strain, peak longitudinal strain measured on apical three chambers; AP4 strain, peak longitudinal strain measured on apical four chambers; BMI, body mass index; EF, ejection fraction; GDM, gestational diabetes mellitus; GLS, global peak longitudinal strain; GWG, gestational weight gain; IVS, ventricle interventricular septal; IVSs, ventricle interventricular septal in systole; IVSd, ventricle interventricular septal in diastole; LVH, left ventricle hypertrophy; LVMI, LV mass index; LVPWd, LV posterior wall in diastole; LVPWs, LV posterior wall in systole; LVDd, LV diameter in diastole; LVDs, LV diameter in systole; RWT, relative wall thickness.

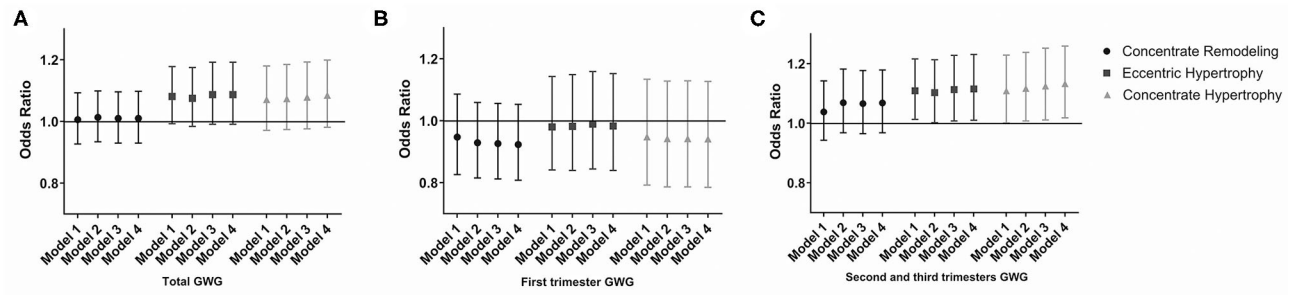

FIGURE 1 | Odds risk (OR) of LVH in offspring with maternal GWG during different gestational trimesters. **(A)** OR of LVH in offspring with maternal GWG in total trimester. **(B)** OR of LVH in offspring with maternal GWG in first trimester. **(C)** OR of LVH in offspring with maternal GWG in second and third trimesters. Data are presented as OR (95%CI). Model 1: adjusted for maternal factors, including age, race, educational level, drinking, passive smoking, pre-pregnancy BMI, GDM and gestational hypertension. Model 2: adjusted additionally for gestational age, sex of offspring, delivery mode, parity. Model 3: adjusted additionally for BMI at 4 years of age. Model 4: adjusted additionally for BP at 4 years of age. BMI, body mass index; GWG, gestational weight gain; LVH, left ventricle hypertrophy.

maternal GWG as an independent risk factor for LV hypertrophy at an early age in the general population.

Our study has several limitations. First, cardiac magnetic resonance imaging is the gold standard to evaluate LV geometry. However, this procedure requires the administration of sedatives in young children. Therefore, in our study, we opted for echocardiography as a more suitable modality for children aged 4 years. Second, data on maternal weight during the second trimester were not available in the medical records, which may need a more detailed investigation in future studies. Third, there was an unequal representation of the populations enrolled in our study regarding the different degrees of GWG and pre-pregnancy BMI, which may have influenced the results of the subgroup analysis.

Conclusion

Excessive GWG, especially during the second and third trimesters, was associated with increased thickening of the IVS. Thus, excessive GWG during the second and third trimesters is a risk factor for LV eccentric and concentric hypertrophy at 4 years of age in the offspring. Our results provide evidence supporting the early screening of LV geometry and function during early childhood, and emphasize the need for additional monitoring and weight management of pregnant mothers during the second and third trimesters.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the research ethics boards of Shanghai Xinhua Hospital (the coordination center, approved on August 23, 2013, ref no. M2013-010). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

JW, BD, and YW drafted and revised the manuscript. JW, BD, YW, ZL, YW, SC, and KS contributed to the conception and design of the work. JW, BD, YW, ZL, and YY contributed to the acquisition of data. JW, BD, YW, QC, XZ, and ZL contributed to the analysis or interpretation of the data. YW, SC, and KS critically revised the manuscript. All authors gave their final approval and agreed to be accountable for all aspects of this work ensuring its integrity and accuracy.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2021.722385/full#supplementary-material

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Body Composition and Physical Fitness Affect Central Hemodynamics in Young Children

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Objective: Central hemodynamics are related to cardiovascular (CV) outcomes in adults, but associations with childhood CV risk remain unclear. The study aimed to investigate the association of obesity, physical activity, and fitness with parameters of central pulse wave reflection in young prepubertal children.

Methods: In this cross-sectional study, 1,324 primary school children (aged 7.2 ± 0.4 years) were screened for parameters of pulse wave reflection such as augmentation index (AIx), central pulse pressure (CPP), body mass index (BMI), and cardiorespiratory fitness (CRF) by standardized procedures for children.

Results: The mean AIx and AIx@75 were 22.2 ± 7.7 and 29.2 ± 9.2%, respectively. With each unit increase in BMI, AIx [−0.226 (−0.328; −0.125)%] and AIx@75 [−0.444(−0.660; −0.229)%] decreased, whereas peak forward pulse wave increased ($p < 0.001$). Increasing BMI was associated with higher CPP, but did not remain significant after adjustment for CRF and heart rate. One unit increase in CRF was associated with lower AIx@75 [−0.509(−0.844; −0.173)%, $p = 0.003$] and lower reflection magnitude [RM: −0.559 (−0.890; −0.227), $p = 0.001$], independent of body weight and height. Girls had significantly higher AIx, AIx@75, peak backward pulse wave, and RM compared with boys.

Conclusion: Childhood obesity was associated with higher CPP but lower augmentation of the reflected pulse wave in children. Assessment of central blood pressures appears to be a valuable asset to childhood CV risk screening. The validity of augmentation indices during childhood development and the association with early vascular aging in children need to be verified in long-term follow-up studies. Physical activity and fitness have the potential to improve vascular hemodynamics in susceptible children and, thus, counteract vascular aging.

Trial registry: ClinicalTrials.gov: Exercise and Arterial Modulation in Youth. **Identifier:** NCT02853747; URL: https://clinicaltrials.gov/ct2/show/NCT02853747.

Keywords: body mass index, physical fitness, central hemodynamics, children, early vascular aging

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INTRODUCTION

Structural and functional changes in large arteries, commonly assessed by arterial stiffness, are related to the pathogenesis of cardiovascular (CV) disease. Aortic augmentation index (AIx), measured by pulse wave analysis (PWA), is a non-invasive and validated vascular biomarker to assess large arterial stiffness (1, 2). AIx values depend on the relative contribution of forward and reflected pulse waves to blood pressure. In addition, the characteristic pulse wave can be separated into forward (Pf) and backward wave (Pb) to calculate reflection magnitude (RM). A previous meta-analysis demonstrated that central hemodynamic wave reflections are the main determinants of cardiovascular (CV) events and all-cause mortality (3). Obesity is one of modern day's main risk factors for the development of CV disease with an increasing global prevalence of physical inactivity and unhealthy dietary intake (4, 5). In adults, body fat has been associated with higher central and peripheral AIx (6). In contrast, body mass index (BMI) was negatively correlated with AIx in a healthy population (6). Fernberg et al. found that young Swedish adults with obesity and low physical fitness had higher AIx compared with peers without obesity (7). A meta-analysis from randomized controlled trials showed that high-intensity aerobic exercise has merits to improve arterial stiffness and wave reflection in adults (8). Childhood obesity and elevated blood pressure (BP) seem to play a key role in mediating a deleterious CV outcome later in life (9, 10). However, the mechanisms of early subclinical hemodynamic changes from childhood until adulthood are still poorly understood. A few studies measured central hemodynamic parameters in children. We recently demonstrated that childhood obesity, hypertension, and low physical fitness were associated with higher pulse wave velocity (11). There is evidence that obesity and elevated BP are predominantly associated with a higher Pf and Pb in children and adolescents (12, 13). Children with obesity have been reported to present with lower AIx compared with normal weight children (12). A negative correlation has been reported between body mass index (BMI) and central AIx (14). However, some studies found no association of childhood obesity with AIx (15, 16). Physical activity and fitness have the potential to counteract the development of childhood obesity and vascular impairments (17, 18). The association of physical activity and fitness with AIx, Pf, Pb, and RM and central BP have never been investigated in young children. Our study, for the first time, aimed to examine the association of body composition and cardiorespiratory fitness (CRF) with central hemodynamic parameters in young children.

METHOD

Study Design and Participants

Data were collected from the large-scale, cross-sectional EXAMIN YOUTH study (19). Inclusion criteria were that children between the ages of 6 and 8 years are allowed to participate in physical education lessons and had a letter of agreement from their parents. Children had to remain fasted in the morning of the medical test day. Physical fitness assessments took place on-site in regular physical education lessons. The study was designed according to the Guidelines for Good Clinical Practice of the Declaration of Helsinki, (20) and ethical approval was obtained by the Ethics Committee of the University of Basel (EKBB, Basel, No. 258/12). The manuscript conforms to The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Guidelines.

Measurements

Central Hemodynamics

PWA was performed using the oscillometric Mobil-O-Graph Monitor, which has been validated in adults (I.E.M. GmbH, Germany) (21–23). The oscillometric assessment of hemodynamic parameters is strongly associated with the conventional tonometric method in adults ($r = 0.71$, $p < 0.001$ for AIx) (21). The measurements were performed in a calm environment in a sitting position. Appropriate small-sized cuffs for children were placed on the left upper arm. After a 5-min resting period, calibration was conducted based on systolic BP. Afterward, PWA was performed and immediately checked for quality and inaccurate data. At least two valid measurements were used to calculate the mean and standard deviation of AIx, AIx corrected for heart rate (AIx@75), Pb, Pf, RM (defined as the ratio of the amplitude of the Pb to that of the Pf), central systolic BP (CSBP), central diastolic BP (CDBP), and central pulse pressure (CPP).

Anthropometrics

Anthropometric parameters were assessed in light sport clothes and no shoes. Body height was measured with a wall-mounted stadiometer (Seca 206; Seca, Basel, Switzerland). To assess body weight and body fat, an electrical impedance device (InBody 170 Biospace device; InBody Co., Seoul, Korea) was used. BMI was calculated as body weight in kilograms divided by the square of height. Children were classified in clinical relevant groups according to cut off points for BMI incorporating age and sex (24). Children with a BMI over the 85th percentile were categorized as overweight and over the 95th percentile as children with obesity.

Blood Pressure

BP was measured with an automated oscillograph (Oscillomate 9002, CAS Medical Systems, Branford, CT, USA), similar models of which have been validated in children (25, 26). After a rest of 5 min in a sitting position, five BP measurements were performed. The mean of the three measurements with the smallest variation was taken for further analysis. According to the population-based German KiGGs study (27) and the 2016 European guidelines (28), children were categorized in systolic and diastolic BP groups defined as normal BP (<90th percentile), high-normal BP (>90th percentile), and hypertension (>95th percentile).

Abbreviations: Aix, augmentation index; AIx@75, augmentation index for a heart rate of 75 bpm; BMI, body mass index; BP, blood pressure; CDBP, central diastolic blood pressure; CPP, central pulse pressure; CRF, cardiorespiratory fitness; CSBP, central systolic blood pressure; CV, cardiovascular; NO, nitric oxide: Pb, peak backward pulse wave; Pf, peak forward pulse wave; RM. reflection magnitude.

TABLE 1 | Population characteristics of the study.

Parameter	Total Mean ± SD	*n*	Boys Mean ± SD	*n*	Girls Mean ± SD	*n*	*p*
Age	7.2 ± 0.4	1324	7.2 ± 0.4	652	7.2 ± 0.3	672	0.164
Height (cm)	124.5 ± 5.6	1324	124.7 ± 5.3	652	124.2 ± 5.9	672	0.113
Weight (kg)	24.7 ± 4.8	1324	24.8 ± 4.5	652	24.6 ± 5.0	672	0.390
BMI (kg/m^2)	15.4 ± 2.2	1324	15.9 ± 2.1	652	15.8 ± 2.3	672	0.695
Percentage body fat (%)	15.4 ± 7.7	1324	13.7 ± 7.0	652	17.0 ± 8.0	672	<0.001
Heart rate (bpm)	85.7 ± 10.4	1324	85.1 ± 10.2	652	86.2 ± 10.5	672	0.060
Systolic BP (mmHg)	103.8 ± 7.8	1324	103.8 ± 7.6	652	103.8 ± 7.9	672	0.998
Diastolic BP (mmHg)	64.1 ± 6.9	1324	64.1 ± 6.9	652	64.1 ± 6.9	672	0.829
Mean arterial BP (mmHg)	77.3 ± 6.5	1324	77.4 ± 6.6	652	77.3 ± 6.7	672	0.880
Central systolic BP (mmHg)	91.6 ± 8.0	1324	91.3 ± 8.0	652	91.9 ± 8.0	672	0.161
Central diastolic BP (mmHg)	61.6 ± 6.7	1324	61.6 ± 6.7	652	61.6 ± 6.6	672	0.880
Central pulse pressure (mmHg)	30.0 ± 6.0	1324	29.7 ± 6.1	652	30.3 ± 5.9	672	0.082
AIx (%)	22.2 ± 7.7	1324	19.5 ± 7.4	652	24.7 ± 7.1	672	<0.001
AIx@75 (%)	29.2 ± 9.2	1324	26.1 ± 9.2	652	32.5 ± 8.2	672	<0.001
Pf (mmHg)	20.4 ± 3.6	1324	20.5 ± 3.7	652	20.4 ± 3.5	672	0.597
Pb (mmHg)	12.2 ± 3.0	1324	12.0 ± 3.1	652	12.4 ± 2.9	672	0.016
RM	59.5 ± 8.8	1324	58.4 ± 9.7	652	60.6 ± 7.7	672	<0.001
20-m Shuttle Run (stages)	3.8 ± 1.5	1324	4.0 ± 1.6	652	3.4 ± 1.3	672	<0.001
Vigorous physical activity (min/day)	67.8 ± 50.5	766	78.1 ± 54.1	307	57.0 ± 44.1	315	<0.001
Screen time (min/day)	55.5 ± 60.4	904	58.1 ± 56.5	356	52.7 ± 64.0	377	0.180

BMI, body mass index; BP, blood pressure; AIx, Augmentation index; AIx@75, Augmentation index normalized for a heart rate of 75 bpm; Pf, peak forward pressure; Pb, peak backward pressure; RM, reflection magnitude; PWV, pulse wave velocity; SD, standard deviation.

Cardiorespiratory Fitness, Physical Activity, and Screen Time

The 20-m shuttle run test was performed to assess CRF. The validated and well-established 20-m shuttle run is an indicator of CRF and maximal endurance exercise capacity (29, 30). After a short warm-up, children had to run continuously between two 20-m lines back and forth as long as possible. The running speed was synchronized with acoustic bleep signals. The running velocity was increased every stage of 1 min by 0.5 km/h from an initial speed of 8 km/h. The test score was achieved if a child failed to cross the lines twice in a row. The number of stages reached (with an accuracy of 0.5 stages) was used for further analysis. Parents were asked to complete a questionnaire about the physical activity level and screen time of their children, based on our previous studies (31, 32). Physical activity was defined by time spent in vigorous physical activity (min/day). Screen time was assessed by questions included watching TV, playing computer or video games, and playing on a smartphone (min/day).

Statistical Analysis

The residuals were analyzed by using Tukey–Anscombe plots and normal QQ plots to assess variance homogeneity and normality. Multilinear regression analysis was performed to analyze central hemodynamic parameters (AIx, AIx@75, Pf, Pb, RM, CSBP, CDBP, CPP) with body composition and physical activity/fitness. Different models were fitted to adjust for age and sex as well as body weight, height, and shuttle run. Univariate analysis of variance (ANOVA) was used to compare differences of central hemodynamic parameters between BMI and BP categories. A two-tailed *p*-value of 0.05 indicates statistical significance, and 95% confidence intervals were presented for measures of effect of uncertainty. Normal density curve was presented as a histogram of continuous variables of AIx and AIx@75. Statistical analyses were performed with an up-to-date version of Stata 15 (StataCorp LP, College Station, TX, USA).

RESULTS

Population characteristics are shown in **Table 1**. Overall, 3,068 school children received an invitation to take part in this large-scale cross-sectional study. From 1,690 children with written consent from their parents, 366 children were ill at the day of examination or had insufficient quality of hemodynamic data. In total, 1,324 children completed all measurements. Parents (420) did not return the questionnaire, leaving 904 children with complete data including questionnaires. **Figure 1** shows the flow diagram. According to the BMI categories, 87% ($n = 1,154$) were of normal weight, 10% ($n = 126$) were overweight, and 3% ($n = 44$) were children with obesity.

Based on peripheral systolic BP, 77% ($n = 1,023$) of the children had a normal BP, 9% ($n = 123$) were classified as children with a high-normal BP, and 13% ($n = 178$) were with hypertension. Based on peripheral diastolic BP, 78% ($n = 1,030$) were categorized as children with a normal BP, 8% ($n = 112$) as high normal, and 14% ($n = 182$) were children

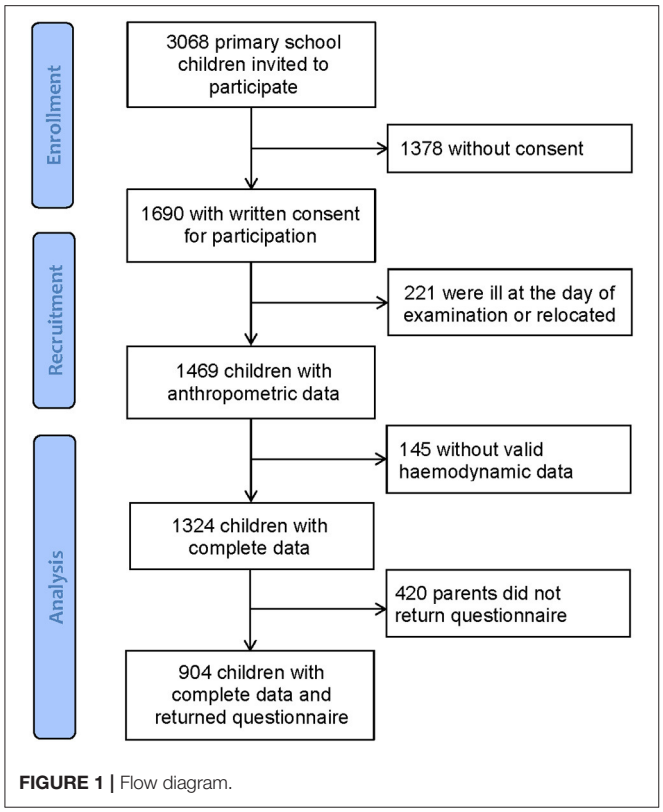

FIGURE 1 | Flow diagram.

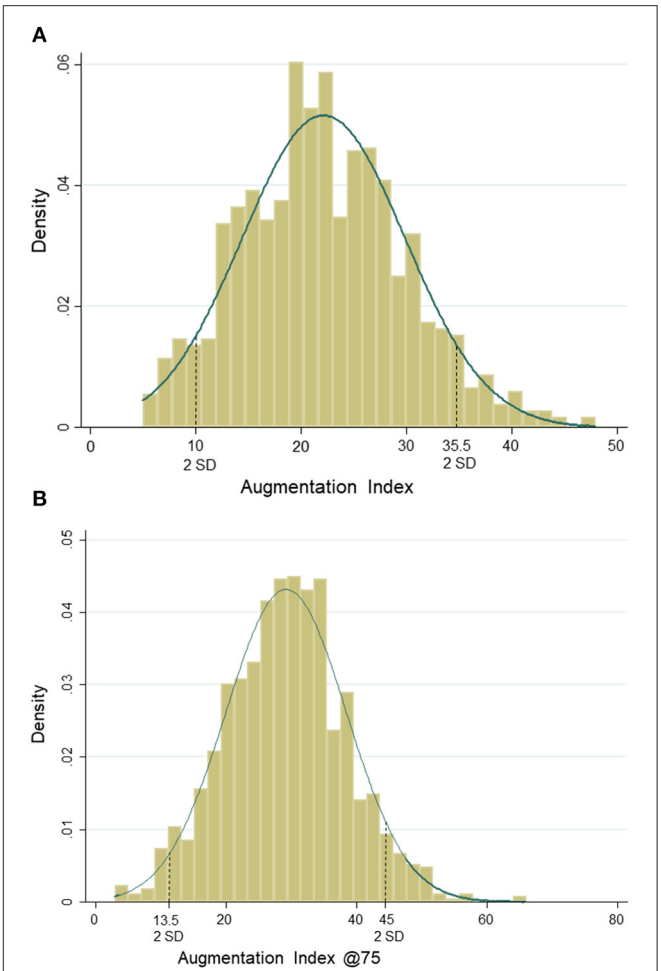

FIGURE 2 | (A) Normal distribution of augmentation index. **(B)** Normal distribution of augmentation index@75.

with hypertension. In total, 7% ($n = 90$) of the children were categorized as children with both systolic and diastolic hypertension. In our study, 89% of children were Caucasian. Girls had significantly higher AIx, AIx@75, Pb, RM, percentage body fat, and lower CRF and vigorous physical activity compared with boys. Mean AIx and AIx@75 were 22.2 ± 7.7 and 29.2 ± 9.2, respectively. Normal density curves of AIx and AIx@75 are presented in **Figures 2A,B**.

Regression Analysis

The regression analysis for parameters of pulse wave reflection is shown in **Table 2**. Lower AIx ($p < 0.001$) and AIx@75 ($p < 0.001$) were found per unit increase in BMI, independent of CRF and heart rate. Higher BMI was associated with higher Pf ($p < 0.001$) and lower RM ($p < 0.001$), even after adjustment for confounders. One-unit increase in body height was independently related to lower AIx, AIx@75, Pb, and RM ($p < 0.001$). After adjustment for body height, body weight was associated with lower AIx@75 ($p = 0.019$), higher Pf ($p < 0.001$), and Pb ($p = 0.028$). One-unit increase in percentage body fat was associated with lower AIx ($p < 0.022$) and higher Pf ($p < 0.001$). After adjustment for body height and weight, higher CRF was associated with lower AIx@75 ($p = 0.003$) and RM ($p < 0.001$).

Linear associations of AIx ($p = 0.044$), AIx@75 ($p = 0.001$), Pb ($p = 0.041$), and RM ($p = 0.004$) with screen time were found. **Table 3** shows regression analysis for the association of body composition, physical activity, and fitness with central BP and CPP. One-unit increase in BMI was independently associated with higher CSBP ($p < 0.001$) and CDBP ($p < 0.001$). Higher CPP was associated with BMI ($p < 0.010$), but not independent of CRF and heart rate. Percentage body fat was associated with higher CSBP ($p < 0.001$) and CDBP ($p < 0.001$) and CPP ($p = 0.003$). One-unit increase in shuttle run (stages) was associated with lower CSBP and CDBP ($p < 0.001$), but the results did not remain significant after adjustment for body height, body weight, and heart rate.

Group Differences Across Clinical Categories

Central hemodynamic parameters of pulse wave reflection in relation to clinical categories of BMI and BP are shown in **Supplementary Table 1**. BMI categories were not associated with AIx and AIx@75. Children with overweight and obesity had higher Pf compared with peers with normal weight ($p = 0.001$). According to systolic BP categories, children with high-normal BP and hypertension showed higher AIx@75 compared with children with normal BP ($p < 0.001$). Pf ($p = 0.002$) and Pb (p

TABLE 2 | Regression analysis for the association of body composition, physical activity, and fitness with parameters of pulse wave reflection.

Parameter	Model	Aix (% change per unit)		AIx@75 (% change per unit)		Pf (mmHg change per unit)		Pb (mmHg change per unit)		RM (change per unit)	
		B (95% CI)	*p*	B (95% CI)	*p*	B (95% CI)	*p*	B (95% CI)	*p*	B (95% CI)	*p*
Body height (cm)	1	−0.292 (−0.364; −0.221)	<0.001	−0.347 (−0.433; −0.262)	<0.001	0.035 (−0.002; −0.071)	0.061	−0.073 (−0.102; −0.043)	<0.001	−0.459 (−0.543; −0.374)	<0.001
	2	−0.226 (−0.328; −0.125)	<0.001	−0.256 (−0.377; −0.135)	<0.001	−0.040 (−0.091; −0.011)	0.123	−0.109 (−0.151; −0.068)	<0.001	−0.423 (−0.542; −0.304)	<0.001
Body weight (kg)	1	−0.285 (−0.368; −0.202)	<0.001	−0.331 (−0.430; −0.232)	<0.001	0.089 (0.047; 0.130)	<0.001	−0.023 (−0.057; 0.012)	0.199	−0.371 (−0.470; −0.272)	<0.001
	3	−0.113 (−0.235; 0.008)	0.068	−0.174 (−0.319;−0.029)	0.019	0.124 (0.063; 0.185)	<0.001	0.056 (0.006; 0.106)	0.028	−0.084 (−0.227; 0.059)	0.249
BMI (kg/m^2)	1	−0.398 (−0.578; −0.217)	<0.001	−0.444 (−0.660; −0.229)	<0.001	0.191 (0.102; 0.280)	<0.001	0.032 (−0.042; 0.106)	0.398	−0.402 (−0.619; −0.185)	<0.001
	4	−0.425 (−0.617; −0.234)	<0.001	−0.563 (−0.791; −0.335)	<0.001	0.198 (0.104; 0.293)	<0.001	0.008 (−0.071; 0.086)	0.849	−0.535 (−0.765; −0.305)	<0.001
Percentage body fat (%)	1	−0.064 (−0.117;−0.011)	0.018	−0.017 (−0.081; 0.047)	0.602	0.050 (0.024; 0.076)	<0.001	0.029 (0.007; 0.050)	0.010	−0.010 (−0.074; 0.055)	0.770
	4	−0.069 (−0.128; −0.010)	0.022	−0.048 (−0.119; 0.022)	0.178	0.054 (0.025; 0.083)	<0.001	0.023 (−0.001; 0.047)	0.061	−0.046 (−0.117; 0.025)	0.205
20-m shuttle run (stages)	1	0.094 (−0.177; 0.365)	0.496	−0.240 (−0.564; 0.083)	0.146	−0.068 (−0.202; 0.065)	0.317	−0.111 (−0.221; −0.5E−3	0.049	−0.314 (−0.640; 0.011)	0.058
	5	−0.104 (−0.385; 0.178)	0.470	−0.509 (−0.844; −0.173)	0.003	0.039 (−0.103; 0.180)	0.592	−0.098 (−0.214; 0.019)	0.100	−0.559 (−0.890; −0.227)	0.001
Vigorous physical activity (min/day)	1	−0.012 (−0.022; −0.001)	0.029	−0.010 (−0.022; 0.002)	0.111	0.001 (−0.004; 0.006)	0.628	−0.001 (−0.006; 0.003)	0.584	−0.013 (−0.025; 0.3E−3)	0.055
	5	−0.009 (−0.019; −0.001)	0.075	−0.007 (−0.019; 0.005)	0.233	0.6E−3 (−0.005; 0.006)	0.827	−0.001 (−0.005; 0.003)	0.641	−0.010 (−0.022; 0.003)	0.126
Screen time (min/day)	1	0.007 (−0.9E−3; 0.015)	0.085	0.014 (0.005; 0.024)	0.003	0.002 (−0.002; 0.006)	0.368	0.004 (0.7E−3; 0.007)	0.018	0.013 (0.004; 0.023)	0.007
	5	0.008 (0.2E−3; 0.016)	0.044	0.016 (0.006; 0.025)	0.001	0.7E−3 (−0.003; 0.005)	0.733	0.003 (0.1E−3; 0.007)	0.041	0.014 (0.005; 0.023)	0.004

Model 1, adjusted for age and sex; Model 2, model 1 plus adjusted for body weight and shuttle run; Model 3, model 1 plus adjusted for body height and shuttle run; Model 4, model 1 plus adjusted for shuttle run; Model 5, model 1 plus adjusted for body height and body weight.

BMI, body mass index; AIx, augmentation index; AIx@75, augmentation index normalized for a heart rate of 75 bpm; Pf, peak forward pressure; Pb, peak backward pressure; RM, reflection magnitude; CI, confidence interval.

TABLE 3 | Regression analysis for the association of body composition, physical activity, and fitness with central blood pressure and pulse pressure.

Parameter	Model	CSBP (mmHg change per unit)		CDBP (mmHg change per unit)		CPP (mmHg change per unit)	
		B (95% CI)	*p*	B (95% CI)	*p*	B (95% CI)	*p*
Body height (cm)	1	0.247 (0.168; 0.327)	<0.001	0.319 (0.254; 384)	<0.001	−0.071 (−0.132; −0.010)	0.022
	2	−0.179 (−0.284; −0.073)	0.001	0.005 (−0.081; 0.091)	0.910	−0.184 (−0.270; −0.099)	<0.001
Body weight (kg)	1	0.579 (0.493; 0.666)	<0.001	0.541 (0.470; 0.613)	<0.001	0.040 (−0.030; 0.109)	0.264
	3	0.721 (0.595; 0.848)	<0.001	0.552 (0.449; 0.654)	<0.001	0.172 (0.069;0.274)	0.001
BMI (kg/m^2)	1	1.273 (1.087; 1.460)	<0.001	1.084 (0.929; 1.239)	<0.001	0.194 (0.044; 0.344)	0.011
	4	1.249 (0.1.052; 1.445)	<0.001	1.093 (0.931; 1.260)	<0.001	0.159 (−0.4E−3; 0.319)	0.051
Percentage body fat (%)	1	0.375 (0.320; 0.430)	<0.001	0.299 (0.253; 345)	<0.001	0.078 (0.034; 0.121)	0.001
	4	0.370 (0.310; 0.430)	<0.001	0.300 (0.248; 0.349)	<0.001	0.073 (0.024; 0.122)	0.003
20-m shuttle run (stages)	1	−0.824 (−1.117; −0.530)	<0.001	−0.618 (−0.863; −0.372)	<0.001	−0.213 (−0.437; 0.012)	0.063
	5	−0.122 (−0.417; 0.172)	0.415	0.007 (−0.232; 0.246)	0.958	−0.133 (−0.371; 0.105)	0.274
Vigorous physical activity (min/day)	1	0.008 (−0.003; 0.019)	0.150	0.009 (−0.001; 0.018)	0.080	−0.3E−3 (−0.009; 0.008)	0.942
	5	0.003 (−0.008; 0.014)	0.564	0.003 (−0.005; 0.012)	0.451	−0.4E−3 (−0.009; 0.008)	0.934
Screen time (min/day)	1	0.014 (0.005; 0.022)	0.002	0.007 (0.5E−8; 0.015)	0.050	0.007 (0.6E−4; 0.013)	0.048
	5	0.007 (−0.002; 0.015)	0.133	0.001 (−0.006; 0.008)	0.737	0.006 (−0.001; 0.012)	0.100

Model 1, adjusted for age and sex; Model 2, model 1 plus adjusted for body weight, shuttle run, and heart rate; Model 3, model 1 plus adjusted for body height, shuttle run, and heart rate; Model 4, model 1 plus adjusted for shuttle run and heart rate; Model 5, model 1 plus adjusted for body height, body weight, and heart rate.
BMI, body mass index; CSBP, central systolic blood pressure; CDBP, central diastolic blood pressure; CPP, central pulse pressure; CI, confidence interval.

= 0.001) increased according to increasing systolic BP categories. Young children with diastolic hypertension showed higher AIx (p = 0.043) and AIx@75 ($p < 0.001$) compared with children with high-normal and normal BP.

DISCUSSION

This study is the first to analyze the association of body composition and physical fitness with central hemodynamics in a large cohort of young prepubertal children. Higher BMI was associated with lower AIx, AIx@75, and RM. Higher body weight and body fat were independently related to higher CSBP, CDBP, and CPP. Physical fitness was associated with favorably lower AIx@75, RM, Pb, CSBP, and CDBP.

In line with the results of our large cohort of young children, a previous smaller-sized study found that a lower AIx and a higher Pf in children with obesity compared with normal weight peers (12). Indeed, findings on the association of BMI with central pulse wave velocity as a marker of arterial stiffness are inconsistent (11, 16, 33, 34). Studies in adolescents found no association of childhood obesity with AIx and AIx@75 (15, 16). Childhood growth and development during puberty and adolescence may be responsible for the loss of associations of body composition with parameters of pulse wave reflection. Indeed, a premature decline of arterial stiffness has been suggested due to accelerated growth and early onset of puberty (35).

We have previously analyzed the association of BMI with central pulse wave velocity in the same population of primary school children (11). One unit increase in BMI was independently related to a higher pulse wave velocity in this cohort of children [0.027 (0.010; 0.034), $p < 0.001$]. In contrast to these previous findings, we have now found an inverse association of BMI with AIx, which appears conflicting and remains to be clarified in future studies.

With respect to the AIx and AIx@75, body height and heart rate need to be discussed as potential influencing factors for lower augmentation in children with higher BMI. We demonstrated that body weight is associated with AIx, but not independent of body height. In our cohort, children with obesity were significantly taller than children with normal weight (data not shown). Body height seems to be a determinant for a lower AIx in children with a higher body weight. Higher Pf and Pb were related to body weight, independent of body height. A lower AIx@75 was independently associated with higher body weight.

Moreover, our findings indicate that not only body height but also heart rate seems to be a key factor for determining AIx, but less so for AIx@75 as this is a set value at a heart rate of 75/min. In our cohort of young children, similar values of AIx and AIx@75 were found compared with values in older adults (36). It may mainly be explained by short stature of children and less so by higher heart rate (mean 85.7 ± 10.4 bpm) in children at a young age. Potential inaccuracy of the oscillometric device in children may add to this phenomenon (37).

Our study is the first to assess AIx and AIx@75 in a large population-based unselected cohort of 6–8 year-old children, offering reliable normal values for young Caucasian children (mean AIx: 22.2 ± 7.7% and AIx@75: 29.2 ± 9.2%). We have recently demonstrated that low physical fitness and sedentary behavior seem to play a key role in the development of micro- and macrovascular impairments in young children (11). These results are in line with our current findings in pulse wave reflection. Higher CRF and lower physical inactivity (screen time) were associated with a favorably lower AIx@75 and RM. A higher AIx and Pf were found per unit increase of screen time. Independent of body composition and physical activity level, elevated peripheral BP affected alterations in central pulse wave parameters. Previous studies showed that elevated BP is associated with a higher Pf and Pb in children and adolescents (12, 13). Similar to these results, we found that children with systolic hypertension had higher AIx@75, Pf, and Pb. A few studies investigated the association of early life conditions with central BP and CPP. In our study, higher body weight and percentage body fat were independently associated with higher CSBP, CDBP, and CPP. We found that CRF was associated with lower CSBP and CDBP, but not independent of body weight and height. Girls had a higher AIx, AIx@75, Pb, RM, and percentage body fat but lower CRF and vigorous physical activity compared with boys. Similar to our findings, a recent study demonstrated that AIx@75 was higher in girls compared with that in boys before puberty (38). The authors argued that early gender differences in aortic geometry and growth are potential reasons for these findings (38). Besides childhood growth, low CRF seems to be a determinant for the development of higher AIx, AIx@75, Pb, and RM in girls. Our results suggest that improving CRF, often accompanied by weight loss, has the potential to ameliorate large-artery hemodynamics in young children.

Some of the potential pathophysiological mechanisms need to be discussed. Central hemodynamic impairments and obesity-related inflammation are mediated through oxidative stress conditions (39). Oxidative stress plays a major role in nitric oxide (NO) bioavailability, a main determinant of vascular tone regulation and vasodilation. Lower levels of NO are, therefore, likely to affect pulse wave reflection. Low CRF and physical inactivity are characterized by reduced mitochondrial capacity and increased oxidative stress, and regular physical exercise has the potential to improve oxidative conditions and NO bioavailability (40).

This study has some limitations that need to be addressed. The study is designed as a cross-sectional investigation and does not examine temporal development of the associations. A long-term follow-up is warranted to confirm causal associations between lifestyle-related risk factors and the development and progression of vascular properties. Only three percent of children in our study were children with obesity. Central hemodynamics may need to be investigated in an obesity-enriched cohort. Our study was performed in a predominant Caucasian population. Future studies have to prove our findings in other ethnic populations. Moreover, it needs to be stressed that measurements of central hemodynamics have been validated in adults but not in children. Recent reports indicated that oscillometric as well as tonometric devices may not be accurate indices of invasively measured AIx in children (37). From the KidCoreBP study, it also appears that MobilOgraph may overestimate peripheral and central BP (41). However, potential inaccuracy of central BP measurement would be systematic in nature, and associations with risk would remain representative in a population-based approach. Our data suggests that using AIx in children should be handled with caution. Finally, a potential selection bias between participants and non-participants cannot be excluded. One strength of the study is the large number of participants. During childhood, age-related changes in vascular structure and function occur rapidly. Growth and developmental effects are minimized by the large sample size and limited age range of children aged 6–8 years.

CONCLUSION

In summary, our results demonstrate that higher BMI in young pre-pubertal children is associated with lower augmentation of the reflected pulse wave. Compared with pulse wave velocity, AIx is more susceptible to factors such as body height and heart rate, which vary greatly throughout inter-individual childhood development. Our results offer normal values of central hemodynamics for a foremost Caucasian population of 6–8-year-old children. Nonetheless, it has to be stated that the use of AIx in childhood needs to be handled with caution. Assessment of central BP appears to be a more valuable asset to childhood CV risk screening. The clinical relevance and predictive value of augmentation indices during childhood development and the association with early vascular aging and CV risk in adulthood remain to be verified in long-term follow-up studies. Based on the available literature and our previous findings, pulse wave velocity seems a more robust marker to be included in childhood CV risk screening. Our results do support recommendations for physical activity programs to be included in childhood primary prevention strategies to improve childhood vascular health and counteract early vascular aging in susceptible children.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

This study was reviewed and approved by Ethics Committee of North-West Switzerland: No.258/12. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

SK performed the recruitment, data collection and analysis, and prepared the manuscript draft. AD, CH, LS, AS-T, and OF revised the manuscript and approved the final draft. HH conceptualized the study, supported the data analysis, revised the manuscript, and approved the final draft. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2021.750398/full#supplementary-material

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Early Vascular Aging in Children With Tuberous Sclerosis Complex

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Objectives: Experimental data indicate that activating mutations in the mTOR (mammalian target of rapamycin) pathway may lead to abnormal arterial wall structure. Vascular anomalies like arterial stenoses are reported in pediatric patients with tuberous sclerosis complex (TSC). In addition, large renal lesions (angiomyolipoma—AML and cysts) are risk factors for arterial hypertension in adult patients with TSC. This study aimed to assess blood pressure, including central blood pressure and arterial damage (early vascular aging—EVA) in children with TSC.

Materials and Methods: In a group of 33 pediatric patients with TSC (11.13 ± 4.03 years, 15 boys, 18 girls), we evaluated peripheral and central office blood pressure, 24-h ambulatory blood pressure, and arterial damage: aortic pulse wave velocity (aPWV) [m/s], [*Z*-score], augmentation index (AIx75HR [%]), common carotid artery intima-media thickness (cIMT) [mm], [*Z*-score], stiffness of common carotid artery (E-tracking), renal lesions in magnetic resonance and ultrasonography, and selected biochemical parameters. The control group consisted of 33 healthy children (11.23 ± 3.28 years, 15 boys, 18 girls).

Results: In TSC group 7 (21.2%) children had arterial hypertension, 27 (81.8%) children had renal angiomyolipomas, 26 (78.8%)—renal cysts, and 4 (12.1%) patients were treated with mTOR inhibitors (2 patients with everolimus and 2 patients with sirolimus) at the moment of evaluation. Children with TSC had higher central systolic blood pressure (AoSBP) (98.63 ± 9.65 vs. 90.45 ± 6.87 [mm Hg], $p < 0.001$), cIMT (0.42 ± 0.05 vs. 0.39 ± 0.03 [mm], $p = 0.011$), cIMT *Z*-score (0.81 ± 1.21 vs. 0.16 ± 0.57, $p = 0.007$), aPWV (4.78 ± 0.81 vs. 4.25 ± 0.56 [m/s], $p = 0.003$) and aPWV *Z*-score (−0.14 ± 1.15 vs. −0.96 ± 0.87, $p = 0.002$) compared to healthy children, without differences in AIx75HR (8.71 ± 15.90 vs. 5.24 ± 11.12 [%], $p = 0.319$) and stiffness of common carotid artery. In children with TSC AoSBP correlated positively with serum cystatin C concentration ($r = 0.377$, $p = 0.030$) and with maximum diameter of renal cyst ($R = 0.419$, $p = 0.033$); mean arterial pressure (MAP) 24 h *Z*-score correlated with serum cystatin C concentration ($R = 0.433$, $p = 0.013$); and aPWV *Z*-score with daily urinary albumin loss [mg/24 h] ($R = 0.412$, $p = 0.029$).

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Conclusions: Children with tuberous sclerosis complex are at risk of elevated central blood pressure and early vascular aging. In children with TSC, blood pressure and arterial stiffness are related to renal involvement.

Keywords: tuberous sclerosis complex, early vascular aging, central blood pressure, arterial stiffness, common carotid artery intima-media thickness, arterial hypertension, children

INTRODUCTION

Tuberous sclerosis complex (TSC, Bournevile-Pringle disease) is an autosomal dominant disorder found in 1:5,800–1:12,500 births. At present, TSC is diagnosed on the basis of recently updated 2021 International Tuberous Sclerosis Complex Consensus Group (ITSCCG) genetic and clinical criteria (1). The mutation in tumor suppressor genes: *TSC1* (chromosome 9q34) or *TSC2* (chromosome 16p13) are found in 85–90% of the TSC patients; remaining individuals were found to have mutations in non-coding regions of the genes or to show genetic mosaicism (2). In most patients, family history is negative (*de novo* mutation); approximately 30% of cases are inherited from one of the affected parents (3). *TSC1* and *TSC2* genes encode natural inhibitors of the mTOR (mammalian target of rapamycin) signaling pathway, i.e., hamartin and tuberin, respectively. Constant activation of the mTOR pathway leads to uncontrolled cell proliferation and formation of hamartomas, benign neoplasms, and rarely, malignant neoplasms in virtually all parts of the body. Thus, the spectrum of TSC-associated disorders includes skin lesions (e.g., depigmented spots, facial angiofibromas, shagreen patches, seen in ∼90% of patients), retinal lesions (87% of patients), central nervous system abnormalities (70–90% of patients), heart (self-limiting rhabdomyoma found in approximately half of the patients), lungs (angiomyolipoma and lymphangioleiomyomatosis present mainly in women) and, finally, diversified renal lesions (1).

Renal abnormalities are present in as many as 50–80% of TSC individuals and are the second cause of mortality (just after brain tumors) in these patients. The spectrum of renal abnormalities involves angiomyolipomas (AML)—present in 55–90% of patients with kidney lesions, renal cysts, glomerulocystic disease, oncocytoma, and renal cell carcinoma (RCC) (4, 5). Angiomyolipomas belong to a family of neoplasms called perivascular epithelioid cell tumors. They can be classified histologically as typical (triphasic or lipid-rich) or atypical or fat-poor (monophasic or epithelioid). Typical, lipid-rich AMLs are benign tumors histologically characterized by (in varying proportions) proliferation of spindle cells, epithelioid cells, and adipocytic cells in concert with many abnormal, thick-walled blood vessels (6). AMLs pose a risk of life-threatening spontaneous bleeding (Wünderlich syndrome) once the tumor's diameter exceeds 30 mm (1, 6).

Experimental data indicate that activating mutations in the mTOR pathway may lead to degenerative phenotype and uncontrolled proliferation of vascular smooth muscle cells (SMC) and abnormal structure of arterial wall (7, 8). Arterial stenoses (e.g., renal artery stenosis, mid-aortic syndrome) or aneurysms have been reported in children and adults with TSC (9–12). In addition, large renal lesions are risk factors for arterial hypertension in adult patients with TSC (3, 13). These factors might theoretically put children with TSC at risk of arterial damage and early vascular aging (EVA), a state of accelerated adverse changes in the biochemical and cellular components of the vascular tree (14, 15). However, to the best of our knowledge, there are no reports on arterial damage in both children and adults with TSC. Thus, this study aimed to assess whether children with TSC are at risk of EVA and evaluate potential arterial damage determinants in this group of patients.

MATERIALS AND METHODS

Study Group

This single-center cross-sectional study included patients with TSC treated in 2018–2021 in one tertiary center of pediatric nephrology. The criterion for inclusion in the study was confirmed TSC following ITSCCG recommendations (1, 16). The exclusion criteria were: height below 120 cm or acute infectious disease (temporary 2-week exclusion). Thirty-three age- and sex-matched healthy subjects were included in the control group. Healthy children were recruited from patients of outpatient University Hospital. Participation in the study was proposed with the following exclusion criteria: height below 120 cm, acute infectious disease, known chronic illness (including kidney, heart, and inflammatory disease), obesity, and arterial hypertension. The flowcharts of the study and control groups are presented in **Figures 1**, **2**, respectively.

The authors obtained approval from the local Bioethical Committee before initiating the research (approval no. KB/145/2017, 4th July 2017). All procedures involving human participants were in accordance with the highest ethical standards of the institutional research committee and were performed according to the Declaration of Helsinki on the treatment of human subjects and its later amendments. Informed consent was obtained from all participants' representatives and participants (≥16 years) before enrolling in the study.

Clinical and Biochemical Parameters

Based on individual medical records, we evaluated the following clinical parameters in all TSC individuals: family history of tuberous sclerosis complex, presence of TSC-related neurological symptoms: developmental delay and epilepsy, presence of arterial hypertension, and medications used, including mTOR inhibitors, antiepileptic and antihypertensive drugs. In all children with TSC, the following basic anthropometric parameters were assessed: age [years], sex, body height [cm], body weight [kg], and body mass index [kg/m^2]. Anthropometric parameters were compared with the standards for the Polish population and

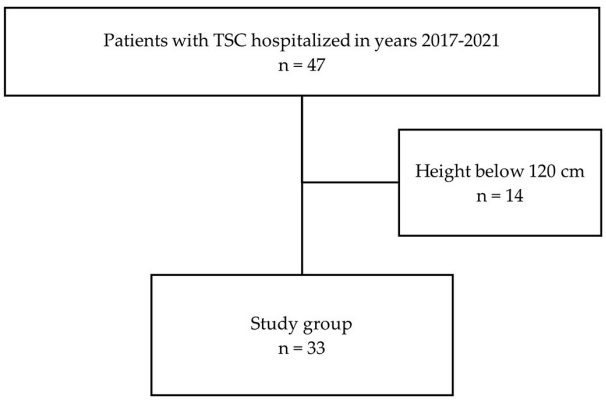

FIGURE 1 | Flowchart of the patients' recruitment (TSC—tuberous sclerosis complex).

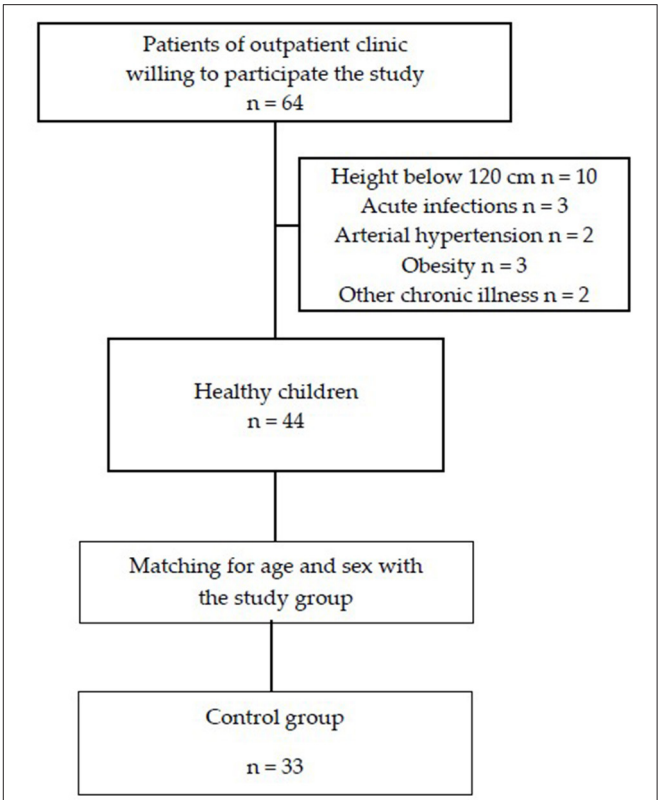

FIGURE 2 | Flowchart of the control group recruitment.

presented in the form of a *Z*-score (17). According to WHO recommendations, overweight and obesity were defined as a BMI *Z*-score above 1 and 2, respectively.

All TSC patients had following biochemical parameters evaluated: serum concentrations of creatinine [mg/dL], urea [mg/dL], cystatin C [ng/mL], uric acid [mg/dL], total, HDL- and LDL-cholesterol [mg/dL], and triglyceride [mg/dL]. Also, daily urinary albumin loss was assessed in all the children. The estimated glomerular filtration rate (eGFR) was calculated in all patients [mL/min/1.73 m^2] according to the revised 2009 Schwartz formula (18). Impaired renal function was defined as eGFR below 60 mL/min/1.73 m^2 [which responses to chronic kidney disease 3G according to (19)], and hyperfiltration was diagnosed when eGFR was equal or exceeded 140 mL/min/1.73 m^2 (20). Normal values of cholesterol and triglycerides were taken from the Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents; National Heart, Lung, and Blood Institute (21), and hyperuricemia was recognized when uric acid was $\geq$5.5 [mg/dL] (22). Elevated urinary albumin loss was defined as daily albuminuria >30 mg/24 h.

Blood Pressure and Parameters of Arterial Damage

Blood pressure measurements were performed using the oscillometric method (Welch Allyn Patient Monitor, Welch Allyn, Skaneateles Falls, NY, USA) in line with ESH recommendations. They were analyzed using pediatric normative values ([mm Hg], *Z*-scores) (23, 24). According to the American Heart Association guidelines, all the TSC children also had 24 h ambulatory blood pressure measurement performed (Oscar 2 Suntech with Sphygmocor Inside, SunTech Medical Inc., Morrisville, NC, USA) (25). Systolic, diastolic, and mean blood pressures (SBP, DBP, MAP, respectively), blood pressure loads, and nighttime blood pressure dipping (DIP) were analyzed. DIP below 10% was considered as disturbed circadian rhythm (25). The blood pressure cuff was chosen following ESH recommendations and the devices' instructions (26).

The assessment for early vascular aging was performed using the following methods: an ultrasonographic examination of the common carotid artery (ALOKA Prosound Alpha 6, Hitachi Aloka Medical Ltd., Tokyo, Japan)—common carotid artery intima-media thickness (cIMT) [mm], *Z*-score (27), and common carotid artery local stiffness (E-tracking); applanation tonometry (Sphygmocor, ATCOR, Sydney, Australia)—central aortic blood pressure, pulse wave analysis, and aortic (carotid-femoral) pulse wave velocity (aPWV) [m/s], *Z*-score (28). All arterial measurements were performed by a single investigator (P.S.) in a quiet room with a controlled temperature (20 $\pm$ 5°C) after 5 min rest. cIMT was measured in all patients in a supine position using a manual method approximately 1 cm proximal to the carotid bulb on the distal carotid wall. Six cIMT measurements were obtained and averaged, three on the left and three on the right side. Peripheral pressure waveforms were recorded from the right radial artery at the wrist in a sitting position, and the transfer function was used to generate the central pressure waveform. aPWV was measured in a supine position and calculated as a difference in the carotid-to-femoral path length divided by the difference in R wave to the foot of the pressure wave taken from the superimposed ECG and pressure tracings. The path length was measured as the distance from the right carotid sampling site to the jugular notch, subtracted from the distance from the jugular notch to the right femoral sampling

site (27). Peripheral pressure waveform and aPWV were obtained three times, and the mean value was analyzed.

Evaluation of Renal Lesions

In all patients, abdominal ultrasonography was performed using a Philips Epiq 5G device (Royal Philips, Amsterdam, The Netherlands) in B-mode. Renal length [mm], echogenicity and corticomedullary differentiation, and the presence of renal parenchymal changes, including typical TSC lesions: angiomyolipoma (AML) and cysts were evaluated. The longest dimension of the largest lesion [mm] was assessed for AML and cysts. In patients diagnosed with arterial hypertension duplex Doppler ultrasonography was performed to exclude renal artery stenosis. In 24/33 (72.7%) patients, magnetic resonance imaging (MRI) of the abdomen was performed with a MAGNETOM Skyra 3T 3-tesla scanner (Siemens AG, Berlin, Germany) in T2-weighted, DWI, and T1-weighted sequences before and after intravenous administration of the contrast agent—Gadovist (gadobutrol) (Bayer AG, Leverkusen, Germany). Renal length [mm], renal cortical signal and differentiation, and presence of renal parenchymal lesions (angiomyolipomas, cysts) with the assessment of the largest dimension of the largest lesion [mm] were evaluated. Fat-poor AMLs were recognized according to the Polish Society of Nephrology recommendations (29). In case of discrepancy between MRI and ultrasound findings, MRI findings were considered conclusive.

Statistical Analysis

The results were statistically analyzed using TIBCO Statistica 13.3 software (TIBCO Software Inc., Palo Alto, CA, USA). The normality of variables was studied using the Shapiro–Wilk test. The numerical data obtained were presented as mean and standard deviation (SD) (normally distributed data) or median and interquartile range (IQR, Q1–Q3) (non-normally distributed data). Normally distributed data were compared with Student *t*-test for independent groups and non-normally distributed data using the Mann–Whitney U test. The relationship between the two groups of variables was analyzed using Pearson correlation or Spearman rank correlation (depending on the distribution). Percentages in both groups were compared using the chi-square test. A p-value <0.05 was considered statistically significant.

RESULTS

Clinical and Biochemical Parameters of Children With Tuberous Sclerosis Complex

Clinical and biochemical parameters in the studied children were presented in **Table 1**. In the group of 33 children with TSC, there were 4 (12.1%) overweight patients and no obese children. Three (9.1%) patients had previously diagnosed arterial hypertension—all these patients were treated with angiotensin-converting enzyme inhibitors (2 with enalapril, 1 with ramipril). In addition, arterial hypertension was diagnosed at the moment of evaluation in 4 patients based on abnormal ambulatory blood pressure monitoring (including three masked hypertension patients). Most of the children had developmental delay and epilepsy. Four (12.1%) patients were treated with mTOR inhibitors. Three children received mTOR inhibitors due to large (>3 cm) renal angiomyolipomas (rapamycin in 2 and everolimus in 1), and one child was treated with everolimus due to the presence of non-operative SEGA tumors. Most patients had kidney involvement revealed in MRI or US. There were only 3 (9.1%) children without renal lesions. The largest AML (71 mm in maximal diameter) was found in a 17-year-old female patient who started rapamycin treatment. Two male patients aged 7.7 and 9.3 years had genetically confirmed contagious genes syndrome (deletion involving TSC2 and PKD1 genes) with very large renal cysts (maximal cyst diameter −35 and 44 mm, respectively). Impaired renal function was found in none of the patients, but in 11 (33.3%) children, hyperfiltration was revealed. Hyperuricemia was found in 6 (18.2%) children with the highest uric acid concentration 6.3 mg/dL. Acceptable total cholesterol (<170 mg/dL) was found in 26 (78.8%), borderline high total cholesterol (170–199 mg/dL) in 5 (15.2%) and high (≥200 mg/dL) in 2 (6.0%) children; acceptable triglycerides (<75 mg/dL in 0–9 years and <90 mg/dl in 10–19 years) was revealed in 24 (72.7%), borderline high triglycerides (75–99 mg/dL in 0–9 years and 90–129 mg/dl in 10–19 years) in 5 (15.2%) and high triglycerides (>100 mg/dL

TABLE 1 | Clinical and biochemical parameters in children with tuberous sclerosis complex.

Analyzed parameters	Children with TSC
Number of patients	33
Age [years]	11.13 ± 4.03
Boys/girls	15/18 (45%/55%)
Positive family history	7 (21.2%)
BMI *Z*-score	0.31 ± 0.97
Arterial hypertension	7 (21.2%)
Developmental delay	22 (66.7%)
Epilepsy*	24 (72.7%)
Angiomyolipoma (*n*, %)	27 (81.8%)
Fat-poor angiomyolipoma (*n*, %)	9 (27.3%)
Angiomyolipoma [mm]	9.5 (6–25)
Renal cysts (*n*, %)	26 (78.8%)
Renal cysts [mm]	7.5 (5–10)
Antihypertensive medications	3 (9.1%)
Antiepileptic medications	17 (51.5%)
mTOR inhibitors	4 (12.1%)
eGFR ac. to Schwartz formula [ml/min/1.73m^2]	130.8 ± 28.1
Cystatin *C* [ng/mL]	0.9 ± 0.2
Uric acid [mg/dL]	4.2 ± 0.9
Total cholesterol [mg/dL]	155 (135–169)
LDL cholesterol [mg/dL]	81.1 ± 28.6
HDL cholesterol [mg/dL]	57.5 ± 19.4
Triglycerides [mg/dL]	78.5 ± 28.7
Albuminuria [mg/24 h]	8.6 (4.6–14.6)

**at present or in anamnesis.*

TSC, tuberous sclerosis complex; BMI, body mass index; mTOR, mammalian target of rapamycin; eGFR, estimated glomerular filtration rate; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

TABLE 2 | Blood pressure in children with TSC and in healthy children.

Parameter	Children with TSC	Control group	*P*
Number of patients	33	33	–
Age [years]	11.13 ± 4.03	11.23 ± 3.28	
Boys/girls	15/18 (45%/55%)	15/18 (45%/55%)	1.000
BMI *Z*-score	0.31 ± 0.97	0.59 ± 1.24	0.170
Office peripheral blood pressure			
SBP [mm Hg]	115.6 ± 10.7	107.6 ± 8.7	0.001
SBP *Z*-score	0.82 ± 0.87	0.00 ± 0.50	<0.001
DBP [mm Hg]	69.1 ± 9.7	62.7 ± 7.3	0.004
DBP *Z*-score	0.9 ± 1.3	−0.02 ± 0.96	0.002
MAP [mm Hg]	85.6 ± 9.1	78.0 ± 6.9	<0.001
PP [mm Hg]	46.5 ± 6.7	44.9 ± 5.9	0.307
Office central blood pressure			
AoSBP [mm Hg]	98.6 ± 9.6	90.4 ± 6.9	<0.001
AoDBP [mm Hg]	71.2 ± 9.9	64.3 ± 7.3	0.002
AoMAP [mm Hg]	85.6 ± 9.1	78.1 ± 6.9	<0.001
AoPP [mm Hg]	27.4 ± 4.4	26.1 ± 4.0	0.227
24-h ambulatory blood pressure			
ABPM SBP 24 h [mm Hg]	114.5 ± 9.9	107.2 ± 5.2	<0.001
ABPM DBP 24 h [mm Hg]	65.5 ± 7.2	60.5 ± 3.9	<0.001
ABPM MAP 24 h [mm Hg]	81.9 ± 7.8	73.0 ± 4.7	<0.001
ABPM MAP 24 *Z*-score	−0.13 (−0.66 to 1.10)	−1.44 (−1.77 to −0.62)	<0.001
PP 24 h [mm Hg]	48.9 ± 5.4	46.7 ± 4.0	0.063
HR 24 h [bpm]	83 (73–91)	80 (75–86)	0.366
SBPL/24 h (%)	10 (2–25)	5 (2–9)	0.037
DBPL/24 h (%)	7 (2–15)	3 (1–4)	0.018
SBP DIP [%]	9.1 ± 5.2	11.2 ± 4.2	0.080
DBP DIP [%]	15.9 ± 7.5	17.4 ± 6.3	0.403

TSC, tuberous sclerosis complex; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure; AoSBP, aortic systolic blood pressure; AoDBP, aortic diastolic blood pressure; AoMAP, aortic mean arterial pressure; AoPP, aortic pulse pressure; ABPM, ambulatory blood pressure monitoring; SBPL, systolic blood pressure load; DBPL, diastolic blood pressure load; DIP, dipping.

in 0–9 years and >130 mg/dl in 10–19 years) in 4 (12.1%) patients. Elevated urinary albumin loss was found in 3 (9.1%) of the children.

Blood Pressure and Arterial Damage in Children With Tuberous Sclerosis Complex

The comparison of blood pressure in children with TSC and healthy children was depicted in **Table 2**. There were no differences between the groups in terms of age, sex, and BMI *Z*-score. In the group of TSC patients, elevated (≥95th percentile) office systolic or diastolic blood pressure was revealed in 10 (30.3%) patients, including two patients with previously recognized arterial hypertension. Elevated blood pressure in ABPM was revealed in 4 (12.1%) patients—all of them were considered as normotensives before the study. Among these four patients, one 13-year-old girl also had elevated office blood pressure. Additionally, ABPM revealed masked hypertension in three patients. All three patients with previously recognized and treated arterial hypertension had normal ABPM results at the moment of the study. The analysis of blood pressure in TSC children was depicted in **Figure 3**. A disturbed circadian blood pressure profile was recognized in 16 TSC patients (including five patients with arterial hypertension). Children with TSC were characterized by significantly higher office peripheral and central blood pressure and 24-h ambulatory blood pressure than healthy peers. The groups did not differ significantly in 24-h pulse pressure, heart rate, and systolic and diastolic blood pressure dipping.

Parameters of arterial damage in both groups were presented in **Table 3**. Patients with TSC were characterized by significantly faster aortic pulse wave velocity (4.76 ± 0.81 vs. 4.25 ± 0.56 [m/s], $p = 0.003$) and thicker common carotid artery intima-media thickness (0.42 ± 0.05 vs. 0.39 ± 0.03 [mm], $p = 0.011$) than healthy individuals. There were no differences between the groups regarding augmentation index, subendocardial viability ratio, local carotid arterial dimension, or stiffness parameters. Central systolic blood pressure, aortic pulse wave velocity, and common carotid intima-media thickness in both groups were presented in **Figures 4–6**, respectively.

When we excluded 7 children with arterial hypertension from the study group, we found that remaining 26 children with TSC were still characterized by significantly faster aortic pulse wave velocity (aPWV: 4.73 ± 0.83 vs. 4.25 ± 0.56 [m/s], $p = 0.011$, aPWV *Z*-score: −0.18 ± 1.18 vs. −0.96 ± 0.87, $p = 0.006$) and thicker common carotid artery intima media thickness (cIMT: 0.41 ± 0.05 vs. 0.39 ± 0.03 [mm], $p = 0.042$). The groups still did not differed in age (10.93 ± 4.07 vs. 11.23 ± 3.28 [years], $p = 0.754$) and sex (boys/girls-−11/15 vs. 15/18, $p = 0.809$).

The Determinants of Blood Pressure and Arterial Parameters in Children With Tuberous Sclerosis Complex

In TSC patients, aortic systolic blood pressure and peripheral office diastolic blood pressure *Z*-score correlated significantly with maximal diameter of the renal cyst ($R = 0.419$, $p = 0.033$ and $R = 0.484$, $p = 0.012$, respectively). Aortic systolic blood pressure and mean arterial pressure during 24 h *Z*-score correlated with serum cystatin C concentration ($r = 0.377$, $p = 0.030$ and $R = 0.433$, $p = 0.013$, respectively).

Boys with TSC did not differ from girls in terms of aortic pulse wave velocity (aPWV [m/s]: 4.85 ± 0.84 vs. 4.72 ± 0.81 [m/s], $p = 0.660$; aPWV *Z*-score:−0.07 ± 1.12 vs.−0.21 ± 1,21, $p = 0.752$) or common carotid artery intima media thickness (cIMT [mm]: 0.43 ± 0.06 vs. 0.41 ± 0.05, $p = 0.383$; cIMT *Z*-score: 0.87 ± 1.14 vs. 0.75 ± 1.30, $p = 0.797$). We found a positive correlation between aPWV *Z*-score and daily urinary albumin loss ($R = 0.412$, $p = 0.029$) and trend toward a positive correlation between aPWV *Z*-score and maximal AML diameter ($R = 0.339$, $p = 0.058$).

As for parameters of local carotid artery stiffness in patients with TSC, pressure strain elasticity modulus (Ep) correlated with mean arterial pressure during 24 h *Z*-score (MAP 24 h *Z*-score) ($R = 0.409$, $p = 0.020$), arterial compliance (AC) correlated with

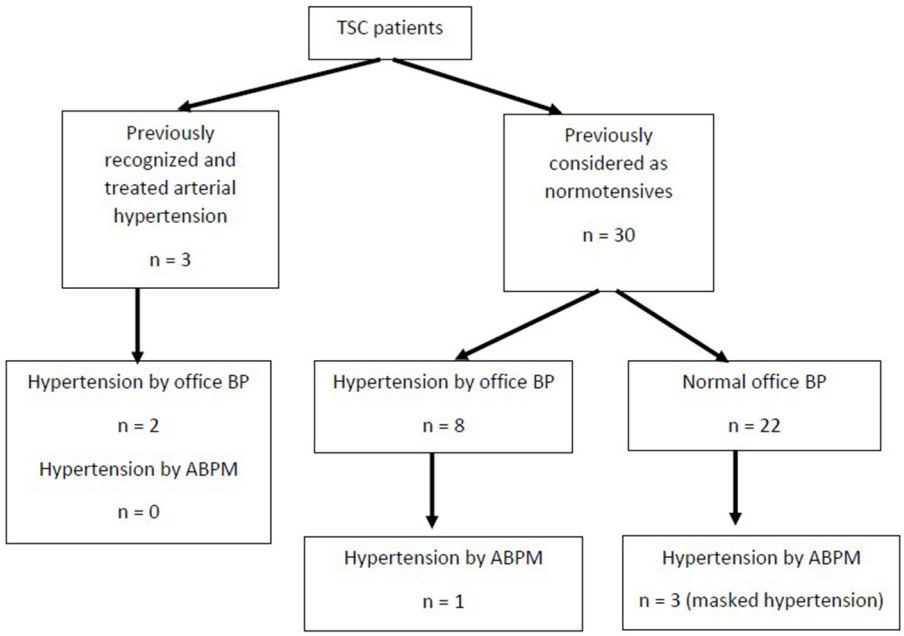

FIGURE 3 | The results of blood pressure measurement in children with tuberous sclerosis complex (TSC, tuberous sclerosis complex; BP, blood pressure; ABPM, ambulatory blood pressure monitoring).

TABLE 3 | Parameters of arterial damage (early vascular aging) in children with tuberous sclerosis complex and in healthy children.

Parameter	Children with TSC	Control group	*P*
aPWV	4.76 ± 0.81	4.25 ± 0.56	0.003
aPWV *Z*-score	−0.14 ± 1.15	−0.96 ± 0.87	0.002
AIx75HR [%]	8.71 ± 15.90	5.24 ± 11.12	0.319
Buckberg SEVR [%]	135.3 (120–159)	145.8 (130–169)	0.115
cIMT [mm]	0.42 ± 0.05	0.39 ± 0.03	0.011
cIMT *Z*-score	0.81 ± 1.21	0.21 ± 0.55	0.007
ET beta	3.4 (2.4–4.1)	3.7 (2.7–4.6)	0.510
ET Ep [kPa]	36 (28–51)	41 (31–50)	0.748
ET AC [mm^2/kPa]	1.14 (0.91–1.52)	1.02 (0.82–1.29)	0.346
ET AIx [%]	0.00 (−6.90 to 17.60)	−3.29 (−5.10 to 0.00)	0.072
ET PWVbeta [m/s]	3.60 (3.20–4.30)	3.75 (3.30–4.20)	0.944
ET D max [mm]	5.85 (5.24–6.16)	5.83 (5.06–6.38)	0.517
ET D min [mm]	4.78 (4.50–5.41)	4.95 (4.32–5.54)	0.807
ET DATmax [ms]	140.00 (123.00–178.00)	128.00 (124.00–142.00)	0.146

aPWV, aortic pulse wave velocity; AIx75HR, augmentation index normalized to heart rate of 75 beats per minute; SEVR, subendocardial viability ratio; cIMT, common carotid artery intima-media thickness; ET, ECHO-tracking; beta, stiffness index; Ep, pressure strain elasticity modulus; AC, arterial compliance; AIx, augmentation index; D max, maximal diameter of right common carotid artery; D min, minimal diameter of right common carotid artery; DATmax, acceleration time to common carotid artery maximal diameter.

systolic blood pressure load during 24 h ($R = -0.367$, $p = 0.036$) and with MAP 24 h *Z*-score ($R = -0.538$, $p = 0.001$) and local pulse wave velocity (PWVbeta) also correlated with MAP 24 h *Z*-score ($R = 0.411$, $p = 0.019$).

DISCUSSION

This is the first study to analyze central blood pressure and the phenomenon of early vascular aging in pediatric patients with tuberous sclerosis complex. We have compared our results with perfectly sex- and age-matched healthy peers. Our analysis revealed that TSC patients were characterized by significantly higher central systolic blood pressure, aortic pulse wave velocity, and common carotid artery intima-media thickness. We have found that blood pressure correlated with serum cystatin C and renal cyst diameter in this group of patients. There was also positive relation between pulse wave velocity and the extent of renal involvement.

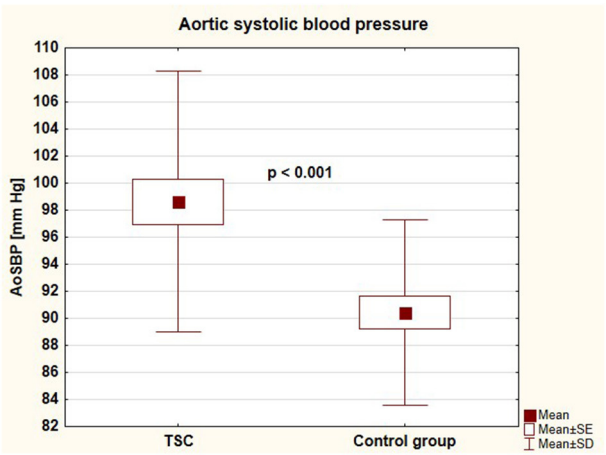

FIGURE 4 | Aortic systolic blood pressure in children with tuberous sclerosis complex and in control group (AoSBP, aortic systolic blood pressure; TSC, tuberous sclerosis complex; SE, standard error; SD, standard deviation).

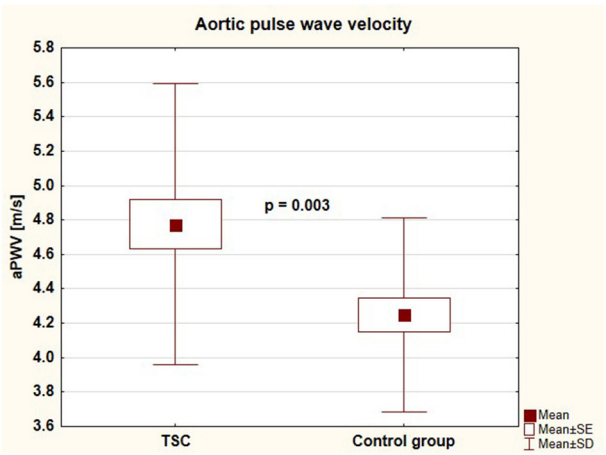

FIGURE 5 | Aortic pulse wave velocity in children with tuberous sclerosis complex and in control group (aPWV, aortic pulse wave velocity; TSC, tuberous sclerosis complex; SE, standard error; SD, standard deviation).

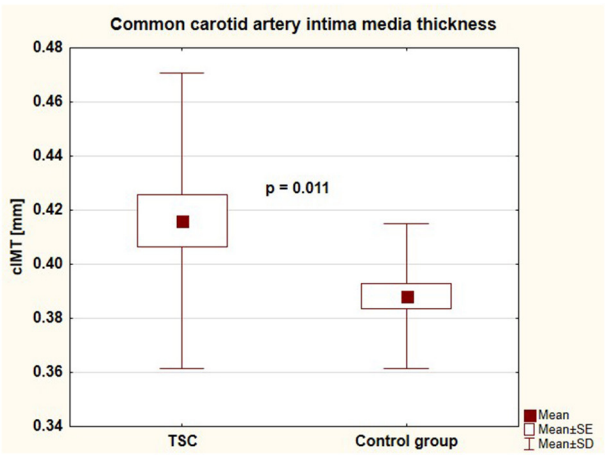

FIGURE 6 | Common carotid artery intima media thickness in children with tuberous sclerosis complex and in control group (cIMT, common carotid artery intima media thickness; TSC, tuberous sclerosis complex; SE, standard error; SD, standard deviation).

Parameters of local artery stiffness correlated with ambulatory blood pressure.

Our analysis has involved 33 children with already diagnosed TSC hospitalized in one tertiary center of pediatric nephrology. Our center has developed a program of nephrological care for pediatric TSC patients in 2017 based on ITSCCG and the Polish Society of Nephrology recommendations (16, 29). The preliminary analysis of our TSC cohort has already been published in a local medical journal (30). In addition, we aimed to analyze blood pressure and vascular phenotype in these patients. The clinical characteristics of our cohort do not differ from those of patients with TSC in large international registries including TOSCA registry (TuberOus SClerosis registry to increase disease Awareness) (3).

Renal lesions including AMLs were more frequently found in our group (90.9% of children) than in other pediatric patients groups—renal involvement is estimated to occur in approximately 38.5−55% of preschool children and 75–80% of schoolchildren (13, 20, 31, 32). The higher incidence of renal manifestations in our group results from the specificity of patients referred to our Department—the main indication was the presence of focal renal lesions found on imaging studies. In the analysis of patients from the TOSCA registry, AML lesions were present in 51.8% of patients (13). Renal cysts in most patients with TSC are small and localized in the subcortical region. Patients with simultaneous deletion of *TSC2* and *PKD1* genes are characterized by a very severe phenotype with large cysts from early childhood. Many of these patients develop a rapid progression to end-stage renal disease already in the second or third decade of life (13, 32). In our study group, renal cysts were found in three-quarters of patients. The largest cysts were found in two boys with a genetically confirmed mutation in the *PKD*1 gene. Of note, in our group of patients, hyperfiltration was found in as many as one-third of the subjects. This frequency is even higher than in the study of Belgian authors (20). It has been postulated that hyperfiltration in patients with TSC is caused by overactivation of the mTOR pathway in the glomerulus and, as in diabetic nephropathy, may be a risk factor for progression of renal disease.

In the analyzed group of patients with TSC, hypertension was present in 21.2% of patients, which is higher than the average prevalence of hypertension in the population of healthy children (3–5%) (33) and is also higher compared to the percentage of patients with hypertension in the Belgian study (13.0%) (20). In adults with TSC, arterial pressure has been shown to depend on the size and number of both AML lesions and cysts (32, 34). Severe hypertension in this group of patients may also be related to the presence of vascular lesions—renal

artery stenosis sometimes accompanied by mid-aortic syndrome (MAS) (10). The ITSCCG recommendations clearly state the need for regular blood pressure measurements in this group of patients (1). The idea of measurement of central blood pressure has gained much attraction in recent years (35). Also, pediatric data indicate that central systolic blood pressure is a significant predictor of target organ damage, as valuable as 24-h peripheral blood pressure monitoring (36). We found that pediatric patients with TSC were characterized by a significantly higher central systolic blood pressure than healthy peers. Cystatin C and renal cyst diameter were significant determinants of elevated blood pressure in our cohort. The latter finding is consistent with the results of a multicenter study in children with autosomal dominant polycystic kidney disease (37) and adult TSC registries (32, 34).

Arterial damage is one of the first alterations observed in pediatric populations with increased cardiovascular risk, such as chronic kidney disease (CKD) (38), primary hypertension (PH) (39), diabetes mellitus (DM) type 1 (40), or familial hypercholesterolemia (41). Elastic properties of arteries in high-risk pediatric patients are similar to vascular changes in the elderly and are defined as early vascular aging. The concept of EVA is based on findings that individuals with PH, DM or CKD present with more advanced signs of arterial aging than their healthy peers (15, 42). Among different indices of EVA, evaluation of common carotid artery intima-media thickness and aortic (carotid-femoral) pulse wave velocity are considered as the most valuable ones with generally accepted pediatric normative values (27, 28, 43) and well-established association with hard-end points in the adult population (44, 45).

Our study is the first to reveal signs of EVA in pediatric patients with TSC. The primary cause of EVA in this group of patients is yet to be solved. Nevertheless, there is some possible explanation for this result. Firstly, vascular senescence in this population could be the effect of blood pressure rise as blood pressure is the primary determinant of cIMT and aPWV in the general pediatric population (27, 43). Moreover, initial functional and morphological vascular changes can be regarded as adaptive in response to increased blood pressure (42). Indeed, office peripheral and central blood pressure was significantly higher in this group of TSC patients compared to healthy age- and sex-matched peers. Nevertheless, blood pressure remained within normal limits in most of these patients, and arterial hypertension was found in approximately 20% of the studied children. In addition, no significant correlation between blood pressure and parameters of arterial damage except for carotid artery stiffness has been revealed in our patients. Also, cIMT and aPWV were still elevated in TSC patients after the exclusion of 7 hypertensive children. Metabolic factors do not seem to play a role either, as these children generally had normal lipid parameters and did not differ in BMI from the control group. Thus, it is possible that other factors might be to blame.

Vascular anomalies have been described in TSC patients for decades (9, 10), including renal artery stenosis (sometimes with the mid-aortic syndrome) and aortal aneurysms also in small children. The youngest reported TSC case is an infant who died of aortic aneurysm rupture at the age of 4.5 months (46). Vascular smooth muscle cells (SMCs) are not terminally differentiated and can transform to proliferating and migrating cells with loss of contractile protein expression and increased synthesis of extracellular matrix proteins. In SMCs, mTOR signaling was found to influence cell differentiation. Cao et al. revealed in an experimental model that the activation of the mTOR pathway with *Tsc2* deficiency leads to SMC proliferation and de-differentiation *in vitro* and *in vivo*, which can be reversed with rapamycin treatment (8). Recently, a disruption of the *Tsc1* gene was also found to induce a degradative smooth muscle cell phenotype (47). The authors hypothesize that the proliferation of degradative SMCs within the media causes arterial dysfunction in TSC patients. A histological study of a thoracoabdominal aneurysm in a child with TSC revealed SMC hyperplasia in the inner media with diminished actin expression and extensive fragmentation of elastic fibers (8). Our preliminary results suggest that the proliferation and degradation of vascular SMCs are already present in children with TSC without macroscopically evident arterial dilations or stenoses.

In addition, the extent of renal involvement might play a role in the development of blood pressure rise and arterial damage as blood pressure was associated with cystatin C and cyst diameter and aortic pulse wave velocity with urine albumin loss and AML diameter. A positive correlation between albuminuria and arterial stiffness has been revealed in adults with CKD (48) and DM 2 (49) and in the general young adult population from the Malmö offspring study (50). Albuminuria is not only a marker of renal damage but is a well-established indicator of endothelial dysfunction, which may result in an altered arterial wall and increased arterial stiffness. Gil-Ortega et al. found that increased albuminuria was associated with abnormalities in arterial wall structure (elastin loss) in an experimental rat model (51). A similar association might also be seen in TSC patients. Inversely, increased urinary albumin loss could be the result of the downstream transmission of pressure pulsatility to the level of renal microcirculation (52).

TSC is one of those rare entities for which there is a targeted treatment that directly affects the mechanism of the disease. Numerous data from both single case reports and multicenter clinical trials indicate high efficacy of mTOR inhibitors (everolimus and sirolimus/rapamycin) in the treatment of virtually all symptoms of the disease, including central nervous system lesions (53), renal AML (54) or lung lesions (55). The timing of initiation of mTOR inhibitors in TSC patients is a matter of debate. Recent multicenter studies have proven the safety and efficacy of mTOR inhibition in TSC patients even younger than 2 years (56, 57). Of note, *in vitro* studies showed that the mTOR inhibitor rapamycin promotes SMC differentiation toward a contractile phenotype (58). Inhibition of the mTOR signaling pathway has been used for many years in medicine in rapamycin-diluting stents that prevent SMC proliferation and artery occlusion after percutaneous interventions (59). There is already some evidence indicating that early treatment in high-risk children and adolescents [e.g.,

in patients with CKD (60) or PH (61)] could reverse EVA and possibly prevent premature cardiovascular events. Whether mTOR inhibitor treatment or any other medical measures (e.g., antihypertensive treatment) would prevent or even reverse negative alterations in arteries of TSC patients is still unknown and should certainly be investigated in years to come.

Some limitations to our research need to be listed. Firstly, the study's cross-sectional nature precludes drawing final conclusions on the relation between blood pressure, arterial damage, and clinical parameters in the studied TSC patients (e.g., the link between aPWV and albuminuria). Secondly, the study population was small and heterogeneous in terms of age, presence of arterial hypertension, the extent of renal involvement, genetic background (*TSC1*, *TSC2*, *TSC2*+*PKD1*), and treatment with mTOR inhibitors. As arterial hypertension was recognized previously in only 3 patients, we could not analyze the influence of duration of hypertension on arterial damage in TSC patients.

CONCLUSIONS

Children with tuberous sclerosis complex are at risk of elevated central blood pressure and early vascular aging. In this group of patients, early vascular aging might be caused by uncontrolled activation of the mTOR signaling pathway in vascular smooth muscle cells. Additionally, in children with TSC, blood pressure and arterial stiffness are related to renal involvement. There is a need for studies on possible vasoprotective measures in TSC patients, including the use of mTOR inhibitors.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Bioethics Committee, Medical University of Warsaw (approval no. KB/145/2017, 4th July 2017). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin and the participants ($\geq$16 years).

AUTHOR CONTRIBUTIONS

PS, AMW, and MS drafted and revised the manuscript. PS, SJ, MB, and MP-T contributed to the conception and design of the work. PS, AMW, MS, MB, AJ-K, and PB contributed to the acquisition of data. PS, AMW, MS, SJ, MB, AJ-K, PB, and MP-T contributed to the analysis or interpretation of the data. SJ and MP-T critically revised the manuscript. All authors gave their final approval and agreed to be accountable for all aspects of this work ensuring its integrity and accuracy.

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Increased Arterial Stiffness Is Associated With Reduced Diastolic Function in Youth With Obesity and Type 2 Diabetes

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Background: Increased arterial stiffness is associated with diastolic dysfunction in adults. Data in youth are lacking, so we examined the impact of arterial stiffness on diastolic function in youth.

Methods: We obtained diastolic function and augmentation index, pulse wave velocity, brachial artery distensibility, and carotid stiffness on 612 youth [10–24 years, 65% female, 38% normal weight, 36% obese, and 26% with type 2 diabetes mellitus (T2DM)]. Participants were classified as compliant (C) vs. stiff (S) arteries based on seven arterial stiffness parameters [Global Stiffness Index (GSI), S = GSI > 4). Mean differences in covariates were evaluated by Student's *t*-tests. A stepwise regression analysis was performed to determine if GSI was an independent predictor of diastolic function.

Results: Lower diastolic function and more adverse cardiovascular disease (CVD) risk factors were present in the S group ($n = 67$) than the C group ($n = 545$) ($p < 0.001$). Covariates that were associated with diastolic dysfunction were higher GSI, male sex, higher body mass index (BMI), and systolic blood pressure (SBP) *z*-score ($R^2 = 0.18$ to 0.25; $p \leq 0.05$).

Conclusion: Adverse diastolic function is seen in youth with increased arterial stiffness independent of CVD risk factors. Interventions to improve arterial stiffness prior to clinical onset of diastolic dysfunction are needed to prevent development of heart failure.

Keywords: arterial stiffness, diastolic dysfunction, pediatrics, obesity, T2DM

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INTRODUCTION

Effective cardiovascular disease (CVD) prevention requires identification of risk factors prior to the onset of clinical burden. While it is commonly understood that adults with obesity or obesity-related type 2 diabetes mellitus (T2DM) are at increased risk for CVD (1), the evidence regarding the extent to which these risk factors impact the pediatric age range is not well-characterized.

Diastolic dysfunction is a risk factor-related measure of target organ damage that predicts heart failure (2, 3) and CV events in adults (4). Emerging evidence suggests that pre-clinical diastolic dysfunction [diastolic dysfunction with normal systolic function and without symptoms of heart

failure (5)] exists in hypertensive adolescents (6) and youth with obesity or T2DM (7). One mechanism for the development of diastolic dysfunction may be increased afterload on the heart induced by increased arterial stiffness (8, 9). Pediatric studies show that arterial damage is associated with higher left ventricular mass (10) and with reduced systolic strain (11). We sought to determine the relationship between arterial damage and diastolic function in healthy youth and those with CV risk factors including obesity and T2DM.

METHODS

The study population consisted of 612 youth (age 10–24 years, mean 18 years, 65% female, 62% non-Caucasian, and 26% with T2DM) who participated in a study comparing cardiovascular parameters among adolescents and young adults who were lean (L), obese (O), or obese with T2DM (T). Pregnant females were excluded from the study. Investigational review board approval was obtained. Written informed consent was obtained from subjects 18 years or older and from the guardian for subjects <18 years old. Written assent was obtained for subjects <18 years old.

CV Risk Factor Measurements

The mean of two measures of height with a calibrated stadiometer (Veeder-Rood, Elizabethtown, North Carolina) and two measures of weight with a Health-O-Meter electronic scale (Jarden Consumer Solutions, Rye, New York) were used in analyses. Body mass index (BMI) was calculated as weight (kilograms) / height (meter) (2). The mean of three resting measures of blood pressure (BP) with mercury sphygmomanometry collected after 5 min of rest according to pediatric guidelines (12) was obtained. After an overnight fast, plasma glucose was measured with a Hitachi model 704 glucose analyzer (Roche Hitachi, Indianapolis, Indiana) with intra-assay and inter-assay coefficients of variation of 1.2 and 1.6%, respectively. Plasma insulin was measured by radioimmunoassay with an anti-insulin serum raised in guinea pigs, indium125-labeled insulin (Linco, St. Louis, Missouri), and a double-antibody method to separate bound from free tracer with a sensitivity of 2 mmol (intra-assay and inter-assay coefficients of variation of 5 and 8%, respectively). Glycated hemoglobin A1c (HbA1c) was measured by use of high-pressure liquid chromatography. Fasting plasma lipid profiles were performed with standardized methods from the National Heart Lung and Blood Institute–Centers for Disease Control and Prevention, and low-density lipoprotein cholesterol concentration was calculated with the Friedewald equation. C-reactive protein (CRP) was measured with a high-sensitivity enzyme-linked immunosorbent assay.

Arterial Stiffness Measurements

Vascular function testing was conducted after 5 min of rest in the supine position. Three measures of brachial artery distensibility (BrachD) were obtained with a DynaPulse Pathway instrument (Pulse Metric, Inc., San Diego, California). This device derives brachial artery pressure curves from arterial pressure signals obtained from a standard cuff sphygmomanometer. Brachial artery compliance is derived from waveform parameters, and then BrachD is calculated as compliance normalized to baseline brachial artery diameter (estimated from a regression equation developed from ultrasound, adjusting for sex and body size). This variable is equivalent to other measures of distensibility, such as those measured with ultrasonography, in that it represents the relative change in volume per unit of pressure and is expressed with the units of %change/mmHg. Repeat measures in our laboratory show coefficients of variability <9%.

Three measures of pulse wave velocity (PWV) were measured and averaged with a SphygmoCor SCOR-PVx System (Atcor Medical, Sydney, Australia). PWV is a measure of the difference in the carotid-to-distal path length divided by the difference in R-wave-to-waveform foot times (m/s). Specifically, electrocardiography (ECG) leads were applied to the carotid artery, the sternal notch, and the distal artery of interest (femoral, radial, and dorsalis pedis). A pressure tonometer the size of a pencil is placed on the proximal artery (carotid) then distal to obtain arterial waveforms gated to the R-wave on the electrocardiography tracing. The ECG recording was used to measure heart rate. Repeat measures in our laboratory show coefficients of variability <7%.

Three measures of augmentation index (AIx) were collected with the SphygmoCor device. The pressure sensor is applied to the radial artery to collect radial artery pressure waves that are calibrated to a non-invasive blood pressure (Pulse Metric, Inc., San Diego, California). A generalized transfer function validated against invasive catheterization data is used to calculate central (aortic) systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), and pulse pressure (PP) and reconstruct the central aortic pressure curve. AIx, adjusted to a heart rate of 75 beats per minute, is calculated utilizing the ascending aorta pressure curve. AIx is the pressure difference between the primary (main outgoing wave) and the reflected wave of the central arterial waveform, expressed as a percentage of the central pulse pressure. Reproducibility studies in our laboratory demonstrated intra-class correlation coefficients between 0.7 and 0.9 for all variables.

Carotid Ultrasonography

Carotid ultrasound studies were performed by a single registered vascular technologist using high-resolution B-mode ultrasonography (GE Vivid 7; GE Healthcare, Milwaukee, Wisconsin) with a high-resolution linear array vascular transducer (7.5 MHz). An optimal two-dimensional (2D) image of the common carotid artery was obtained, where both the near and far wall intima/media complex were well-visualized. The M-mode cursor was then placed 1 cm proximal to the beginning of the carotid artery bulb. Multiple image loops were digitally transmitted by use of the Camtronics Medical System (Camtronics Medical Systems, Hartland, Wisconsin) for off-line reading. The maximal and minimal lumen diameters were read from the M-mode tracing for calculations of carotid stiffness. Calculations included arterial compliance (AC), beta stiffness index (β), circumferential arterial strain (CAS), Peterson's elastic modulus (PEM), and Young's elastic modulus (YEM). Because of pulse-wave amplification along the arterial tree, which results

in overestimation of brachial SBP, the central BP calculated from the radial artery pressure curve using the SphygmoCor device (obtained no more than 30 min before the carotid scan) was used in the calculations of carotid artery stiffness.

Echocardiographic Technique

Echocardiograms were obtained with a GE Vivid 5 or 7 (Milwaukee, WI, USA) or Philips Sonos 5500 (Andover, MA, USA) ultrasound system. A complete 2D pulsed Doppler, tissue Doppler, and color Doppler echocardiographic examination was performed on each participant. All images were obtained with the participant in the left lateral decubitus position to acquire parasternal long-axis, parasternal short-axis, and apical four-chamber views for a total of three cardiac cycles. Left atrial diameter (LAD) was measured in the long axis and indexed to height (LAD/ht). Measurement was performed off-line by either of two sonographers using a Cardiology Analysis System (Digisonics, Houston, TX, USA).

The assessment of mitral inflow velocity was obtained with pulsed wave Doppler parallel to mitral inflow in the apical four-chamber view, and maximal velocity measured at the mitral valve leaflet tips. The mitral peak *E* (early filling) and *A* (inflow with atrial contraction) waves were measured off-line, and an *E*/*A* ratio was calculated. Tissue Doppler imaging of myocardial flow velocities was acquired in the apical four-chamber view. The peak and late velocities of mitral annular flow were recorded at both the septal annulus (e'-sept, a'-sept) and lateral annulus (e'-lat, a'-lat). The e'/a' ratios were calculated in addition to E/e'-lat and E/e'-sept ratios. The E/e' ratio corrects for myocardial relaxation in transmitral flow (*E*) and has been shown to correlate with left ventricular (LV) end-diastolic pressure (7). In adults, an E/e'-lat of >10 is predictive of elevated LV filling pressures, and <6 is normal. In addition, the left atrial size was assessed by two-dimensional-directed M-mode and indexed to height.

Statistical Analysis

All analyses were performed with Statistical Analysis Software (SAS®, version 9.1.3, Cary, North Carolina). Variance-stabilizing measures to transform non-normal values were performed as needed. The 95th percentile for each of the seven arterial stiffness measures (BrachD, AIx, PWV, AC, β, CAS, PEM, and YEM) for lean subjects without diabetes was determined. Subjects were given a score of 1 for the parameter if $\geq$95th percentile for the lean group ($\leq$5th percentile for AC and BrachD) and 0 if below the cutpoint (overall, a total of 7 points are possible). Global Stiffness Index (GSI) was calculated as the sum of the stiffness points for each of the four measures of carotid artery stiffness and the three non-ultrasound measures of arterial stiffness. The GSI has been shown to be linearly related to LV mass index in a previous study (10). Subjects were stratified into either "compliant arteries" (CA) or "stiff arteries" (SA) based on their GSI score (a score of 4 or greater, which was the 95th percentile for GSI for the lean, healthy group, qualified as stiff). Average values for demographic, anthropometric, BP, and laboratory values were obtained for each group. Student's *t*-tests were performed to determine differences by stiffness classification. The χ^2 analyses were performed for categorical variables. Bivariate correlations were calculated for GSI, covariates, and diastolic function variables. Variables that were significant in the bivariate analysis were included as potential independent predictors in the general linear model analyses. The full model contained the following data: demographic (age, race/ethnicity, sex, and presence of T2DM), anthropometric (BMI *z*-score), hemodynamic (SBP *z*-score, DBP *z*-score, and HR), and laboratory (fasting glucose, fasting insulin, HbA1c, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, and CRP). The significance of each covariate in the initial model was assessed, and non-significant terms were removed by backward elimination until all remaining covariates or their interaction terms were significant. Robustness of the models was assessed with the use of the maximum *R*-square technique.

TABLE 1 | Demographics and metabolic profile of study participants stratified by Global Stiffness Index category ($n = 612$, mean ± SD or frequency).

Variable	Compliant ($n = 545$)	Stiff ($n = 67$)	*P* value
Age (years)	17.8 ± 3.3	19.5 ± 3.2	<0.01
Sex (% male)	192 (35.2%)	18 (26.9%)	NA
Race (% non-Caucasian)	335 (61.5%)	46 (68.6%)	NA
Presence of T2DM (%)	127 (23.3%)	30 (44.8%)	NA
Weight (kg)	87 ± 31	113 ± 26	<0.01
Height (cm)	167 ± 11	168 ± 10	NS
BMI (kg/m^2)	31 ± 10	40 ± 8	<0.01
SBP (mmHg)	114 ± 12	124 ± 12	<0.01
DBP (mmHg)	63 ± 12	71 ± 15	<0.01
HR (beats/min)	66 ± 11	72 ± 11	<0.01
TChol (mg/dl)	169 ± 35	184 ± 43	<0.01
LDL-C (mg/dl)	99 ± 29	114 ± 40	<0.01
HDL-C (mg/dl)	50 ± 13	46 ± 11	<0.01
TG (mg/dl)	96 ± 67	126 ± 73	<0.01
Fasting glucose (mg/dl)	103 ± 43	123 ± 70	<0.01
Fasting insulin (mU/ml)	18 ± 15	23 ± 12	<0.01
HbA1c (%)	5.97 ± 1.8	6.72 ± 2.3	<0.01
hsCRP (mg/l)	4 ± 6.5	6.5 ± 7.3	<0.01

T2DM = type 2 diabetes mellitus; BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate; TChol = total cholesterol; LDL-C = low density lipoprotein concentration; HDL-C = high density lipoprotein concentration; HbA1c = glycoselated hemoglobin; hsCRP = high sensitivity C-reactive protein.

RESULTS

The population included 612 youth (10–24 years, 65% female, and 62% non-Caucasian) enrolled in one of three groups (38% lean, 36% obese without T2DM, and 26% obese with T2DM). When stratified as having compliant or stiff arteries (**Table 1**), participants with stiff arteries were older and more obese and had higher peripheral BP and heart rate, a more adverse lipid and metabolic profile, and more evidence of inflammation (all $p \leq 0.01$). Specifically, lipids in the stiff group were within normal limits, but glucose, insulin, and HbA1c were elevated, since more diabetics were included in the group. Additionally, the

stiff group had a mean SBP in the elevated BP category according to BP guidelines (12).

Consistent with stratification by GSI, the stiff cohort had arterial function measures (AIx, PWV, β, PEM, YEM, AC, CAS, and BrachD) that were in the direction of higher arterial stiffness (**Table 2**). The stiff group also had significantly lower E/A ratio and e'/a', as well as higher E/e', all suggesting pre-clinical diastolic dysfunction (**Table 2**). There was a linear relationship between increasing levels of GSI (0–7) and lower diastolic function including increased LAD/ht (**Figure 1**) and between lower E/A ratio (**Figure 2**) and e'/a' (**Figure 3**). There was a similar increase in E/e' across GSI score (data not shown).

General linear models demonstrated that GSI was independently related to diastolic function ($p \leq 0.0001$ for LAD/ht, E/A, and e'/a'). Other important covariates associated with lower diastolic function were male sex, higher BMI and SBP z-score, age, LDL, CRP, and HR ($R^2 = 0.16$ to 0.40; model $p \leq 0.001$ and all parameters $p \leq 0.05$) (**Table 3**).

TABLE 2 | Cardiovascular parameters stratified by arterial stiffness category (mean ± SD).

Variables	Compliant ($n = 545$)	Stiff ($n = 67$)	P value
Stiffness variables (means)			
AIx (%)	1.65 ± 11	8.27 ± 13	<0.01
BrachD (%Δ/mmHg)	6.15 ± 1.3	4.73 ± 0.6	<0.01
PWV (m/s)	5.9 ± 1	7.3 ± 1.2	<0.01
AC (mm/mmHg)	0.27 ± 0.07	0.20 ± 0.06	<0.01
Beta (unitless)	2.2 ± 0.5	2.9 ± 0.9	<0.01
CAS (unitless)	0.19 ± 0.04	0.16 ± 0.05	<0.01
PEM (mmHg)	192 ± 65	209 ± 77	NS
YEM (mmHg/mm)	256 ± 112	403 ± 150	<0.01
Diastolic variables			
E/A ratio	1.99 ± 0.55	1.76 ± 0.43	<0.01
e′/a′ avg	2.36 ± 0.65	1.91 ± 0.48	<0.01
E/e′ avg (LVEDP)	6.47 ± 1.43	7.29 ± 1.68	<0.01
LA diameter/ht (cm)	1.98 ± 0.32	2.21 ± 0.33	<0.01

DISCUSSION

Our study findings demonstrate that higher arterial stiffness, independent of traditional CVD risk factors, is associated with lower diastolic function in youth. Importantly, these changes in youth are pre-clinical and represent an early form of cardiac disease that is measurable before most other traditional determinants of cardiac disease, such as overt heart failure.

Clinical symptoms of heart failure are common in the adult population, experienced by at least 6.2 million Americans according to American Heart Association data (1). However, many more adults may have asymptomatic diastolic dysfunction, with the prevalence in the Framingham Heart Study reported to be 36% (1). This is relevant since diastolic dysfunction is predictive of incident heart failure (3), reduced quality of life (13), and all-cause mortality (3, 5, 14).

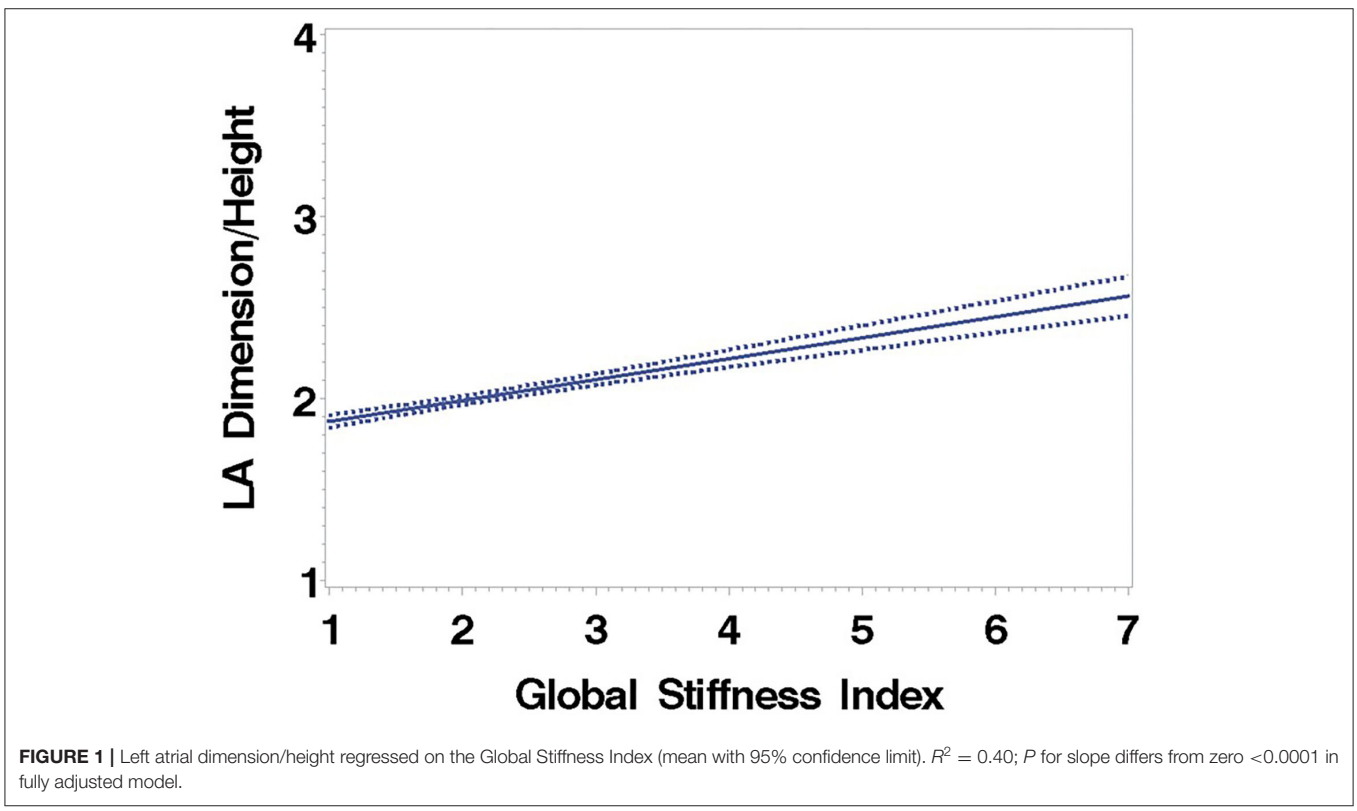

FIGURE 1 | Left atrial dimension/height regressed on the Global Stiffness Index (mean with 95% confidence limit). $R^2 = 0.40$; P for slope differs from zero <0.0001 in fully adjusted model.

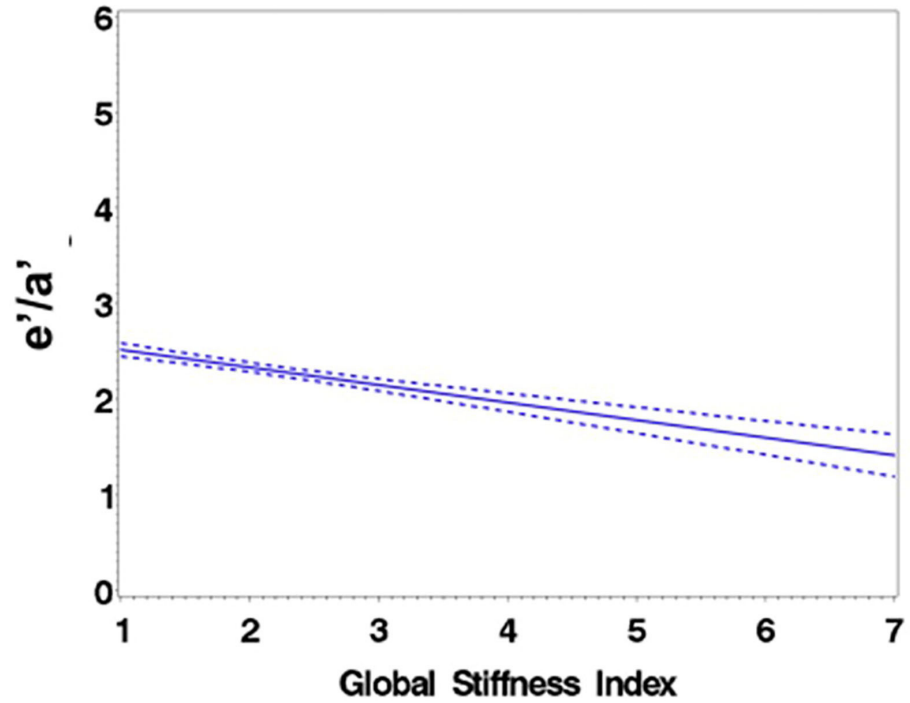

FIGURE 2 | Regression of *E/A* ratio on the Global Stiffness Index (mean with 95% confidence limit). $R^2 = 0.16$; *P* for slope differs from zero <0.0001 in fully adjusted model.

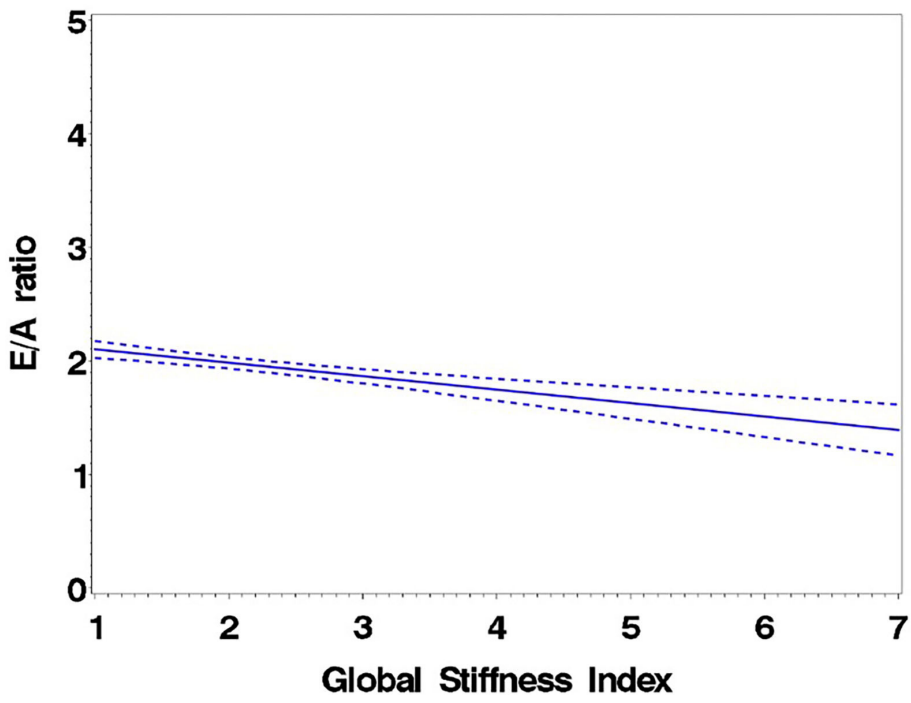

FIGURE 3 | Regression of *e′/a′* average on the Global Stiffness Index (mean with 95% confidence limit). $R^2 = 0.29$; *P* for slope differs from zero <0.0001.

TABLE 3 | Independent determinants of diastolic function.

Variable	LAD/ht	*E/A*	*e′/a′**	*E/e′**
Intercept	1.73	1.48	1.73	1.67
Presence of T2MD		−0.079		0.072
GSI	0.029	−0.049	−0.032	NS
Male sex			0.058	
BMI *z*-score		−0.029	−0.051	0.060
Age (years)		−0.013	−0.017	
SBP *z*-score				0.072
HR (beats/min)		−0.0067	−0.0075	
LDL (mg/dl)			−0.00075	
CRP (mg/dl)	0.0052			
R^2	**0.40**	**0.16**	**0.29**	**0.23**

All models $p \leq 0.0001$ and all parameters $p \leq 0.05$.
**Average of septal and lateral TDI velocities.*
All measures of diastolic function are unitless ratios.

Adult studies have demonstrated that increased pulse pressure (a crude surrogate for arterial stiffness) is independently predictive of not only diastolic dysfunction (15–17) but also heart failure with preserved ejection fraction (18, 19). In addition, carotid artery wall stiffness (20, 21) and aortic compliance, a similar parameter, positively correlate with LV diastolic function (22). Similar to our results, increased PWV is independently associated with diastolic dysfunction in patients with hypertension (23), type 2 diabetes (24), clustered CV risk factors (25), and suspected coronary artery disease (26, 27). Measures of wave reflection including augmentation index are also associated with diastolic dysfunction (28) and LV filling pressure (E/e') (9). Although an association cannot prove causality, investigators have proposed that as cardiac output falls with worsening diastolic function, neurohumoral activation, and vasoconstriction increase vessel tone to maintain mean arterial pressure and thereby increase vascular smooth muscle mass, tone, and fibrosis, resulting in increased stiffness (29). A direct relationship between neurohumoral activation and increased carotid stiffness has been demonstrated in subjects with heart failure (30). It is also possible that increased pulse wave velocity generates an earlier reflected wave in the cardiac cycle, increasing late systolic afterload, affecting thick–thin myofilament interactions and crossbridge dissociation, and leading to impaired relaxation (31, 32). The importance of increased arterial stiffness in determining diastolic function is seen in studies of normo- and hypertensive adults, where relaxation assessed with tissue Doppler varies inversely with afterload and vascular stiffness (31). Measurement of ventricular–arterial coupling (VAC = ratio of arterial elastance to end-systolic elastance) is also finding increasing usage as VAC predicts outcomes in adults with cardiac disease and heart failure (33). Our study provides a different method to evaluate the relationship between arterial and cardiac function.

Few data are available examining the relationship between arterial stiffness and diastolic function in adolescents. Our previous work demonstrated that increased left ventricular mass was associated with higher arterial stiffness (9), and carotid intima media thickness was associated with reduced systolic strain in healthy youth and those with obesity and T2DM (11). One small study found a relationship between left atrial strain (reflecting diastolic dysfunction) and measures of insulin resistance in obese children (34). Bradley et al. found both increased arterial stiffness and diastolic dysfunction in a group of adolescents with type 1 diabetes mellitus, but did not evaluate the association between the two factors (35). In a later study of children with type 1 diabetes, endothelial function (brachial flow-mediated dilation), which is associated with arterial stiffness, was inversely correlated with isovolumic relaxation time, another echocardiographic measure of diastolic function (36). Arterial stiffness has also been associated with elevated LVM in youth after repair of coarctation (37–39). Altered wave reflections leading to increased afterload on the heart has been proposed as a mechanism explaining this observation in youth with a history of coarctation repaired at a young age (40).

Adult studies have also examined the impact of metabolic syndrome (41) and T2DM (42) on arterial stiffness and diastolic function. Roes et al. (41) used MRI to evaluate diastolic dysfunction and found increased PWV and impaired LV diastolic function in subjects with metabolic syndrome, regardless of blood pressure. However, the relationship between PWV and diastolic dysfunction was not examined. Sharman et al. (1) found central pulse pressure, reflecting central arterial stiffness similar to PWV, but not brachial pulse pressure, reflecting stiffness of medium muscular artery, independently predicted diastolic dysfunction in subjects with T2DM. They concluded that increased central stiffness, possibly due to amplified pressure wave reflections, was one potential etiology of the observed abnormalities in LV diastolic function in patients with T2DM. Our work extends the observations of a relationship between arterial aging and diastolic function to youth who are healthy, have uncomplicated obesity, or have obesity-related T2DM.

Many studies have employed exercise interventions to improve arterial parameters. Adult studies have shown a positive association between exercise training and improvement in endothelial dysfunction in adults with both insulin resistance (43, 44) and T2DM (44, 45). The study by Okada et al. actually saw a decreased rate of cardiovascular events in those participating in the exercise program (45). Similarly, exercise training has been found to improve endothelial function (as measured by FMD) in adolescents with obesity (46, 47) and T2DM (48). Some studies have attempted to reverse cardiac dysfunction, with a few demonstrating improved left ventricular diastolic function in obese adults following successful weight loss (49, 50). The effect of lifestyle modification on diastolic function has not been studied extensively in youth. However, the above findings suggest that the implementation of an exercise program in obese and diabetic patients may be an appropriate investment of health care dollars to decrease future risk of cardiovascular disease.

LIMITATIONS

Our cross-sectional design does not allow us to determine the time sequence for the development of changes in arterial stiffness and cardiac diastolic function. As a result, we cannot speculate about causality and cannot precisely determine whether increased arterial stiffness preceded the development of diastolic

dysfunction or if the reverse is true. In addition, we do not know if they developed simultaneously.

Because of the original study design, our population contains a large proportion of obese subjects and subjects with T2DM that may limit the generalizability of our findings to other populations. Furthermore, both adiposity and the presence of T2DM were important determinants of diastolic function. We were neither able to assess the duration of obesity nor is the duration of T2DM certain, as the earliest phase may be asymptomatic and go unrecognized.

There may also have been other non-measured confounders (for example, activity pattern and fitness level) that affected the vascular-cardiac relationship. However, our findings are similar to results obtained in adults with known cardiovascular risk factors. Finally, equipment and expertise in collecting ultrasound measures of carotid artery stiffness and non-ultrasound measures of arterial stiffness may not be readily available to many pediatric care providers, thus limiting the applicability of the GSI calculation to the clinical setting.

CONCLUSIONS

We conclude that lower diastolic function is seen in youth with increased arterial stiffness independent of traditional CVD risk factors. Arterial stiffness likely contributes to reduction in diastolic function by increased pulse pressure and LV afterload. Screening for arterial stiffness and diastolic dysfunction in obese or T2DM adolescents may identify youth at increased risk for developing early CVD and provide the temporal opportunity for normalization of pre-clinical disease.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by IRB committee, Cincinnati Children's Hospital Medical Center. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

PK and EU designed the study, collected the data, performed data analyses, and contributed to the manuscript. NM, JH, and RM created the initial draft of the manuscript and contributed to final edits. All authors contributed to the article and approved the submitted version.

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REVIEW
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An Overview of Vascular Dysfunction and Determinants: The Case of Children of African Ancestry

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The balance between dilatory and constrictive factors is important as it keeps blood vessels in a homeostatic state. However, altered physiological processes as a result of obesity, hypertension, oxidative stress, and other cardiovascular risk factors may lead to vascular damage, causing an imbalance of vasoactive factors. Over time, the sustained imbalance of these vasoactive factors may lead to vascular dysfunction, which can be assessed by non-invasive methods, such as flow-mediated dilation, pulse wave velocity, flow-mediated slowing, retinal vessel analysis, peripheral vascular reactivity, and carotid intima-media thickness assessment. Although there is increasing prevalence of cardiovascular risk factors (obesity and hypertension) in children in sub-Saharan Africa, little is known about how this may affect vascular function. This review focuses on vasoactive factors implicated in vascular (dys)function, highlighting the determinants and consequences of vascular dysfunction. It further describes the non-invasive methods used for vascular (dys)function assessments and, last, describes the impact of cardiovascular risk factors on vascular dysfunction in children of African ancestry.

Keywords: vascular dysfunction, obesity, cardiovascular risk factors, African children, vascular function

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INTRODUCTION

Cardiovascular diseases (CVDs) are a major cause of morbidity and mortality worldwide. In 2019, an estimated 17.9 million people died from CVDs, representing 32% of all global deaths (1). In sub-Saharan Africa (SSA), the disability-adjusted life years (DALYs) due to CVDs increased from 90.6 million in 1990 to 151.3 million in 2017 (2). CVDs in SSA are of major concern as they pose a challenge on an already strained health system (3). Although the prevalence of CVDs is higher in adults, the risk factors for CVDs, including obesity and hypertension, are increasing among children in SSA (4).

There is evidence that risk factors for CVDs, including obesity, hypertension, and hyperglycemia, begin early in life and may be associated with vascular dysfunction (5, 6). Also, it is reported that vascular dysfunction, an early initiator of CVD, begins in childhood and may lead to CVDs and associated complications in adulthood (7). Vascular dysfunction, which includes endothelial dysfunction, microvascular dysfunction, and stiffening of large arteries, results when the homeostatic function of relaxation and contraction of blood vessels is affected (8).

The endothelium is a major layer of blood vessels, and it is regulated by the release of potent vasodilators, such as nitric oxide (NO), prostaglandin I2, hydrogen sulfide,

endothelium-derived hyperpolarizing factor as well as contracting factors, such as endothelin, prostacyclin, and thromboxane (9). A balance between vasodilatory and vasoconstrictive factors is important as it keeps blood vessels in a homeostatic state (10). Changes in the release of vasoactive factors, such as decreased bioavailability of NO, may lead to endothelial dysfunction. Endothelial dysfunction, along with other risk factors, such as aging, inflammation, obesity, increased salt intake, smoking, and alcohol consumption, could contribute to the development of arterial stiffness (8). Sustained arterial stiffening may predispose the intima layer of the affected blood vessels and may contribute to the development of atherosclerosis (11). Obesity is one of the major risk factors for the development of vascular dysfunction and CVDs (12). It increases the concentration of circulating free fatty acids and alters anti-inflammatory and pro-inflammatory cytokines that are released from visceral fat. These functional and structural changes affect the microvasculature, leading to vascular dysfunction and possibly CVDs (13). Also, oxidative stress is reported to affect vascular function as free radicals are shown to affect the availability of NO, leading to endothelial dysfunction (14). Free radicals can equally affect enzymes implicated in the regulation of the extracellular matrix of the blood vessel wall, leading to arterial stiffness (15).

It is reported that vascular dysfunction is central to the origin of CVDs (16). Moreover, there is increasing prevalence of cardiovascular risk factors, such as obesity and hypertension, in African children. A study conducted among adolescents in Fetakgomo Municipality, Limpopo Province of South Africa found that the prevalence of obesity was 35% (17). Another study carried out in the Eastern Cape Province of South Africa documented a 19.8% prevalence of obesity in children aged 6–9 years old (18). A recent meta-analysis study reports an increased prevalence of hypertension among African children aged 2–19 years (19). Although the prevalence of cardiovascular risk factors, such as obesity and hypertension, in children in SSA is on the rise, little is known about how these factors may affect vascular function. Hence, this review intends to give an overview of bioactive factors in the regulation of vascular function. It also discusses the causes of vascular dysfunction along with the methods used for assessment. It further highlights the key determinants of vascular dysfunction and the associated consequences and provides evidence of vascular dysfunction in children and adolescents of African ancestry.

Vascular Function

The vascular system is made up of blood vessels, such as arteries, veins, and capillaries (20). Blood vessels are organized in hierarchal levels with complex and different configurations designed to ensure efficient exchange of nutrients and waste in and between tissues throughout the body. Large arteries with diameters above 6 mm transport oxygenated blood from the heart to smaller arteries ranging between 1 and 6 mm in diameter, then to the arteriolar network with diameters of 100–1,000 μm, and last into capillary beds of 10–15 μm in diameter (21). The arterial wall is an organized structure composed of matrix proteins (collagen fibers oriented in various directions and elastic lamellae), vascular smooth muscle cells (VSMCs), and other matrix components, such as glycosaminoglycans and endothelial cells in the inner layer (22). The cross-sectional layers of the arterial wall are shown in **Figure 1**. The endothelium is a thin monolayer of simple squamous cells lining the inner surface of the whole cardiovascular system (23, 24). It was once thought to be just an inert layer wrapping all endovascular surfaces. However, over the last four decades, research on the endothelium has become enormous, and its results have led to an understanding of its complex functions (25). It forms a biocompatible barrier between the circulating blood and all the underlying tissues (26). The endothelium plays an essential role in vascular function through several mechanisms, including the synthesis and release of substances that act in an autocrine and/or paracrine form. It controls all cardiovascular activities by releasing several vasoactive agents (27). The endothelium-derived dilating and contracting factors are balanced under physiological conditions so that vascular homeostasis is maintained in favor of vasodilation. Dilatory factors include NO, hydrogen sulfide, prostacyclin (prostaglandin I2), and endothelium-derived hyperpolarizing factor, whereas contracting factors include endothelin, thromboxane, and asymmetric dimethyl arginine (ADMA) (27). Microcirculation is the terminal vascular network of the systemic circulation comprising microvessels with a diameter of $<20\,\mu m$. These microvessels consist of arterioles, postcapillary venules, and capillaries. Microcirculation is regarded as the last destination of the cardiovascular system and is ultimately accountable for the transfer of oxygen from the red blood cells in the capillaries to the parenchymal cells where oxygen is delivered to fulfill the energy requirements of the tissue cells (28). The capillaries consist of a single layer of endothelial cells (29). The distensibility and elasticity of arteries keep a relatively fixed blood pressure regardless of the pulsating nature of blood flow by each heartbeat. Arteries expand as a result of receiving blood expelled from the heart during systolic contraction and eject it to the periphery during diastole to supply the peripheral circulation with a steady flow of blood during systole and diastole cycles (30). Some of the major vasoactive factors implicated in the vascular function of blood vessels are discussed below.

Vasoactive Factors

Thromboxane and Prostacyclin

Prostacyclin and thromboxane are vasoactive factors implicated in the regulation of blood vessel relaxation and contraction. Although prostacyclin also known as prostaglandin I2 is a vasodilator, thromboxane is a vasoconstrictor. Prostacyclin and thromboxane are produced from the endothelium of blood vessels (31). Prostaglandin H2 is produced following the enzymatic degradation of phospholipid membrane in the endothelium by phospholipase enzyme to release arachidonic acid (AA) (32). AA is then metabolized by cyclooxygenase-1 (COX-1) or cyclooxygenase-2 (COX-2) to produce prostaglandin H2, which is a precursor for thromboxane synthase, prostaglandin synthase, and prostacyclin synthase. Under physiological conditions, COX-1 is expressed in most

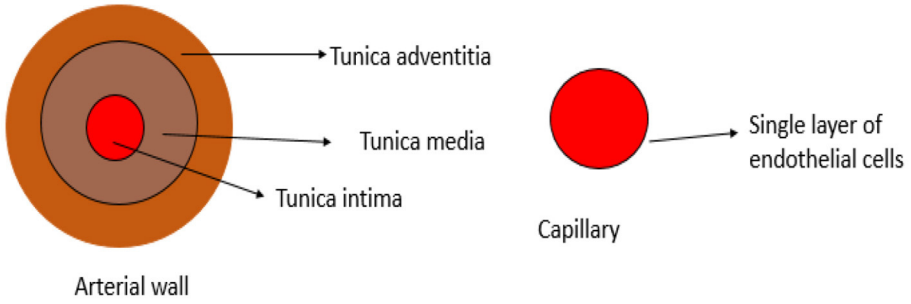

FIGURE 1 | Cross-section of layers in the arterial wall.

tissues, whereas COX-2 is expressed by inflammatory cells, such as macrophages, and it leads to the production of thromboxane, which plays a role in platelet aggregation, vasoconstriction, and proliferation (33, 34).

The platelets remain in their inactive state as they circulate through the blood vessels of the intact endothelium. This inactivated state is sustained by continuous secretion of prostacyclin as well as the absence of pro-inflammatory factors that can activate COX-2. Once there is a break in the endothelium, platelets become activated by thromboxane, which initiates the aggregation of platelets into a growing thrombus through the activation of G-protein. This activates phospholipase C to hydrolyze phosphatidylinositol phosphate to diacylglycerol and inositol triphosphate as well as increases calcium ion accumulation to directly heighten VSMC contraction (35, 36). Following the release of prostacyclin, it acts on VSMCs through prostacyclin receptors linked to the activation of membrane-bound adenylate cyclase, which converts adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP). Accumulation of cAMP as a result of prostacyclin leads to vasodilation and inhibition of platelets aggregation (37).

NO

The most important vasoactive factor is NO as it plays a crucial role in the vasculature stimulating VSMC relaxation and, thus, controlling vascular resistance and blood pressure. It also eliminates free radicals and prevents build-up of plaque (38). As blood flows through the vessels, endothelial cells detect shear stress exerted by the pressure of blood and respond by releasing acetylcholine to act on its endothelial receptor, which triggers excessive release of calcium ions from the endogenous storage sites (39). The released calcium ions attach to calmodulin protein in the cytoplasm of the cell to form a calcium-calmodulin complex, which activates the endothelial nitric oxide synthase (eNOS). The active form of this enzyme catalyzes the conversion of L-arginine and oxygen to citrulline and NO molecule. There are three isoforms of mammalian NOS, namely, neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS) of which the latter is the main source of NO in the endothelium (40). To apply its dilatory effects, NO diffuses to adjacent VSMCs, where it binds to the heme moiety of cytosolic guanylate cyclase (GC). This active enzyme, in turn, activates guanosine triphosphate to its active form, that is, cyclic guanosine monophosphate (cGMP) (41). It is the cGMP that facilitates the dephosphorylation of the myosin light chain, and this process induces the dissociation of myosin and actin filament resulting in VSMC relaxation (41).

Endothelin

Endothelin is a vasoconstrictor that exists in three isoforms, namely, endothelin-1 (ET-1), endothelin-2 (ET-2), and endothelin-3 (ET-3). Three different genes encode endothelin, which gives rise to three different precursors of pre-pro-endothelin (42). Pre-pro-endothelin-1 is the first product encoded by the ET-1 gene (43). This precursor is transformed into pro-ET-1 by removal of a short sequence by a signal peptidase. The pro-ET-1 is then converted to big ET-1 through the activity of furin, a maturing enzyme. Mature ET-1 is obtained by proteolytic cleavage of big ET-1 by endothelin converting enzyme into a small active 21 residue ET-1 (44). Once ET-1 is formed and released from the endothelium, it acts through two types of receptors, namely, endothelin A (ETA) and endothelin B (ETB) receptors. Currently, ET-1 and ET-2 are known to have the strongest affinity for both receptors, whereas ET-3 binds only on ETB (42). ET-1 binds to these receptors on the VSMCs. ETA and ETB are coupled to G-protein to form inositol triphosphate (IP3). This IP3 accumulates in the sarcoplasmic reticulum, leading to the secretion of calcium ions, which, in turn, results in the contraction of VSMCs (45). It is documented that ET-1 is the most potent vasoconstrictor. Moreover, ET-1 is suggested to decrease endothelium-dependent vasodilation. This may be due to the combined effect of ET-1–induced vasoconstriction and, to a lesser extent, ET-1–mediated inhibition of NO production, which together affect the balance between dilatory and constrictive factors in favor of the latter (46). The normal vascular endothelium is considered as a gatekeeper of cardiovascular health, whereas harmful stimuli, such as oxidative stress and inflammation, alter the normal endothelium function, leading to the development of vascular dysfunction (45).

ADMA

Dimethyl arginines are formed during the methylation of L-arginine residues within specific proteins, a process that is catalyzed by arginine methyltransferase. ADMA is released following a cleavage of methylated proteins during physiological

protein turnover (47). Under physiological conditions, ADMA is excreted in urine. However, under pathological conditions, its elimination may be blocked due to hypertension, hypercholesterolemia, diabetes mellitus, and chronic kidney failure (48). As such, there is increased ADMA concentrations in the circulation, which, in turn, competes with L-arginine for the NOS binding site, thereby inhibiting the production of NO (49). Furthermore, both ADMA and L-arginine are transported into the cell through a cationic amino acid transporter; therefore, they compete with each other at the transporter to enter the cell where they are being catalyzed by NOS. As such, the production of NO depends on the balance between L-arginine and ADMA because they both compete for NOS and cell transport (50).

Endothelium-Derived Hyperpolarizing Factor

Endothelial-derived hyperpolarizing factor (EDHF) plays an important role in controlling the vascular tone in the microvasculature (51). Whereas blood vessel relaxation is easily impaired as a result of decrease in NO, EDHF activity of relaxation is enhanced to preserve the homeostasis of blood vessels. This activity of EDHF induces the formation of a disulfide bond between two cysteine 42 residues of each of the adjacent chains in protein kinase G (PKG) (51). This leads to the opening of large Ca^{2+}-dependent channels, resulting in hyperpolarizing and vasodilation (52). The vasoactive factors and their functions are summarized in **Table 1**.

Vascular Dysfunction

Vascular dysfunction comprises dysfunction of the endothelium (endothelial dysfunction), microvascular dysfunction, and large artery dysfunction due to arterial stiffness (9). Endothelial dysfunction is characterized by an imbalance between constrictive factors and dilatory factors, increased concentration of reactive oxygen species (ROS), pro-inflammatory factors, and decreased NO bioavailability (41). The production of NO depends on its precursor, L-arginine, which is synthesized in healthy humans from l-citrulline by endogenous synthesis. This means that reduced levels of L-arginine and l-citrulline contribute to NO insufficiency. Also, free radicals, such as superoxide ($O_2 \cdot^-$), may react with NO to form peroxynitrite ($ONOO^-$) radicals, thereby reducing NO levels (40). A variety of ROS-producing systems, such as NADPH oxidase, xanthine oxidase, eNOS, and enzymes of the mitochondrial respiratory chain, are found within the vascular wall. Moderate levels of ROS have important signaling roles under physiological conditions. Excessive and persistent production of ROS, however, when exceeding the present antioxidant defense enzymes, leads to oxidative stress and decreased NO production (53). It is documented that NO production can also be decreased by ADMA, which competes with the substrate of eNOS, L-arginine, thus inhibiting NO production (54). Endothelial NO is one of the major dilatory factors, and its insufficiency contributes to elevated vascular constriction (55). A study documents that deterioration of NO results in increased levels of ET-1, which is a major vasoconstrictor, leading to a decrease in endothelial dilatory capacity (56). A study conducted in South Africa finds that ADMA is inversely correlated with carotid intima-media thickness (57). Another study documents that black men and women have higher central systolic blood pressure, higher plasma ADMA, and lower urinary nitrate than their white counterparts. This suggests potential increased chances for vascular damage and large arterial stiffness in people of African ancestry in the future as a result of endothelial dysfunction (58).

Microvascular dysfunction is a condition characterized by impaired endothelium-dependent dilation of isolated arterioles. It is documented that microvascular dysfunction precedes and predicts the development of conduit artery atherosclerosis and its determinants (59). Abnormal microvascular function may occur as a result of structural alterations in small arteries due to inward eutrophic remodeling without overall growth of the cell, leading to decreased vasodilator reserves and changes in distensibility of arterioles (60). A study reports that remodeling (damage) of the small artery plays a crucial role in the increase of vascular resistance. This damage in the small arteries, characterized by the thickening of the carotid intima, may be considered as the first manifestation of target organ damage before it occurs in the large arteries (61). More direct impairment of microvascular function occurs as a result of persistent ischemia, manifesting as reduced maximal flow on computerized tomography without the presence of conduit stenosis (59). Microvascular dysfunction is linked to several conditions, such as smoking, obesity, hypertension, and diabetes (62). As such, microcirculatory alteration noted in the renal and retinal systems are extensively studied to investigate the predictive role of glycemic variations early in diabetes (60).

The loss of arterial elasticity, also called arterial stiffness, describes the mechanical property of artery resistance to deformation (63). The stability, compliance, and resilience of the vascular wall are dependent on the activity of two major scaffolding proteins, namely, elastin and collagen (64). The content of these proteins is usually made stable by a dynamic but slow process of their synthesis and degradation. Dysregulation of this balance between their production and degradation commonly stimulated by inflammatory molecules leads to the overproduction of collagen at abnormal levels, which diminishes the normal elastin content. This affects the elasticity and resistance of the arteries, contributing to vascular stiffness (63). With every heartbeat, a pulse wave generated by the arteries travels through the vascular bed until it reaches peripheral resistance or any bifurcation point, producing a new reflected wave back to the heart (65, 66). The reflected wave velocity and the stage of the cardiac cycle in which it happens (during systole or diastole) depends on the peripheral vascular resistance, elasticity primarily of the large arteries, and central blood pressure (66). In healthy individuals, arteries are compliant, and therefore, the reflected wave is slow and returns to the heart during the diastole cycle. However, in individuals with arterial stiffness, the reflected wave reaches the heart early during systole cycle. As a result, this increases the systolic blood pressure with a subsequent increase in cardiac workload to overcome the augmented systolic blood pressure (30, 66).

Assessment of Vascular Function

Vascular function constitutes endothelial function and functioning of the microcirculation and macrocirculation.

TABLE 1 | Vasoactive factors and their functions.

Vasoactive factors	Functions	Citation
Endothelium-derived hyperpolarizing factor	Vascular relaxation in the microvascular beds	(52)
Nitric oxide	Stimulates vascular smooth muscle relaxation, modulate vascular tone and, controls blood pressure	(38)
Thromboxane	Powerful vasoconstrictor and stimulate platelet aggregation	(35)
Prostacyclin	Inhibit platelet aggregation and is a potent vasodilator	(36)
Endothelin	Potent vasoconstrictor and counteracts nitric oxide	(45)
Asymmetric dimethylarginine	Inhibitor of nitric oxide synthesis	(49)

Endothelial function is mostly assessed by flow mediated dilation (FMD) techniques, which require occlusion. Retinal imaging is mostly used to assess the functioning microcirculation, and the macrocirculation function can be assessed by measuring the pulse wave velocity (PWV) as discussed below (67, 68).

FMD

Vascular function can be assessed by numerous methods, including invasive and non-invasive techniques (69). Among the non-invasive techniques, FMD is one of the validated methods for the assessment of vascular function. The method involves ultrasound imaging in stages, at baseline (before occlusion) and during reactive hyperemia (5 min after occlusion of the artery) (70). Endothelial cells lining the artery sense an increase in blood flow and react by generating NO, which causes the diameter of an artery to increase to accommodate the increased demand (71). Such a response is known as FMD. In this technique, a blood pressure cuff is inflated in the forearm to temporarily occlude the brachial artery for a few minutes. This is followed by deflation of the pressure cuff to restore blood flow to the forearm and using an ultrasound to measure the increased diameter of the brachial artery caused by the sudden increase in blood flow (69, 71).

Impaired FMD is linked with conditions predisposing CVDs and is known to be the earliest step in developing subclinical target organ damage (72). In addition, assessment of FMD can classify individuals at low, moderate, or high risk for future clinical events (69). FMD provides valuable prognostic data and is considered the gold standard for assessing endothelial dysfunction (72). However, it has a few limitations that are worth consideration. First, the absence of standardization and differences in placement or positioning of the cuff/probe makes comparison of results difficult. Results may be operator-dependent as the technique requires expertise in the placement of the probe on the arm to identify the pulsating artery. Moreover, changes in structure of the arteries and impaired dilation may be a limiting factor during an FMD test (69).

Flow-Mediated Slowing

Flow-mediated slowing (FMS) can be described as the minimum PWV during reactive hyperemia representing endothelial function (73). A vicorder device is used to perform this test, in which the participant is requested to rest in a supine position for at least 20 min before oscillometric cuffs are wrapped around the upper arm and wrist. FMS assessment commences with baseline measurement of PWV for 4 min followed by 5 min of blood pressure occlusion and finally, 4 min of a postocclusion in which the pressure cuff is released (74). At the end of the test, minimum PWV (m/s) during hyperemia is recorded. PWV is calculated by dividing the arterial length by transit time between the upper arm and wrist. Particularly, the length is measured directly using the device to bypass body contours between the two midpoints of the two cuffs (73). FMS is easier to perform than FMD and is less operator-dependent. As a result, some studies report that FMS seems to be a promising and feasible method for endothelial function assessments (75, 76).

Peripheral Vascular Reactivity Assessment

Endothelial dysfunction can also be measured non-invasively by using a quantitative magnetic resonance imaging (MRI) technique that measures the peripheral vascular reactivity in the superficial femoral artery and vein (77). In this method, participants are required to lie in a supine position on the imager table whereby an eight-channel extremity transmitter–receiver coil is used for assessment. Following 2 min of a baseline period, a sphygmomanometer cuff is applied to the upper right thigh proximal to the targeted vessels, and then it is quickly inflated with a pneumatic pump for a 5-min occlusion period to the target pressure of 220 mmHg. This is followed by a post-occlusion period of 5 min (78). Vessel-wall imaging is done at baseline, occlusion, and post-occlusion to quantify superficial femoral artery luminal flow-mediated dilation, venous oxygen saturation, and arterial blood flow velocity (78). A study reports that methods of quantitative MRI can detect endothelial dysfunction in the presence of overt cardiovascular disease. However, so far, the use of this instrument is limited to research to identify biomarkers for disease progression (77).

Retinal Microvasculature Assessment

The retina is rich with blood vessels and, thus, shares similar anatomical features and physiological properties with blood vessels in the body. As such, visualization of the retinal vasculature allows direct non-invasive assessment of the microvasculature in relation to health and diseases of the vascular system (79). Retinal microvascular changes, such as arteriolar narrowing, arteriovenous nicking, focal arteriolar narrowing, and changes in static retinal vascular caliber, are reported to be early signs of hypertensive retinopathy and atherosclerosis (80). Analysis of the retinal image is of importance as it assists in early diagnosis of diabetic and hypertensive retinopathy and CVDs (80). A portable and easily movable fundus camera is a tool used

to assess changes in the retina, retinal vasculature, and macula of the eye using a low-power intricate microscope in a cost-efficient manner (80, 81). Furthermore, dynamic measurements, such as maximal retina vessel dilation, can also be used to further assess retinal microcirculation (77). The digital interior imaging of the eye through a fundus camera has sensors that convert a light signal into an electric signal, and the result is stored in the form of a pixel (80). Static digital photographs of the retina are taken from both eyes, and computer-based software is used to measure the diameter of arterioles and venules (79). The diameter of the central retinal artery (CRAE) and central renal vein equivalent (CRVE) are calculated. Also, other structural changes, including arteriovenous nicking (AVN) and focal arteriolar narrowing (FAN), are assessed (79). To perform this test, the patient is required to sit in front of the camera with the patient's forehead against the bar. The trainer focuses and aligns the fundus camera on the pupil, and the shutter button is released, thus, firing a flash that forms a photograph of the interior surface of the eye (82). A fundus camera can assist health workers to control vascular diseases affecting both the central and peripheral retina, and it can help patients understand the extent of their cardiovascular health condition (82). An observational study among 40- to 60-year-old adults in the United Kingdom shows that retinal fundus imaging alone may predict multiple cardiovascular risk factors, such as age, gender, and systolic blood pressure (83).

Pulse Wave Velocity

At the end of the ventricular ejection phase, a pressure wave generated from the heart propagates along the arterial tree (69). PWV is defined as a measure of the speed of the arterial pressure wave traveling from the heart along the aorta to the large arteries. It is calculated as the distance of the pressure wave between the arteries/transit time. PWV is the most widely used measure for arterial stiffness (84). There are different types of PWV measurements with carotid-femoral PWV (cfPWV) and brachial-ankle (baPWV) being the most commonly used methods in clinical settings and research (84). PWV can be assessed non-invasively using a vicorder device, and it is referred to as the "gold standard" measurement for arterial stiffness because it is a reliable, inexpensive, and simple non-invasive tool to identify or detect CVD risk in its earliest stages (84). A study finds that the 10th, 50th, and 90th percentiles of cfPWV assessed using a vicorder were, respectively, 4.8, 5.57, and 6.6 m/s as reference values for adolescents aged 18 years old (85).

Apart from the vicorder, the sphygmocor cardiovascular management suite (CvMS) has been used in the field as a non-invasive method for PWV and aortic pressure waveform assessment. This device depends on applanation tonometry to detect radial, carotid, and femoral blood pressure waveforms (86). Studies utilize this device to measure PWV (87, 88). A study in South Africa has equally utilized this device to assess PWV in pre-eclamptic women (89). Although this device is reported to be effective in assessing PWV, its major disadvantage is difficulty in obtaining the peripheral waveform. Also, the device is technically difficult to use, and it is operator-dependent in identifying the peripheral signal (86, 90).

Recently, a new device called the Sphygmocor XCEL, which makes use of the volumetric displacement (cuff-based) technique to obtain pulse information, was developed (86). It is used to measure arterial stiffness and wave reflection strength (91). A study in South Africa reports that further studies are required to investigate the accuracy of PWV measurements by Sphygmocor XCEL (89). This device is preferable over the Sphygmocor CvMS because it is not operator-dependent (92). Furthermore, there is no need for an electrocardiogram to be aligned sequentially to acquire signals when assessing cfPWV using Sphygmor XCEL. However, Sphygmocor CvMS is more suitable in research than Sphygmocor XCEL in measuring high-frequency components of the waveform (86).

Another device for the measurement of PWV and central systolic blood pressure is the Complior. This device measures the PWV between the carotid and radial arteries using piezoelectric clips (sensors) placed around the neck and the wrist (93). This device is suggested to be accurate and reliable in the non-invasive assessment of PWV and is utilized in studies in South Africa to measure PWV (94–97). However, one of the limitations of this device is that it is operator-dependent in accurately positioning the sensors in the various arteries to measure the waveform. This may lead to discrepancies between the distance measured between the sensors and the actual path length traveled by the pulse wave. Furthermore, the sensors are highly sensitive to motion and may be affected by the positioning of the arteries (94, 98).

Carotid Intima-Media Thickness Assessment

Carotid intima-media thickness (cIMT) is the thickness of the intimal and medial layers of the carotid arterial wall, and it can be measured non-invasively using a scanner imaging device (99). The test is performed using a sonography with a high frequency of 7.5 MHz linear array transducer. The patient is required to lie in a supine position, and the common carotid artery is visualized at 1 cm proximal to its bifurcation (100). The cIMT is described as the length between the leading edge of the luminal echo to the leading edge of the adventitia of the media (101). It is documented that cIMT >0.9 mm is denoted as a marker of asymptomatic organ damage. Moreover, intima media thickness (IMT) is accepted as an earliest marker of atherosclerotic vascular disease, and screening of IMT can help physicians to classify patients with cardiovascular risk into lower or higher risk categories (102). A study conducted in South Africa reveals that cIMT is elevated in females with HIV aged 35–45 years old in Elandsdoorn, Limpopo (103). A study among a group of individuals from Johannesburg and Limpopo, South Africa, finds that increased cIMT is associated with cholesterol (104). In the North West Province of South Africa, lower cIMT was associated with physical activity among female teachers (105).

Determinants of Endothelial Dysfunction

It is known that risk factors for CVDs begin early in life (5, 6). A study finds that carotid bifurcation regions depicted widespread intimal lipid accumulation among newborn cadavers (106). Moreover, bifurcation anatomy affects blood flow, which causes endothelial injury (106). This indicates that endothelial

dysfunction begins early in life. A study confirms that offspring have a distinct endothelial regulatory micro RNA profile at birth, which is associated with altered endothelial cell behavior during the first 3 months of life (107). It is documented that maternal total cholesterol (TC) concentrations increase in human pregnancy to meet the demands of the growing fetus (108). In some pregnancies, however, TC increases excessively mainly due to low-density lipoprotein cholesterol levels, a condition called maternal supraphysiological hypercholesterolemia in pregnancy, which is associated with endothelial dysfunction of the umbilical vein and early development of atherosclerosis in the fetal aorta (109). Furthermore, endothelial dysfunction is associated with various obstetrical syndromes, such as fetal growth restriction (FGR) (110). Evidence shows that FGR fetuses alter their cardiovascular function *in utero* to adjust to persisting suboptimal conditions, mainly chronic hypoxia (111). Changes in cardiovascular function secondary to utero-placental deficiency may result in permanent alterations in vascular structure (112). Fetal growth restriction leads to low birth weight. Children born with low birth weight experience catch up growth during their first years of life, thus, accumulating greater visceral adiposity, exposing them to an adverse metabolic outcome (110). All these findings suggest that maternal cardiovascular risk factors may affect the vascular function of the fetus and neonates.

Obesity, a multifactorial condition characterized by excess adipose tissue is a major determinant of vascular dysfunction and constitutes a serious worldwide health problem (113). The adipose tissue, where fat is stored in the body, is a type of connective tissue comprising lipid-filled cells (adipocytes) surrounded by a matrix of collagen fibers, blood vessels, immune cells, and fibroblasts. It consists of several cells with adipocytes being the most abundant. Other cells include stromal vascular fraction (SVF), endothelial cells, macrophages, stem cells, fibroblasts, and lymphocytes (114). Persistent accumulation of fat in the adipose tissue leads to adipocyte hypertrophy and hyperplasia (113). Adipose tissue hypertrophy (adipocyte cell size increases) and hyperplasia (increase in adipocyte number) occurs in childhood (115). The expansion of adipocytes leads to an increased release of free fatty acids and necrotic cell death due to hypoxia and inflammation (116). During physiological conditions, inflammation is regarded as a protective mechanism. However, obesity is accompanied by some degree of inflammation called low-grade inflammation (117) whereby the adipose tissue secretes high levels of pro-inflammatory adipocytokines, including tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), resistin, and leptin, due to cell death by necrosis following hypoxia (113). This causes an infiltration of neutrophils, eosinophils, monocytes, and lymphocytes to clean up the dead cells (117). The resident macrophages in the adipose tissue release chemo-attractants for macrophages, which results in the persistent nature of chronic inflammation. This, in turn, promotes the inhibition of the production of adiponectin, an anti-inflammatory adipokine (117). Adiponectin is regarded as a beneficial adipokine in relation to metabolism with plasma concentration indirectly associated with trunk obesity, type 2 diabetes risk, and insulin resistance, whereas leptin positively correlates with waist circumference and is associated with the onset of insulin resistance (95, 118). TNF-α is known to trigger insulin resistance in obese individuals. IL-6 is known to be implicated in the pathways of insulin sensitivity, lipoprotein lipase downregulation and triglyceride synthesis (119). Persistent release of these pro-inflammatory markers, such as TNF-α and IL-6 results in decreased production of adiponectin (120). Decreased plasma levels of adiponectin promote the synthesis of arginase, a metalloprotease that catalyzes the conversion of L-arginine to L-orthinine and urea. The increased concentrations of arginase compete with eNOS for the substrate L-arginine. Increased arginase activity uncouples eNOS for the synthesis of NO, thereby leading to reduced production of NO (121). A decreased bioavailability of NO leads to endothelial dysfunction. Defect in the synthesis of NO can also be caused by high concentrations of ADMA in the plasma (122). ADMA is an endogenous competitive inhibitor of L-arginine for all three isoforms of NOS. High levels of ADMA block the synthesis of NO and limit the cellular uptake of L-arginine, thereby further disrupting the production of NO. In this manner, ADMA further affects the endothelial function (123).

Secreted inflammatory molecules, including pro-inflammatory cytokines, contribute to the generation of ROS (124). Since adipose tissue are known to secrete pro-inflammatory cytokines, they may promote the generation of ROS. As such, adipose tissue is regarded as an independent factor for the development of oxidative stress (125). ROS are highly reactive radicals derived from molecular oxygen, such as O^{2-}, hydrogen peroxide (H_2O_2), hydroxyl radical (OH·), and $ONOO^-$, that impair structural conformation of protein, DNA, and RNA in the cell, resulting in cellular dysfunction and cell death (126). Under physiological conditions, ROS contribute to cellular growth regulation, differentiation, and apoptosis (114). Furthermore, they are produced from endothelial cells by several enzymes, including NADPH oxidases, xanthine oxidoreductase (XOR), and mitochondrial enzymes, among many other sources (127). It is known that H_2O_2 has vasodilatory effects, whereas O^{2-} is a vasoconstrictor and leads to endothelial dysfunction (128). High levels of O^{2-} may react with NO to form an unstable free radical called $ONOO^-$ (129). Furthermore, ROS can be produced from the uncoupling of eNOS (129). eNOS uncoupling may occur due to limited availability of the substrate L-arginine (128). As a result, eNOS may produce O^{2-} instead of NO, leading to more defect in the synthesis of NO and, hence, endothelial dysfunction (129). Also, small, dense, low-density lipoprotein (LDL) in the lumen is deposited into the subendothelial space where it becomes oxidized by ROS to become ox-LDL, which activates endothelial cells, causing expressed receptors for white blood cells on the surface (130). It is reported that ox-LDL induces the expression of ICAM-1 and VCAM-1, increasing the adhesive properties of the endothelium. The production of NO by endothelial cells is inhibited by ox-LDL. It is documented that ox-LDL leads to oxidative stress, producing high amounts of O^{2-}, which inactivates NO to form $ONOO^-$ (131). The decrease in NO as a result of ox-LDL leads to endothelial dysfunction.

Although hypertension is generally known be a consequence of endothelial dysfunction (132, 133), recent data suggest that

hypertension may be a cause of endothelial dysfunction. There are reports that hypertension-induced endothelial dysfunction may be a result of hypertension-induced oxidative and inflammation (134). Hypertension-associated oxidative stress regulated by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and mitochondria show reductions in endothelium-dependent vasodilation to acetylcholine in carotid arteries of mice exposed to increasing intraluminal pressure as a result of increase in NADPH oxidase activity and vascular O^{2-} production (135). Also, obese hypertensive rats with perivascular inflammation show impaired endothelial function (136). Further, the activation of the innate immunity complement pathway, which regulates inflammation, is negatively associated with vascular endothelial function in hypertensives (137). All these studies support the notion that hypertension may be the cause of endothelial dysfunction.

Consequences of Vascular Dysfunction

Endothelial dysfunction is a crucial risk factor for the development of high blood pressure as it not only impairs the control of the vascular tonus, but also alters structural function, such as the tunica intima of blood vessels (138). LDL as a result of hyperlipidemia, which is associated with obesity, may be deposited into the intima of blood vessels where they may be oxidized by ROS. This oxidized LDL (ox-LDL) activates the endothelial cells to induce monocyte recruitment into the endothelial wall (139). The recruited monocytes differentiate into macrophages that take up the ox-LDL via scavenger factors, resulting in intracellular lipid accumulation and subsequently the formation of foam cells (139, 140). Foam cells produce growth factors that cause the synthesis of collagen and VSMC to migrate into the intima, which begins to proliferate and secrete extracellular matrix, resulting in thickening of the arterial intima. Thickening of the intima can lead to severe CVDs, such as stroke, ischemic disease, and congestive heart failure later in life (139, 141).

It is known that early endothelial dysfunction decreases vascular relaxation and causes the infiltration of inflammatory cells, leading to mild inflammation in blood vessels (142). eNOS is formed in high concentrations in endothelial cells, specifically in the renal medulla, where it maintains medullary blood flow in response to renal vasoconstrictors, such as angiotensin II. Impaired activity of eNOS may be due to endothelial damage or extrinsic free radical activity altering NO activity (143). ROS may influence the effects of dilatory and constrictive factors, thus leading to elevated vascular resistance and acute kidney injury (144).

Sustained damage by hyperglycemia or other factors, such as hypertension in the microvessels of the retina results in diabetic retinopathy (145). Diabetic retinopathy is the main cause of blindness in high- and middle-income countries (109). Hyperglycemia increases hypoxia induced factor 1 (HIF-1) and insulin-like growth factor-1 (IGF-1). The overexpression of HIF-1 and IGF-1 and other factors activate Müller cells to transform into chronic inflammatory cells. Moreover, this induces overexpression and buildup of vascular endothelial growth factor (VEGF) causing fibroblast growth, thereby initiating fibrosis (146). VEGF is documented to stimulate angiogenesis and neovascularization, which are involved in the pathogenesis of proliferative retinopathy (145). Microvascular dysfunction can also result from arterial stiffness (147). Arterial stiffness is associated with normal and accelerated aging (147). The consequence of arterial stiffness includes augmented systolic blood pressure, which is characterized by pulse pressure (30, 148). Greater pulsatile pressure increases the pulsatile flow to penetrate deeper into the periphery and damage the microvasculature specifically in the brain and kidney (30).

Vascular Dysfunction in Children and Adolescents of African Ancestry

The increasing prevalence of cardiovascular risk factors, such as hypertension, in SSA children has implications on their vascular health (4). However, very few studies assess the vascular function of children of African ancestry. A study in Kwa-Zulu Natal Province of South Africa shows that age and resting heart rate were positively associated with arterial stiffness among children aged 10–13 years old (149). Age could play an important role when assessing arterial stiffness (150). However, for a deeper understanding, it should be examined in conjunction with growth and maturation, given that body height at the transition from childhood to adolescence is documented to affect arterial stiffness. An association between resting heart rate and arterial stiffness in children is still lacking in the literature (149). A study conducted in the Eastern Cape Province, South Africa, among 6- to 9-year-old children finds that blood pressure parameters, such as mean arterial and diastolic blood pressure, increased with increasing PWV (151). This suggests that hypertension may result in vascular impairment in children. Another study conducted in Potchefstroom, North West Province of South Africa, in 6- to 8-year-old boys shows that oxidative stress is positively associated with cfPWV and carotid dorsalis pedis PWV in boys exposed to maternal cardiovascular risk compared with the non-maternal risk group (152). This suggests that oxidative stress may be an early mediator of vascular changes in children exposed to maternal cardiovascular risk. PWV significantly correlates with ADMA and systolic blood pressure (SBP) in a study conducted among 13- to 16-year-old children in the Eastern Cape Province of South Africa, suggesting that ADMA might be considered as a major risk factor of vascular dysfunction in adolescents (153). The PWV increased with cumulative time on ART in children living with HIV among primary school children in Cape Town, Western Cape Province of South Africa (154). In Mozambique, a study conducted among children with perinality-acquired HIV finds that PWV is higher in participants with increased visceral fat, elevated lipids, and insulin resistance (155). A study carried out in Egypt among 74 obese children aged 6–18 years finds a significant positive correlation between cIMT and BMI. cIMT equally shows a significant positive correlation with triglycerides and TC (156). Another study conducted in Egypt among 5- to 14-year-old children finds that cIMT is higher in obese children as compared with non-obese children. Further, obese children with elevated LDL and TC show increased risk for endothelial dysfunction and early signs of atherosclerosis

TABLE 2 | Vascular dysfunction and their associated risk factors in African children.

Age	Number of children	Country	Type of study	Measure of vascular function	Outcome	Citation
10–13	59	South Africa	Cross-sectional	PWV	Arterial stiffness was associated with age in boys.	(149)
6–9	303	South Africa	Cross-sectional	PWV	PWV increased with an increase in arterial pressure	(151)
6–18	74	Egypt	Cross-sectional	cIMT	cIMT correlated with BMI	(156)
13–16	244	South Africa	Cross-sectional	PWV and ADMA	PWV significantly correlated with ADMA	(153)
5–14	82	Egypt	Cross-sectional	cIMT	Increased cIMT in obese children	(157)
6–8	81	South Africa	Cross-sectional	PWV	High PWV observed in black boys as compared to their white counterparts	(158)
6–8	81	South Africa	Cross-sectional	PWV	Lipid peroxidation correlated with cfPWV	(152)
6–12	77	Mozambique	Cross-sectional	PWV	PWV higher in children with increased visceral fat, insulin resistance and increased lipids	(155)

ADMA, Assymetric Dimethyl arginine; PWV, Pulse wave velocity; CIMT, Carotid intima-media thickness; BMI, Body mass index; cfPWV, Carotid-femur PWV.

(157). Thus, higher cIMT in obese children denotes increased risk for early vascular dysfunction. Exposure to risk factors of CVDs, such as hypertension and hyperlipidemia in obese children may induce alterations in the arteries, thus contributing to impaired endothelial function (156, 157). Higher PWV (carotid-radial, carotid-femoral, and carotid-dorsalis), diastolic blood pressure, and cIMT are reported in black boys than in white boys aged 6–8 years old in Potchefstroom, North West Province of South Africa. Moreover, black boys had increased levels of pentosidine, which is a biomarker for microvascular complications. However, arterial stiffness was not associated with pentosidine in both groups of boys, suggesting that vascular aging begins early in black population (158). Risk factors associated with vascular dysfunction in African children are summarized in **Table 2**.

CONCLUSION

Cardiovascular risk factors, such as obesity and hypertension, are known to be major contributors to the development of vascular dysfunction in children of African ancestry. Parameters of vascular function, such as PWV, cIMT, and ADMA, are used to assess cardiovascular risk in children of African ancestry. The presence of vascular dysfunction triggered by obesity, hypertension, oxidative stress, and inflammation in these children suggest a future risk of CVDs, such as stroke and heart attack in adulthood. However, only a few studies assess vascular changes in children of African ancestry, and such assessments are mostly limited to arterial stiffness and cIMT, as non-invasive methods along with a few vasoactive factors. Moreover, limited or no studies utilize FMD, FMS, retinal vascular assessments, and other recent PWV techniques to assess vascular function. These findings are, therefore, not sufficient to clearly describe the state of vascular dysfunction in children of African ancestry, and thus, additional studies with more robust methods for the assessment of vascular function, such as FMD and retinal microvasculature measurements are needed to provide sufficient information on vascular function in children of African ancestry and its implication.

AUTHOR CONTRIBUTIONS

GE and BN-C were involved in the development and conceptualization of this review. EM developed the literature with the assistance from GE, BN-C, and CS-R. All authors mentioned contributed to the final manuscript.

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Cross-Talk Between Large Artery Stiffness and Retinal Microvasculature in Children: The ExAMIN Youth SA Study

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Background: Cross-talk between the macro-and microvasculature is considered an important contributor to target organ damage. Previous findings were predominantly in adult populations and investigation into this mechanism in children may provide insight into the development of early adverse vascular changes. Whether any ethnic differences in cross-talk is evident, also remains to be determined.

Objective: To determine whether retinal microvascular diameters are associated with large artery stiffness in young children and whether ethnic differences are evident.

Materials and Methods: In this cross-sectional study, 730 black ($n = 437$) and white ($n = 293$) school children aged 5-9 years were included. Pulse wave velocity (PWV) was measured and the central retinal arteriolar equivalent (CRAE) and central retinal venular equivalent (CRVE) diameters were calculated from fundus images. The arterio-venous ratio (AVR) was subsequently calculated.

Results: Pulse wave velocity was lower ($p \leq 0.001$) in the black group when compared to the white group. The black group had a narrower CRAE, wider CRVE and lower AVR (all $p < 0.001$). Pulse wave velocity associated negatively with CRAE ($r = –0.141$, $p = 0.003$) and AVR ($r = –0.185$, $p \leq 0.001$) in the black group only. A positive association between PWV and CRVE was seen in the black ($r = 0.174$, $p \leq 0.001$) and white ($r = 0.119$, $p = 0.043$) group.

Conclusion: Large artery stiffness is associated with retinal arterial narrowing and venular widening in children, suggesting cross-talk between the macro-and microvasculature. Ethnic differences in these associations are also evident. Our findings warrant further investigation into environmental and sociocultural risk factors contributing to premature cardiovascular disease development.

Keywords: arterial stiffness, central retinal artery equivalent, central retinal vein equivalent, arterio-venous ratio, ethnicity, pediatric

INTRODUCTION

Cardiovascular disease (CVD) remains a major health challenge globally. This challenge is not limited to adult populations as an increasing trend in hypertension and the subsequent future development of CVD in childhood populations has become evident (1–3). A number of cardiovascular risk factors track from childhood into adulthood which may initiate early onset of CVD and related mortality later in life (4, 5), highlighting the importance of primary and even primordial prevention of CVD (6).

The importance of large artery stiffness in cardiovascular (CV) risk prediction in adults is well established, with studies showing associations with end organ damage and clinical outcomes (7). Arterial stiffening is a natural consequence of aging and the associated natural biological deterioration of vascular structure and function, however in any given population some individuals are at higher risk of accelerated biological aging, placing them on a trajectory of early vascular aging (EVA) (8, 9). As central arterial stiffness is also considered one of the earliest detectable manifestations of vascular compromise (10), adverse changes in measures of arterial stiffness in children may provide insight into possible early vascular compromise and create opportunity for interventions.

In addition to the prognostic value of large artery stiffness parameters in cardiovascular risk prediction, the retinal microvasculature enables the investigation of the manifestation of systemic vascular diseases on a microvascular bed (11). Indeed, retinal microvascular alterations such as retinal arteriolar narrowing, venular widening and the resulting lower arteriolar to venular ratio (AVR) predict the risk of hypertension and stroke (12, 13) and is associated with cardiovascular mortality (14) in adults. It is proposed that cross-talk between large and small arteries occurs and that CV risk factors affect both vascular beds, even in children (15). Cross-talk between these vascular beds is also supported by the findings from a study including Swiss children (aged 6–8 years, $n = 1171$), where large artery stiffness, as measured by pulse wave velocity (PWV), was associated with retinal arterial narrowing as well as venular widening (15). Aside from these findings, data regarding cross-talk between large and small arteries in children is limited. An important factor of consideration is whether ethnic differences in the cross-talk between large and small arteries will be evident, as higher aortic stiffness has been extensively reported in black pediatric population groups compared with age-matched white groups independent of traditional risk factors (16–19). Data on ethnic differences in microvascular function in children is limited, however studies in adult populations have established that microvascular dysfunction is more evident in black groups when compared to their white counterparts (11, 20).

The investigation of preclinical measurements in children may provide novel insight into accelerated vascular deterioration, also coined EVA (21), a significant contributor to hypertension and CVD. We therefore aimed to determine whether retinal microvascular diameters are associated with large artery stiffness, as determined by PWV, in young children and whether ethnic differences in these associations are at play.

MATERIALS AND METHODS

The Exercise, Arterial Modulation and Nutrition in Youth South Africa (ExAMIN Youth SA) study was designed to investigate the interplay between body composition, dietary intake, physical fitness, and physical activity, psychosocial stress, cardiovascular function as well as urinary and salivary biomarkers. In this cross-sectional study we included data from 730 apparently healthy children (aged 5–9 years) that included black ($n = 437$) and white ($n = 293$) girls and boys, after the exclusion of participants with missing data for arterial stiffness and retinal vessel diameters ($n = 332$).

The study population and protocol for the ExAMIN Youth SA study has been described elsewhere (22). Briefly, children (aged Five to Nine years) of both sexes and all ethnicities attending public primary schools within two of the southern municipal areas of the Dr. Kenneth Kaunda district, namely JB Marks (Potchefstroom) and Matlosana (Klerksdorp) in the North West province, South Africa, were invited to participate voluntarily with parental permission. There were no specific exclusion criteria; however, children were excluded if no informed consent from the parent was obtained or if the child did not want to participate. On the day of participation, no children presented with any known illnesses.

The study was conducted in line with the ethical principles of the Declaration of Helsinki (23), was approved by the Health Research Ethics Committee of the North-West University and is registered at ClinicalTrials.gov (NCT04056377). All participants and their parents were fully informed about the objectives of the study and written informed consent/assent was obtained from each participant.

Anthropometric Measures

All anthropometric procedures were performed according to specific guidelines set out by the International Society for the Advancement of Kinanthropometry (ISAK) (22, 24). Waist circumference (cm) was obtained in triplicate using standard protocol (Lufkin® Executive thin line 2 mm steel tape; Apex Tool Group B.V.; AK Emmen, Netherlands). The body mass index (BMI) [weight (kg)/square height (m^2)] of each participant was calculated (SECA portable 213 stadiometer; SECA 813 electronic scale; Birmingham, UK). Body mass index z-scores and percentiles were calculated according to child growth reference data based on their age and sex (25).

Cardiovascular Measures

Blood Pressure

Participants were required to remain in a relaxed chair-seated position for 3–5 min prior to blood pressure (BP) measurements. With the use of a validated automated oscillometric pediatric BP monitor (Omron HBP-1100-E; OMRON HealthCare Co., LTD. Kyoto, Japan) and the correctly sized BP cuff, brachial BP was measured with the participants' feet on the floor and their back and right arm supported (26, 27). Measurements were conducted five times with one-min intervals on the right arm (28, 29). The three measurements with the smallest variation were used to calculate a mean (30). Systolic blood pressure

(SBP) and diastolic blood pressure (DBP) were captured from each measurement. Mean arterial pressure (MAP) was calculated using the following formula (DBP) + (0.4*pulse pressure) [35]. Prior to BP measurements, participants were also required to avert from using any stimulants (food and/or drugs).

Pulse Wave Analysis

Arterial pulse wave analysis was performed with the use of the validated oscillometric Mobilo-O-Graph monitor (I.E.M GmbH, Germany) and integrated ARCSolver software. Participants were in a seated position and using a correctly sized cuff on the mid-upper right arm. Measures of the central systolic- (cSBP) and diastolic blood pressure (cDBP), stroke volume, cardiac output, total vascular resistance, and arterial PWV were determined. Participant data was downloaded using the HMS Client-Server software package version 4.7.1 (I.E.M GmbH, Germany).

Retinal Vessel Analysis

Static retinal blood vessel images were captured using a Static Retinal Vessel Analyzer (SVA-T, Imedos Systems GmbH, Jena, Germany). The system consists of a fundus camera (Topcon TRC NW8) and analyzing software (Visualis 2.80, Imedos Systems GmbH, Jena Germany), allowing non-invasive and non-mydriatic assessment of retinal vessel diameters. Two valid images from the retina of both the left and right eye with an angle of 45° and with the optic disc in the center were taken per child. Retinal arterioles and venules, coursing through an area of 0.5–1 disc diameter from the optic disc margin, were semi-automatically identified at higher magnification using the Vesselmap 2, Visualis, Imedos Systems GmbH software. The examiner differentiated all retinal arterioles and venules in the outer ring-zone and measured them with the software tools. Vessel diameters were averaged to central retinal artery (CRAE) and vein equivalents (CRVE), using the ParrHubbard formula [41] and the arterio-venous ratio (AVR) was subsequently determined (CRAE/CRVE). For the CRAE and CRVE the mean of the right eye results was used.

Statistical Analysis

For statistical analyses, IBM® SPSS® version 27 (IBM Corporation, Armonk, New York) and GraphPad Prism version 5.03 for Microsoft® Windows (GraphPad Software, San Diego, California, USA) were used to analyze and plot the data. Variables were tested for normality using the Kolmogorov-Smirnov test and QQ-plots. Data was expressed as mean ± standard deviation.

For comparisons between the groups, independent *t*-tests were used. Analysis of covariance was also used to determine differences in brachial blood pressure measures with adjustments for age, sex, and body height. Pulse wave velocity was additionally adjusted for MAP while CRAE was adjusted for CRVE and vice versa. Pearson and partial correlations (adjusted for age, sex and MAP) were used to determine the relationships of large artery stiffness with retinal vessel calibers.

RESULTS

The general characteristics of the study population, stratified by ethnicity are presented in **Table 1**. The ethnic groups were comparable in terms of age ($p = 0.96$), while the black group had lower body height, body weight and standardized BMI (all $p \leq 0.001$) when compared to the white group. The black group presented with higher brachial DBP ($p \leq 0.001$) while the central blood pressure measures were comparable (all $p \geq 0.36$). In terms of vascular function, the black group showed a higher total vascular resistance ($p \leq 0.001$), while PWV was lower ($p \leq 0.001$) when compared to the white group. The black group had a narrower CRAE, wider CRVE and subsequently a lower AVR (all $p \leq 0.001$).

In single regression analyses (**Figure 1**), PWV associated negatively with CRAE ($r = -0.189$, $p \leq 0.001$) and positively with CRVE ($r = 0.148$, $p = 0.002$) in the black group only. A negative association between PWV and AVR was evident in the black ($r = -0.296$, $p \leq 0.001$) and the white group ($r = -0.168$, $p = 0.004$).

The results from the single regression analyses were confirmed in partial regression analyses (**Table 2**) with adjustments for age and sex and MAP. PWV associated negatively with CRAE ($r = -0.141$, $p = 0.003$) and AVR ($r = -0.185$, $p \leq 0.001$) in the black group only. In addition, a positive association between PWV and CRVE was also observed in the black group ($r = 0.174$, $p \leq 0.001$). In the white group, PWV associated positively with CRVE ($r = 0.119$, $p = 0.043$).

DISCUSSION

We aimed to investigate the cross-talk between large and small arteries in 730 primary school children by determining whether retinal microvascular calibers are associated with PWV. We further aimed to establish whether any ethnic differences in these associations are evident. Measures of microvascular function were more adverse in the black children, with this group showing a narrower CRAE, wider CRVE, and subsequently a lower AVR. In terms of macrovascular function, we found against expectations, that PWV was lower in the black children when compared to their white counterparts. Ethnic differences in the associations between retinal microvascular calibers and large artery stiffness were also evident and our most prominent finding was that large artery stiffness was associated with retinal arterial narrowing and venular widening in the black group, independent of age, sex, and MAP. In the white children, large artery stiffness was associated with venular widening only.

The concept of large and small artery cross-talk is nested in findings that showed a strong relationship between arterial stiffness and microvascular damage in various organs such as the heart, brain, retina, and kidneys (31–33). Cross-talk is described as a vicious circle of events with adverse changes such as increased wall-lumen ratio and rarefaction of small arteries (22, 34) driving an increase in blood pressure; which in turn, increases large artery stiffness through the process of vascular remodeling. Ultimately, increased large artery stiffness is a major determinant of increased pulsatile pressure, which damages small arteries (23) and favors the development of target organ damage (24). Data to support

TABLE 1 | General characteristics of children stratified according to ethnicity.

	Black children ($n = 437$)	White children ($n = 293$)	*p*
Age (years)	7.46 ± 0.967	7.58 ± 0.809	0.96
Sex, boys (*n* %)	213 (42.0)	163 (52.6)	**0.003**
Body composition			
Waist circumference (cm)	53.16 ± 5.86	58.3 ± 7.89	**<0.001**
Body height (cm)	121 ± 7.46	127 ± 6.99	**<0.001**
Body weight (kg)	23.6 ± 5.50	27.1 ± 6.48	**<0.001**
BMI (kg/m²)	15.9 ± 2.40	16.7 ± 6.48	**<0.001**
BMI z-score	−0.182 (−2.13; 1.75)	2.41 (−1.43; 2.06)	**<0.001**
Cardiovascular measures			
Brachial systolic blood pressure (mmHg)*	101 ± 10	104 ± 10	0.44
Brachial diastolic blood pressure (mmHg)*	65 ± 8	64 ± 7	**<0.001**
Brachial mean arterial pressure (mmHg)*	80 ± 8	80 ± 7	**0.001**
Central systolic blood pressure (mmHg)	96 ± 10	96 ± 8	0.61
Central diastolic blood pressure (mmHg)	65 ± 8	65 ± 8	0.36
Pulse wave velocity (m/s)†	4.42 ± 0.328	4.53 ± 0.295	**<0.001**
Stroke volume (ml)	46.9 ± 7.99	51.2 ± 9.56	**<0.001**
Cardiac output (L/min)	4.10 ± 0.557	4.37 ± 0.646	**<0.001**
Total vascular resistance (mmHg/ml/s)	1.23 ± 0.133	1.19 ± 0.153	**<0.001**
Retinal calibers			
Central retinal artery equivalent (MU)‡	198 ± 14.1	200 ± 15.0	**<0.001**
Central retinal vein equivalent (MU)‡	241 ± 15.2	230 ± 15.6	**<0.001**
Arterio-venous ratio	0.825 ± 0.063	0.870 ± 0.057	**<0.001**

Values are arithmetic mean ± standard deviation or geometric mean (5th and 95th percentiles) for logarithmically transformed variables.
Bold values denote statistical significance ($p < 0.05$).
**Brachial blood pressure measures were adjusted for age, sex, and body height.*
†Pulse wave velocity was adjusted for age, sex, and mean arterial pressure.
‡Central retinal artery equivalent was additionally adjusted for central retinal vein equivalent and vice versa.
n, number of participants; MU, measuring units.

this concept has largely been published from studies including adult populations, especially within the setting of hypertension (25). The negative association between CRAE and PWV in the black group, as well as the positive association between CRVE and PWV in both ethnic groups in our study is suggestive of cross-talk between different vascular beds. Our results are in line with the findings from Salvetti et al. where a positive association between the wall-lumen ratio of retinal arterioles and PWV was reported in an adult cohort which included treated and non-treated hypertensive individuals (26). More recently, it was shown that the progression of microvascular dysfunction was associated with higher PWV after 4 years in a pediatric population (27), while a significant but weak inverse association between CRAE and PWV was also reported in a Swiss cohort of children aged 6–8 years (15).

In terms of the possible contribution of ethnicity to differences observed in cross-talk between the macro- and microvasculature, the black children also had a narrower CRAE and wider CRVE. Although data regarding ethnic differences in microvascular function is limited, our results are in line with those of a study that included young black and white adults (20-30 years) that also showed smaller CRAE values in the black group compared to their white counterparts, taking into account that the 24 h BP and anthropometric profiles were similar. Furthermore, data from the Atherosclerosis Risk in Communities Study (28) showed that black participants tended to have narrower retinal arteries and wider retinal venules, although this study included older individuals. Against expectations, in our study PWV was lower in the black children when compared to the white children. Our results contradict the findings of a number of previous adult studies that showed black population groups to have increased large artery stiffness, as measured by various markers such as PWV (20), pulse pressure amplification (29), and augmentation index (30). Despite the lower PWV observed in the black children, the associations between the macro-and microvascular parameters were more pronounced, with all retinal vessel calibers showing adverse associations with PWV in this group. These findings may infer that even at lower levels of arterial stiffness, cross-talk between these vascular beds is more significant in black children. A further important finding was the higher total vascular resistance observed in the black children, as it is known that vascular resistance in the large arteries is closely associated with the progression of arterial stiffness. Recent findings have also suggested that an increase in BP in childhood seems to be driven by peripheral resistance resulting in increased arterial stiffness and at a later stage; the manifestation of high BP might

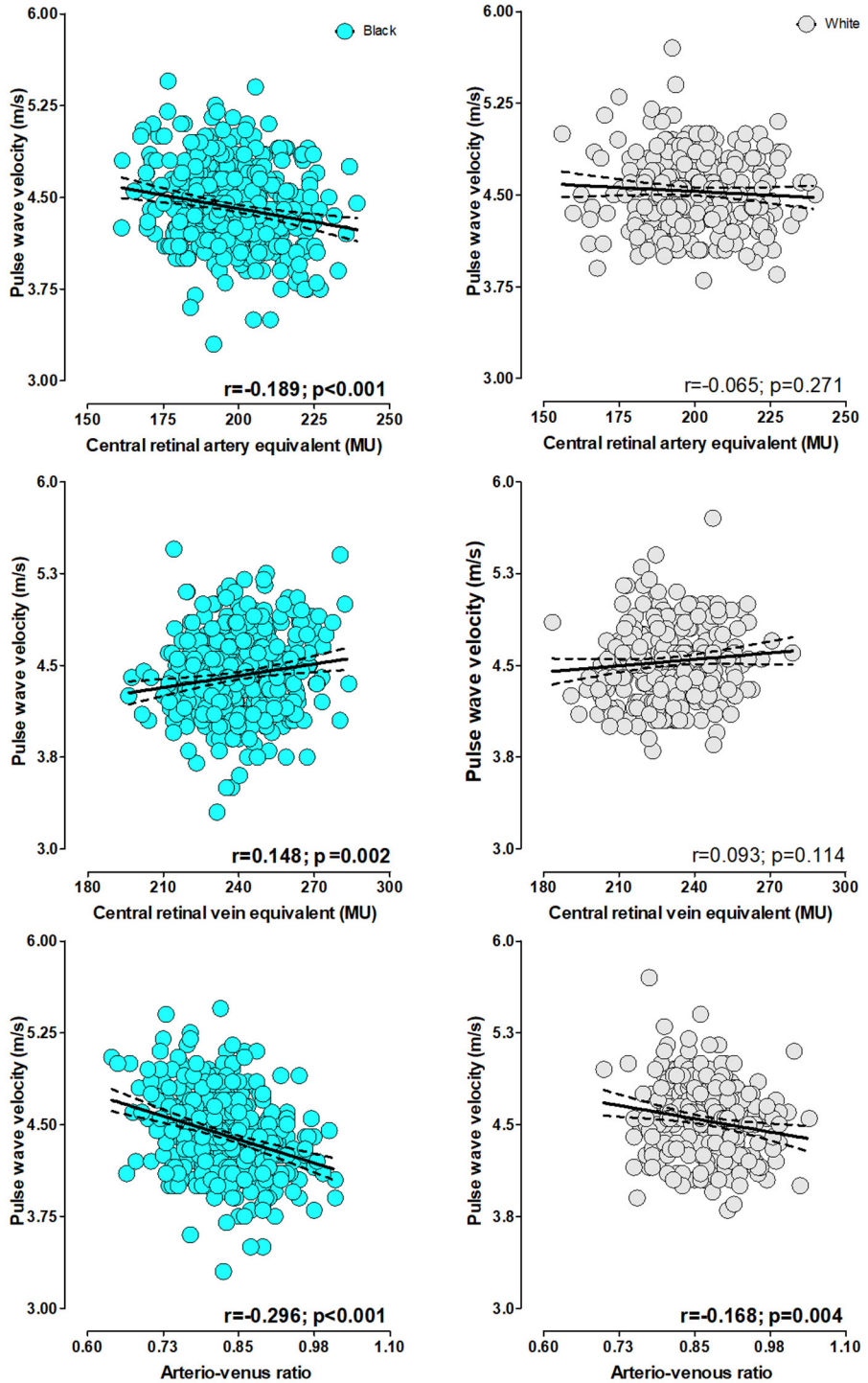

FIGURE 1 | Single regression analyses pulse wave velocity with retinal calibers stratified by ethnicity.

be mediated by arterial stiffness. Although numerous ethnic differences in arterial stiffness and microvascular function have been previously reported, whether ethnicity in itself can be regarded as a risk factor for EVA remains debatable. It is likely that multiple differences in environmental and sociocultural risk factors which may adversely influence biological aging could explain these differences and warrants further investigation.

This study must be interpreted within the context of its strengths and limitations. This study is limited by its cross-sectional design; hence cause and effect cannot be inferred.

TABLE 2 | Partial correlations between large artery stiffness and retinal calibers in children stratified according to ethnicity.

	Central retinal artery equivalent (MU)		Central retinal vein equivalent (MU)		Arterio-venous ratio	
	Black ($n = 437$)	**White ($n = 293$)**	**Black ($n = 437$)**	**White ($n = 293$)**	**Black ($n = 437$)**	**White ($n = 293$)**
Pulse wave velocity (m/s)	**$r = -0.141$; $p = 0.003$**	$r = -0.055$; $p = 0.348$	**$r = 0.174$; $p < 0.001$**	**$r = 0.119$; $p = 0.043$**	**$r = -0.185$; $p < 0.001$**	$r = -0.090$; $p = 0.125$

Variables included in the models were age and sex. Pulse wave velocity was adjusted for mean arterial pressure. Central retinal artery equivalent was additionally adjusted for central retinal vein equivalent and vice versa.
Bold values denote statistical significance ($p < 0.05$).

We only included children from the North West Province of South Africa and our sample may therefore not be representative of the population of the entire country. Although PWV was measured by brachial oscillometry, which is a method that has not been validated in pediatric populations, it has previously been associated with CV risk in children. This study was the first to report associations between PWV and retinal microvascular calibers in a large sample of children including different ethnic groups. For retinal vessel imaging, duplicate images were taken from the eye, allowing for a high accuracy in retinal vessel diameter detection. To expand on the present findings, future studies are warranted to determine differences in environmental and sociocultural risk factors in children from a multi-ethnic point of view that may ultimately impact EVA differently among ethnic groups. Moreover, our findings necessitate the screening of black pediatric subjects for early signs of vascular deterioration as well as determining the impact of lifestyle behaviors (physical fitness/activity, dietary intake, and psychosocial factors) involved in early vascular aging. Such data could aid in the aid in the development of primary prevention programs.

In conclusion, large artery stiffness is associated with retinal arterial narrowing and venular widening in children, with these findings being more pronounced in black children. Our findings suggest that cross-talk between the large and small arteries are already evident at an early stage in the life course and may provide insight into the development of early vascular aging. These results warrant further investigation into environmental and sociocultural risk factors contributing to premature cardiovascular disease development.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Health Research Ethics Committee of the North-West University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

RK and HH conceptualized and designed the study. YB and AC was responsible for data analysis and YB wrote the original draft. AC, WS, SB-L, LG-M, SB, JR, HH, and RK contributed to the interpretation of data and critical review of manuscript. All authors gave final approval of the version to be submitted.

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Early Vascular Aging in Children With Type 1 Diabetes and Ambulatory Normotension

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Background: Preliminary data suggest that target organ damage (TOD) and early vascular aging (EVA) may occur in children with normal blood pressure (BP).

Objectives: To analyze TOD and EVA in normotensive (BP <95th percentile on ambulatory BP monitoring) type 1 diabetes children (T1D) in comparison to healthy controls (C).

Subjects: 25 T1D aged 13.9 ± 2.6 years and 22 C aged 14.0 ± 3.4 years.

Methods: We analyzed age- and height-related pulse wave velocity (PWV) Z-scores and expected PWV based on age, height, and mean arterial pressure (MAP). Expected vascular age based on measured PWV was calculated from pooled pediatric and adult PWV norms. Left ventricular mass index (LVMI), estimated glomerular filtration rate (eGFR), and urinary albumin/creatinine ratio (ACR) were obtained as markers of TOD.

Results: T1D and C groups did not differ in anthropometry, ambulatory, LVMI, and ACR. However, median age- and height-related PWV Z-scores were higher in T1D compared to C (1.08 vs. 0.57, $p = 0.006$; 0.78 vs. 0.36, $p = 0.02$, respectively). Mean (±SD) difference between measured and expected PWV was 0.58 ± 0.57 in T1D vs. 0.22 ± 0.59 in C, $p = 0.02$. The mean (±SD) difference between chronological and expected vascular age was 7.53 ± 7.74 years in T1D vs. 2.78 ± 7.01 years in C, $p = 0.04$.

Conclusion: Increased arterial stiffness and increased intraindividual differences between expected and measured PWV as well as between chronological and expected vascular age indicate that EVA may develop in T1D children even at normal ambulatory BP levels.

Keywords: ambulatory blood pressure monitoring (ABPM), arterial stiffness, children, diabetes type 1, early vascular aging

INTRODUCTION

Elevated blood pressure (BP) represents an important cardiovascular risk factor with a direct relationship between the BP level and rates of stroke, myocardial infarction, and the risk of end-stage renal disease (1).

In children, the BP cutoffs for increased cardiovascular risk are not clearly defined due to the lack of longitudinal studies linking childhood BP levels to long-term outcomes in adulthood (2). However, there is a high probability that a hypertensive child would become a hypertensive adult, a well-known phenomenon called BP tracking (3). The risk of CV complications is low during childhood, but children can develop target organ damage (TOD) as a consequence of untreated hypertension (4, 5).

Recent studies suggest that even mild elevation of BP, below the hypertension threshold, or white coat hypertension (WCH) can lead to heart and vascular damage (6–8). In children with kidney disease and/or diabetes mellitus, the vascular injury is further potentiated by the underlying disease, leading to a much higher risk of hypertension-related TOD (9, 10).

Pulse-wave velocity (PWV) can be used for non-invasive assessment of vascular function (i.e., arterial stiffness) and evaluation of vascular aging; it can be successfully applied to children for whom there are age- and height-specific normative data (11–15). Increased PWV is considered an early marker of hypertension-related TOD and marker of early vascular aging (EVA) (16, 17).

The goal of our study was therefore to analyze BP and PWV in children with diabetes mellitus type 1 in comparison with healthy controls.

We hypothesized that diabetic ambulatory normotensive children would show functional cardiovascular changes (increased arterial stiffness) and a higher vascular age compared to healthy controls even in the absence of structural changes [increased left ventricular mass (LVM), microalbuminuria] and that the functional changes would occur with only mild elevation of the BP level.

METHODS

Patients

All consecutive patients with type 1 diabetes who were referred for assessment of hypertension to Pediatric Nephrology & Hypertension Clinic, Department of Pediatrics, University Hospital Ostrava, Czechia, from January 2017 to December 2019 were enrolled in a prospective study. Out of a total of 29 patients enrolled, 25 patients with T1D were diagnosed with ambulatory normotension based on ambulatory BP monitoring (ABPM) criteria (see below), and they were included in the current study.

The control group consisted of 22 healthy children (12 boys) recruited from hospital co-workers' family members.

All subjects completed the office BP measurements, ABPM, echocardiography, and pulse wave velocity (PWV) measurements. No patient was treated with antihypertensive therapy at the time of the investigation. All patients had normal renal function without any significant proteinuria except for one diabetic patient with albuminuria (albumin-to-creatinine ratio, 13.4 mg/mmol). The demographics of both groups is shown in **Table 1**.

Inclusion criteria were as follows: written informed consent and age 10–19 years. For children with diabetes type 1, the additional inclusion criterion was diabetes duration ≥1 year. The exclusion criteria were as follows: history of other serious disorders or history of diseases affecting BP (especially heart and kidney diseases), current antihypertensive medications, or other BP-affecting issues such drug abuse or smoking.

The study was conducted in accordance with the Declaration of Helsinki and was approved by the local ethics committee. Written informed consent was obtained from all parents and patients of both study groups, as appropriate.

Anthropometric Measurement

At the time of the office and ABPM measurements, the body height and weight were recorded. The body mass index (BMI) was calculated as kg/m^2; BMI, weight, and height were converted into standard deviation scores (SDS), e.g., Z scores, based on reference values for healthy Czech children; see http://www.ojrech.cz/lesny/kompendium/index.htm. Weight and height were measured by a single trained nurse with precision electronic scales and fixed stadiometer.

TABLE 1 | Basic characteristic of both study groups.

Parameter	T1D ($n = 25$)	C ($n = 22$)	p
Gender (female/male)	13/12	10/12	NS
Age	13.9 ± 2.6	14.0 ± 3.4	NS
Height (cm)	159.9 ± 13.2	159.8 ± 14.7	NS
Height-SDS	−0.3 ± 0.9	−0.02 ± 1.3	NS
Weight (kg)	55.1 ± 20.0	48.9 ± 13.8	NS
Weight-SDS	0.3 ± 1.3	−0.1 ± 1.0	NS
BMI (kg/m^2)	19.7 (18.2, 23.7)	18.5 (16.6, 20.4)	NS
BMI-SDS	0.4 (−0.3, 1.5)	−0.2 (−1.0, 0.6)	NS
Diabetes duration (years)	5.1 ± 2.9	-	ND
Obesity + overweight, n (%)	6 (24)	1 (4.6)	NS
T-Cholesterol (mmol/l)	4.4 ± 0.7	4.2 ± 0.6	NS
HDL-cholesterol (mmol/l)	1.6 (1.4, 1.7)	1.3 (1.2, 1.5)	NS
LDL-cholesterol (mmol/l)	2.6 ± 0.5	2.7 ± 0.4	NS
Triglycerides	0.9 (0.7, 1.3)	0.9 (0.7, 1.1)	NS
HbA1C (mmol/mol) /n, %/	67.0 ± 7.4/8.3 ± 2.8/	-	ND
eGFR/creatinine (ml/s/1.73 m^2)	1.8 (1.6, 2.1)	1.6 (1.4, 1.7)	0.005
eGFR/Cystatin C (ml/s/1.73 m^2)	1.6 ± 0.2	1.5 ± 0.1	0.027
ACR (mg/mmol)	0.7 (0.4, 0.9)	0.5 (0.4, 0.7)	NS

ACR, albumin/creatinine ratio; BMI, body mass index; eGFR, estimated glomerular filtration rate; NS, nonsignificant; ND, not done; SDS, standard deviation score. Data are expressed as mean ± SD or median [interquartile range (IQR)] as appropriate.

Office Blood Pressure Measurement

The office BP was measured by a single trained nurse on the same day as the ABPM (before initiating the ABPM device), according to the current European guidelines (9). After 10 min of rest, the BP measurement was done with an automatic oscillometric Omron 705IT device[1] (OMRON Healthcare Europe B.V., Hertogenbosch, Netherlands). The oscillometric device was validated for BP measurement in children[2] The measurements were taken using the right arm, in the sitting position with the elbow at the level of the right atrium, using one of three cuff sizes (child: 6–12, medium: 12–23, or adults: 17–38.6 cm). The appropriate cuff size was determined by measuring the mid-arm circumference and was ∼40% of the arm circumference (an inflatable bladder width). The first BP reading was used for analysis. The obtained absolute systolic BP (SBP) and diastolic BP (DBP) values were subsequently converted into Z-scores (SDS) based on age- and height-related normative values for children.

Twenty-Four-Hour Ambulatory Blood Pressure Monitoring

The ABPM was performed using the oscillometric device SpaceLabs 90217 (SpaceLabs Medical Inc., Redmond, Washington, USA). The monitor was programmed to measure the BP every 20 min during the day (6 A.M.−10 P.M.) and every 30 min during the night (10 P.M.−6 A.M.). The parents and children were instructed to keep a diary of daily activities during the ABPM measurement. However, in order to compare our results with the normative values for ABPM (18), we defined the daytime period as 8 A.M.−8 P.M. (12 h) and the nighttime period as 12 P.M.−6 A.M. (6 h). The cuff size was determined by measuring the mid-arm circumference and was ∼40% of the arm circumference. In all patients, the length of the cuff covered 100% of the arm circumference. The cuff was placed on the non-dominant arm. The patients were instructed to avoid vigorous physical exercise during the ABPM measurement but to follow their usual daily activities. A minimum of 40 ABPM recordings were required to consider the ABPM valid.

For the study purposes, the following ABPM parameters were obtained and analyzed: mean arterial pressure (MAP), SBP, and DBP measured over 24 h, daytime and nighttime periods. The average absolute values for MAP, SBP, and DBP for all time periods were subsequently converted into Z-scores (SDS) using the ABPM normative data (18). Night-time BP dipping was calculated using the ratio of mean daytime/mean nighttime MAP, SBP, and DBP. Non-dipping (absence of nocturnal BP fall at least 10%) was defined as day/night (D/N) ratio <1.1 in MAP and/or SBP and/or DBP. ABPM raw data were also used to estimate the ambulatory arterial stiffness index (AASI), which was calculated as 1 minus the regression slope of DBP on SBP values over 24-h period (19).

[1]http://www.dableducational.org/sphygmomanometers/devices_1_clinical.html#ClinTable; and http://www.dableducational.org/pdfs/equivalence_declarations/E15%20Omron%20M4-I%20ESH-BHS.pdf

[2]http://www.dableducational.org/accuracy_criteria.html

Definition of Ambulatory Blood Pressure Monitoring Normotension

Normotension on ABPM was defined as mean SBP and DBP and MAP <95th percentile (i.e., <1.645 SDS) during 24 h, daytime and nighttime periods. Hypertension on ABPM was defined as mean SBP or DBP or MAP value ≥95th percentile (i.e., ≥1.645 SDS) at any time period. Similarly to adult and European Society of Hypertension (ESH) guidelines, the BP load was not included in the definition of ABPM hypertension (9).

Definition of White Coat Hypertension

Patients with ABPM normotension (as defined above) and office SBP or DBP SDS >1.645 were categorized as patients with WCH.

Arterial Stiffness

Arterial stiffness was assessed by carotid femoral pulse wave velocity (cfPWV) *via* applanation tonometry using validated PulsePen device (DiaTecne s.r.l.) (16, 20) as described previously (11, 20). All measurements were performed by one trained physician.

Prior to cfPWV measurement, patients were placed in the supine position and rested for 15 min in quite temperature-comfortable room. Three electrocardiographic leads were attached. The right carotid artery was palpated and marked (proximal site), and the procedure was repeated for the right femoral artery (distal site). To assess pulse wave travel distance, surface tape measurements were performed between the carotid site and the jugular notch and between the jugular notch and the femoral site. The difference between these two distances was considered the pulse travel distance (11, 16). PWV was examined by sequential recordings of the arterial pressure wave at the carotid and femoral arteries and was defined as the distance of the sampling sites divided by the time difference between the rise delay of the distal and proximal pulses according to the R wave belonging to the ECG QRS complex calculated by the software. The pulse wave was calibrated by measuring the BP immediately before each recording. The measurement of transit time was discarded and repeated if BP and HR varied by >10%, the variability between consecutive systolic or diastolic waveforms was >10%, and/or when the amplitude of the pulse wave signal was 80 mV. All of PWV measurements were performed 3–4 times in each participant, and the average of the two lowest PWV measurements with inter-measurement divergence ≤0.5 m/s was taken in analysis. The absolute PWV values were subsequently converted into age- and height-adjusted Z-scores (SDS) using normative pediatric data, which were obtained using the same PWV device (PulsePen) (11).

Expected Pulse Wave Velocity and Vascular Age Calculation

To calculate the expected PWV in each child based on age, height, and MAP, we used the equation proposed by Reusz et al. (11) expressed as follows:

$$\text{“}PWV(m/s) = 0.049 \times age(years) + 0.008 \times height(cm) + 0.024 \times MAP(mmHg) + 1.129.\text{”}$$

The expected PWV results were then compared with obtained PWV results (PWV difference) in each individual patient and subsequently compared between T1D and C groups.

To assess vascular age of our children, we combined normative age-based PWV data for children aged 7–18 years (11) obtained with applanation tonometry device (PulsePen) with normative age-based data for adults aged 19–40 years (21) obtained with two different devices (SphygmoCor and Complior) for PWV measurement using applanation tonometry for males and females separately. There was a significant linear relationship between chronological age (in years) and 50th percentile PWV (m/s) across the whole chronological age range from 7 to 40 years: PWV in males (m/s) = 3.99 + 0.08 × age (years), $r^2 = 0.95$, $p < 0.0001$; PWV in females (m/s) = 4.01 + 0.08 × age (years), $r^2 = 0.96$, $p < 0.0001$. The expected age based on measured PWV (vascular age) for individual patients was then calculated as follows: patient's measured PWV – 3.99 for males (4.01 for females)/0.08. The intraindividual differences between the chronological age and the vascular age were then compared between groups.

Echocardiography

Echocardiography was performed using a General Electric Vivid 9 and 95e (General Electric, Milwaukee, WI) ultrasound systems. Measurements were performed off-line by a single experienced physician according to the guidelines of the American Society of Echocardiography (22). Two-dimensional echocardiography images were obtained for the analysis of left ventricular (LV) volumes on three consecutive beats from apical four- and two-chamber views. Wall thickness and chamber dimensions are obtained from the two-dimensional parasternal long axis or M-mode short axis at the midventricular level. The LVM was calculated by using the Devereux Equation (23) and indexed to the height$^{2.7}$ [left ventricular mass index (LVMI)]. From the study purposes, LVMI was expressed as LVMI ratio, which was obtained by dividing the measured LVMI value by the 95th percentile of LVMI for healthy children (24). LVH was defined as LVMI ratio >1.0 (>95th percentile).

Laboratory Parameters

All the laboratory investigations were performed on the day of ABPM. Blood draws of the patients were performed in the morning after overnight fasting. Biochemical analysis in whole blood [glycated hemoglobin (HbA1C)], serum (creatinine, lipid profile, cystatin C), and the first morning urine (albumin and creatinine) was measured by routine laboratory methods immediately after collection. These blood samples except HbA1C were centrifuged at 2,500 g for 6 min at 4°C. Serum concentration of total cholesterol (T-cholesterol), high-density lipoprotein cholesterol (HDL-cholesterol), low-density lipoprotein cholesterol (LDL-cholesterol), and triglycerides were measured by enzymatic methods on AU5420 analyzer (Beckman Coulter, Inc., USA). HbA1C was measured using high-performance liquid chromatography (HPLC; Tosoh G8, Tosoh Bioscience, Inc., CA, USA). The blood was collected in ethylenediaminetetraacetic acid (EDTA) anticoagulant tubes.

Serum and urine concentrations of creatinine were determined by the enzymatic method on AU5420 analyzer (Beckman Coulter, Inc., USA). Serum concentration of cystatin C and urine albumin was measured by immunonephelometric method (BN ProSpec, Siemens Healthcare, USA). The estimated glomerular filtration rate for creatinine (eGFR/creatinine) was calculated using the updated Schwartz formula (25). We also calculated eGFR based on the cystatin C equation developed by the Chronic Kidney Disease Work Group (26). The albumin/creatinine ratio (ACR) was analyzed from a first morning urine sample. Pathological albuminuria was defined as ACR >2.2 mg/mmol.

Statistical Analysis

The distribution of continuous data was analyzed with the d'Agostino & Pearson omnibus test, normally distributed data are presented as mean ± SD, non-normally distributed data are shown as median and interquartile range (IQR; i.e., 25th and 75th percentile). Continuous variables were compared using Student's unpaired *T*-test (if normally distributed data) or Mann–Whitney test (if non-normally distributed data). In addition to the classic null hypothesis significance testing (NHST), we used estimation statistics with permutation on 5,000 resamples; results are shown as mean difference between groups, bias-corrected and accelerated confidence intervals, and permutation *p*-values. The magnitude of the difference between groups was estimated using Cohen's D effect size with bias-corrected accelerated 95% confidence intervals (27). Thresholds for Cohen D effect size include 0.2 (small effect), 0.5 (medium effect), and 0.8 (large effect). The categorical variables (proportion of patients between groups) were compared using a chi-square test or the Fisher's exact test.

The relationship between PWV, diabetes, and BP was analyzed using linear regression analysis. We also performed multivariate regression analysis with only selected, deemed as most clinically important, variables ($n = 3$) as predictors of PWV (dependent variable).

Results were considered statistically significant if the *p*-value was below 0.05. Statistics were performed using the GraphPad software, version 6.0, for Windows and Python (Jupyter Lab, package dabest version 0.3.1) (27).

RESULTS

Patient Characteristics

Basic characteristics of T1D and healthy controls (C) are presented in **Table 1**. All children were of Caucasian origin. The average (± SD) duration of diabetes was 5.1 ± 2.9 years in T1D group. There were no significant differences between T1D and C patients in age, gender, anthropometric parameters, or proportion of obesity. There were also no differences in metabolic parameters such as T-cholesterol, HDL- and LDL-cholesterol, triglycerides, and ACR between both groups. However, the Schwartz eGFR and cystatin C eGFR was significantly higher in T1D group, most likely due to hyperfiltration (**Table 1**).

TABLE 2 | Blood pressure values.

	T1D ($n = 25$)	C ($n = 22$)	p-value
Office SBP-SDS	0.9 ± 1.1	0.07 ± 1.1	0.006
Office DBP-SDS	0.2 ± 1.5	−1.0 ± 1.3	0.004
24-h SBP-SDS	−0.4 ± 0.8	−0.5 ± 0.8	NS
24-h DBP-SDS	−0.2 ± 0.7	−0.5 ± 0.9	NS
24-h MAP - SDS	0.2 ± 0.7	−0.2 ± 0.7	NS
Day SBP-SDS	−0.5 ± 0.8	−0.3 ± 0.9	NS
Day DBP-SDS	−0.3 ± 0.8	−0.4 ± 0.9	NS
Day MAP-SDS	0.1 (−0.7, 0.4)	−0.2 (−0.8, 0.6)	NS
Night SBP-SDS	−0.1 ± 0.7	−0.4 ± 0.8	NS
Night DBP-SDS	0.1 ± 0.8	−0.3 ± 0.8	NS
Night MAP-SDS	0.3 ± 0.6	−0.0 ± 0.6	NS
Number of people with WCH, n (%)	7 (28)	1 (5)	NS
SBP D/N	1.1 ± 0.1	1.2 ± 0.1	NS
DBP D/N	1.3 ± 0.1	1.3 ± 0.1	NS
MAP D/N	1.2 (1.1, 1.3)	1.2 (1.1, 1.2)	NS
Number of people with non-dipping, n (%)	11 (44)	6 (27)	NS
24-h PP	44.1 ± 5.2	45.0 ± 5.5	NS
24-h AASI	0.27 ± 0.1	0.25 ± 0.1	NS

AASI, ambulatory arterial stiffness index; DBP, diastolic blood pressure; HR, heart rate; MAP, mean arterial pressure; n, number; NS, nonsignificant; PP, pulse pressure; SBP, systolic blood pressure; SDS, standard deviation score. Data are expressed as mean ± SD or median [interquartile range (IQR)] as appropriate.

Blood Pressure

The absolute and SDS values of office SBP and DBP were significantly higher in diabetic children compared to healthy controls (**Table 2**). However, ABPM (absolute and SDS) parameters of 24 h, day and night SBP, DBP, and MAP did not differ between both groups except for absolute day MAP (**Table 2**). There was also no difference in BP dipping. The proportions of patients with non-dipping hypertension or WCH were not significantly different between groups (**Table 2**). There were also no differences in pulse pressure and AASI.

Echocardiography

We did not find any significant differences between diabetic children and healthy controls in LVM, LVMI, and LVMI/95th percentile [median (25th, 75th percentiles): T1D 72.60 (62.45; 105.50) vs. C 70.80 (53.35; 100.20), T1D 22.00 (18.48; 25.00) vs. C 20.40 (18.25; 25.80), and T1D 0.52 (0.46; 0.59) vs. 0.50 (0.46; 0.59), respectively].

Arterial Stiffness and Left Ventricular Mass

Children with diabetes type 1 had higher absolute values of PWV (m/s) ($p = 0.037$) and significantly higher age-related PWV SDS on standard NHST comparison (**Table 3**) and on estimation statistics: mean difference = 0.57; 95% CI = 0.1–1.02, permutation $p = 0.02$; Cohen's D effect size = 0.69, 95% CI = 0.00–1.24 (**Figure 1A**). Height-related PWV SDS was also significantly higher in T1D (**Table 3**): mean difference = 0.57; 95% CI = 0.11–1.04, permutation $p = 0.03$; Cohen's D effect size = 0.68, 95% CI = 0.02–1.22 (**Figure 1B**).

TABLE 3 | Vascular parameters.

Parameters	T1D	C	p-value
Measured PWV (m/s)	5.48 (5.25; 6.05)	5.16 (4.85; 5.84)	0.037
Measured PWV-SDS$_{age}$	1.08 (0.71; 1.39)	0.57 (0.02; 0.96)	0.006
Measured PWV-SDS$_{height}$	0.78 (0.39; 1.19)	0.36 (−0.23; 0.81)	0.022
Measured vs. predicted PWV difference (m/s)	0.58 ± 0.57	0.22 ± 0.59	0.020
Predicted vascular vs. chronological age difference (years)	7.53 ± 7.74	2.78 ± 7.01	0.04

C, controls; PWV, pulse wave velocity; SDS, standard deviation score; T1D, diabetes type 1. Data are expressed as mean ± SD or median [interquartile range (IQR)] as appropriate.

Using the formula for PWV prediction based on age, height, and MAP published by Reusz et al. (11), the PWV difference (measured vs. expected PWV) was significantly higher in T1D patients compared to controls (**Table 3**) with the mean difference of 0.36 m/s, 95% CI = 0.03–0.68, permutation $p = 0.04$; Cohen's D effect size was 0.63; 95% CI = 0.05–1.18 (**Figure 2A**).

In children with diabetes type 1, the difference between the PWV expected and actual chronological age was significantly higher in T1D patients as compared to the control group (**Table 3**); the mean difference was 4.75 years, 95% CI = 0.51–8.75, permutation $p = 0.04$, Cohen D effect size was 0.64 (95% CI = 0.02–1.2) (**Figure 2B**).

The comparison of LVM and LVMI did not show any significant differences between the groups (**Table 3**).

The relationship between 24-h SBP SDS and PWV height-related SDS is shown in **Figure 3**. While there is no significant correlation between of 24-h SBP SDS and PWV SDS (height-related) in controls, the correlation was significant ($r = 0.42$, $p = 0.04$) in diabetic children (**Figure 3**). Diabetic children also had a higher intercept and steeper slope of the regression line compared to controls (**Figure 3**).

A limited multivariate analysis (due to the low number of patients) including age, presence/absence of diabetes, and 24-h SBP showed age and diabetes as significant predictors of PWV ($r^2 = 0.39$, $F = 9.327$, $p < 0.0001$) (**Table 4A**). In children with diabetes type 1, the 24-h SBP SDS and HbA1C value were significant predictors of PWV with a good overall correlation coefficient ($r^2 = 0.372$, $F = 6.520$, $p = 0.006$) (**Table 4B**).

DISCUSSION

Our study showed that ABPM normotensive diabetic children and adolescents had significantly increased absolute PWV as well as age- and height-related PWV SDS compared to their normotensive controls. Moreover, the intraindividual differences between measured and predicted PWV were significantly higher in T1D patients compared to healthy controls. Similarly, children with diabetes type 1 had

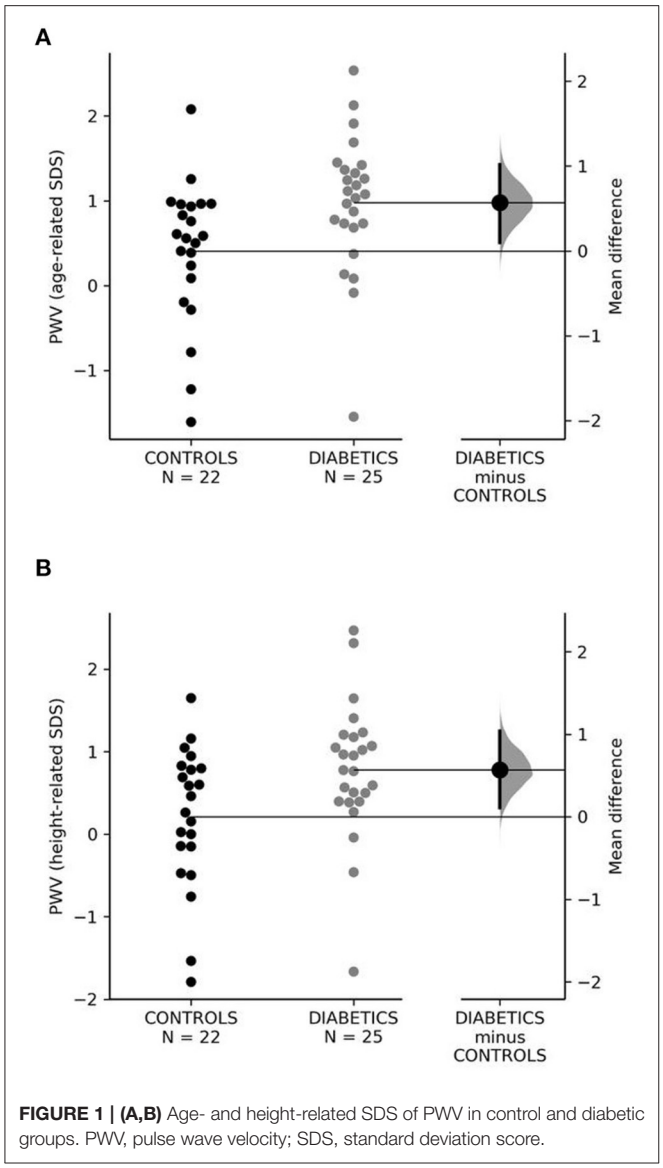

FIGURE 1 | (A,B) Age- and height-related SDS of PWV in control and diabetic groups. PWV, pulse wave velocity; SDS, standard deviation score.

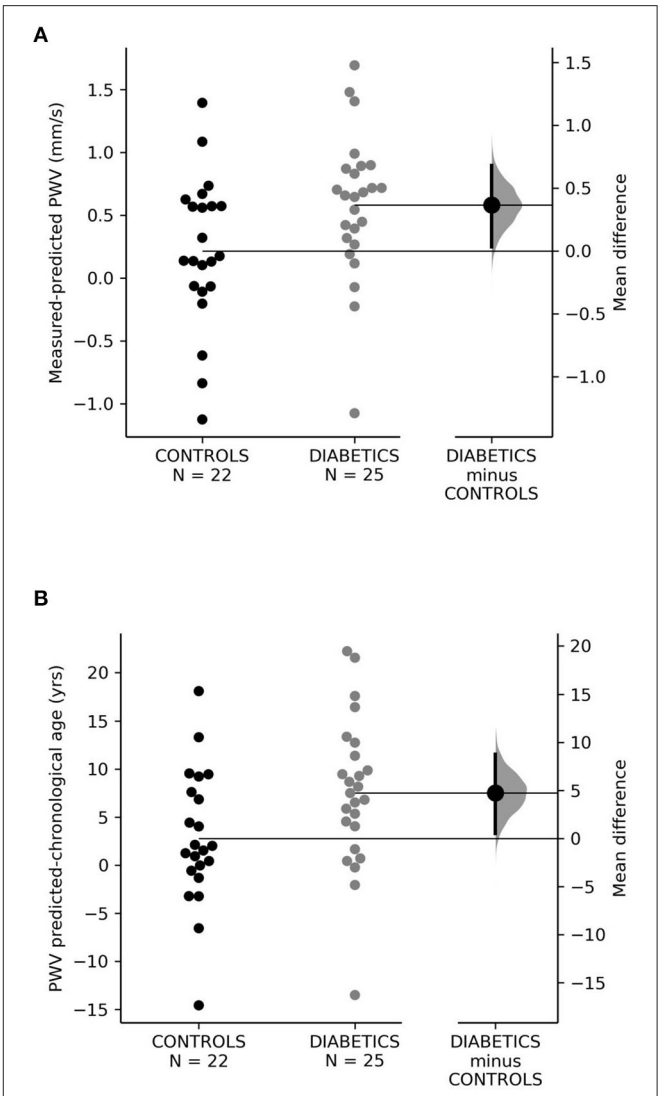

FIGURE 2 | (A,B) Vascular age. yrs, years.

significantly higher intraindividual differences between chronological and PWV-predicted vascular age as compared to controls, suggesting early vascular aging (EVA) in T1D patients. This is a novel finding, not previously described. There was no difference in LVM index and microalbuminuria between patients with diabetes type 1 and healthy controls.

Diabetes mellitus has been proven to be a major risk factor for the development of cardiovascular disease (CVD) (28). Recent ESH guidelines (9) and some studies have consequently pointed out that children with diabetes type 1 have increased prevalence of hypertension (29) and are at higher risk of an early-onset CVD (10, 30–33). Children and adolescents with diabetes type 1 generally do not have manifest clinical signs of CVD. They may however suffer from a subclinical cardiac and vascular damage that can occur at BP levels that are even below the hypertension threshold (7). In pediatrics, the most frequently used indirect and non-invasive subclinical CVD markers are increased PWV and LVM.

PWV is the most widely accepted method for assessment of arterial stiffness and vascular age (34, 35) in both children and adults. In adults, PWV is related mainly to age and BP; further determinants are male gender and diabetes (36). Similarly to adults, children's PWV increases with age and is also dependent on sex (11–15). Furthermore, at the age of 18 years, the 50th (10th, 90th) percentiles of PWV appear to merge into reference values obtained in adults with optimal BP levels (12, 21).

In adults with diabetes type 1, the increased PWV is associated with adverse cardiovascular outcomes and increased likelihood of early cardiovascular morbidity and with all-cause and cardiovascular mortality, often in connection with various comorbidities (hypertension, chronic kidney disease, etc.) (31–33). Importantly, according to recent data from observational

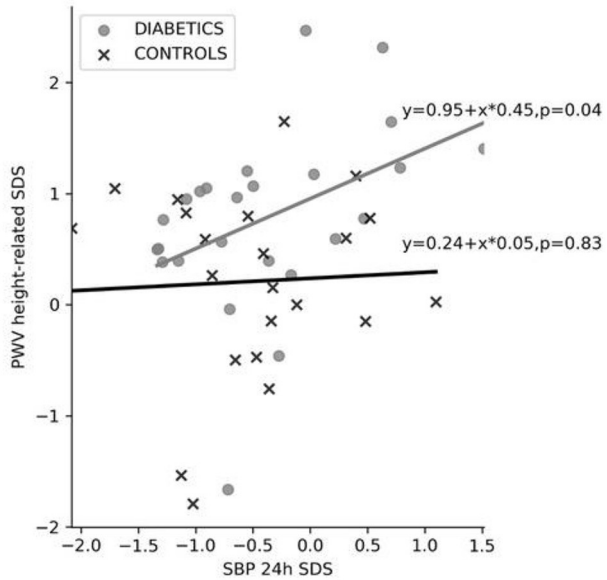

FIGURE 3 | Relationship between 24-h SBP SDS and height-related PWV SDS. PWV, pulse wave velocity; SBP 24-h SDS, 24-h systolic blood pressure standard deviation score.

studies, the CV morbidity and mortality are seen in young adults and are associated with a shorter life span of 9.4–17.7 years of life (31). Comorbidities such as hyperglycemia, hypertension, dyslipidemia, diabetic kidney disease, insulin resistance, and obesity are still the strongest risk factors for CVD and mortality in type 1 diabetes.

The extent of cardiovascular morbidity in children with diabetes type 1 is not well-understood. However, many children and adolescents with diabetes type 1 fail to meet the American Diabetes Association (ADA) and International Society for Pediatric and Adolescent Diabetes (ISPAD) targets for HbA1C, SBP and DBP, LDL-cholesterol, and triglycerides (37), and many youth with diabetes type 1 are not treated or undertreated for hypertension, dyslipidemia, and microalbuminuria. They may therefore be at risk for CVD complications even in the absence of comorbidities and obvious clinical signs of CV injury. An early identification of subclinical CV injury may help with management of diabetes type 1 and other contributing factors, mainly arterial hypertension and proteinuria.

While the diagnosis of hypertension and its various forms (white coat, masked, true hypertension) and proteinuria (urine albumin/creatinine) is well-established and used by most physicians, the assessment of subclinical CV injury is still not routinely done in all children with diabetes type 1. Most centers use echocardiography to assess LVM, but the assessment of vascular stiffness using PWV is done mainly for research purposes.

There are several studies describing PWV (applanation tonometry) in children and adolescents with diabetes type 1. The recent meta-analysis (38), which included four age-matched case-control PWV studies with 1,491 children with a mean age of 15.2 years (975 patients with diabetes type 1 average duration of 7.1 years and 516 controls), showed significantly increased carotid–femoral PWV (absolute) values (m/s) in diabetic children compared with controls. The significant determinants of PWV in diabetic children were diabetes duration, age, and presence of diabetes; other important variables were gender and MAP.

In the prospective study with a 5-year follow-up (38), achievement of office BP <90th percentile for age, sex, and height was associated with significantly lower PWV during the follow-up (5.5 vs. 5.7 m/s, $p = 0.04$). Another recent study (39) including 1,809 youth with diabetes type 1 found an association of PWV mainly with diabetes duration and HbA1C but also with other determinants—adiposity, higher (office) BP, and adverse lipid levels, i.e., traditional CV risk factors. In none of the studies was ABPM used.

Our study is in agreement with the abovementioned studies. We found significantly increased arterial stiffness in ABPM-normotensive diabetic children compared with controls. Age, presence of diabetes type 1, and 24-h SBP were the strongest predictors of PWV in the whole group (**Table 4A**); in children with diabetes type 1, the predictors of PWV were the 24-h SBP SDS and HbA1C (**Table 4B**). These findings are similar to previously published studies (38–40). In most of the studies (except for two recent studies), BP and PWV were expressed in absolute values only. In contrast, we show all BPs and particularly PWV in absolute values and sex-, age-, or height-related Z-scores (SDS). This allowed for a more detailed quantification of observed results and direct comparison with sex- and age-/height-specific normative data. Moreover, most other studies (39, 41, 42) [except for (5)] used office BP for correlation with PWV, whereas we used ABPM 24-h BP, which is in general a better predictor of CV risk than office BP. Although we measured the office BP in our children, we did not consider it as a valid assessment of BP in our study population given the high variability of office BP, white coat effect, etc. Indeed, 28% of our ABPM normotensive diabetic children had an elevated office BP suggesting WCH. Although all diabetic children had normal BP on ABPM (as per inclusion criteria) (**Table 2**), the ABPM BP Z-scores were slightly/non-significantly increased (mean ± 24-h SBP and DBP Z-scores = −0.4/-0.2 SDS) (**Table 2**) compared to the control group (-0.5/-0.5 SDS). This mild increase of 24-h BP, albeit clinically not noticeable, significantly contributed to the increase of PWV/arterial stiffness in the presence of diabetes type 1, as suggested by the multivariate analysis (**Table 3**).

Because PWV increases with age, increased values of arterial stiffness in children can lead to premature vascular injury and EVA. Children with diabetes type 1 would be theoretically at a higher risk of EVA due to the cumulation of EVA risk factors (diabetes type 1 + hypertension). Our results confirmed our hypothesis that children with diabetes type 1 had higher intraindividual differences between chronological and predicted vascular age (**Figure 2**), suggesting an accelerated vascular aging as compared to healthy normotensive children. Our results show that children with diabetes type 1 suffer from EVA, which can be considered a novel finding, not previously described in pediatric

TABLE 4 | Multivariate analysis.

Names	coef	SE	T	*p*-value	R^2	Adjusted R^2	97.5% CI	Relimp	Relimp %
(A) Model 1 for all children.									
Intercept	1.29	1.40	0.92	0.363	0.39	0.35	−1.54; 4.11	N/A	N/A
Age	0.10	0.03	3.15	0.003	0.39	0.35	0.04; 0.17	0.21	52.39
Diabetes	0.39	0.17	2.32	0.025	0.39	0.35	0.05; 0.72	0.08	19.45
24-h SBP	0.02	0.01	1.63	0.111	0.39	0.35	−0.01; −0.01	0.11	28.17
(B) Model 2 for T1D only.									
Intercept	8.61	1.04	8.31	0.000	0.37	0.32	6.46; 10.75	N/A	N/A
24-h SBP-SDS	0.35	0.15	2.37	0.027	0.37	0.32	0.04; 0.66	0.16	42.32
HbA1C	−0.04	0.02	−2.76	0.011	0.37	0.32	−0.07; −0.01	0.22	57.68

(A) Model 1: F-statistic = 9.327, p (F-statistic) < 0.0001.
(B) Model 2: F-statistic = 6.520, p (F-statistic) = 0.006.
HbA1C, current glycated hemoglobin; 24-h SBP, 24-h systolic blood pressure; SDS, standard deviation score.

literature. Children with EVA may be at an increased risk of future cardiovascular complications later in life, as observed in recent observational studies (31–33).

We also measured LVM in our study population and found no difference between children with T1D and control group. This is different from some other authors who found various abnormalities in LV geometry, LVM, and LV function (43, 44). The discrepancy between our and literature results may be due to the differences in BP levels, duration of diabetes type 1, indexation of LVM, use of normative values, and the fact that the healthy children may have a physiologically higher LVM due to physical activity regardless of the BP level. It would be reasonable to assume that morphological/structural LVM changes occur later in the course of the disease and at a higher BP level, whereas all our children with diabetes type 1 were normotensive on ABPM. While some minor/functional changes on echocardiography such as diastolic dysfunction can be expected and were described in children with diabetes type 1 (42–44), we did not measure diastolic function on echocardiography due to technical limitations in our institution.

Limitations and Strengths/Advantages

Firstly, the number of study subjects is limited. However, we compared the results of diabetic children with a control group of healthy children using absolute values as well as sex-, age-, and height-related reference values (Z-scores). In addition, we used estimation statistics with permutation *p*-values and effect size estimation, which allowed us to draw conclusions from differences derived from a relatively small sample but estimated and confirmed on a large permutated sample (n = 5,000). The use of modern statistical estimation methods can be considered a strength of our study. Secondly, in our study, we used a different applanation tonometry device (PulsePen) than most other studies in diabetics (SphygmoCor). However, the obtained PWV results from both devices are comparable, with excellent concordance between these two devices (45). Moreover, the normative data for children were generated by the same device as the one used in our study (PulsePen). Thus, the use of applanation tonometry can be considered another strength of our study. Thirdly, as already discussed above, we did not measure the diastolic function on echocardiography.

CONCLUSION

In conclusion, children with diabetes type 1 and ambulatory normotension have significantly increased arterial stiffness and PWV-predicted vascular age (EVA) while having normal LVM and no significant albuminuria. The main predictors of increased vascular stiffness in children with diabetes type 1 were the HbA1C levels and systolic 24-h BP. It appears that even a small increase in ambulatory BP, even within the normal range, can contribute to the development of vascular injury and EVA in the presence of diabetes type 1.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee, University Hospital Ostrava, 17. Listopadu 1790/5 708 52 Ostrava-Poruba e-mail: eticka.komise@fno.cz. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

TŠu: author of the supporting grant, responsible for the data collection, controls selection, office BP measurement, ABPM, PWV measurement, calculation of Z-scores, descriptive statistics, NHST, and writing a manuscript. JS: office BP measurement, diabetic patients selection, blood, and urine samplings. JP: echocardiography. RP: multivariate analysis. TSe:

author of the supporting grant and revisions of manuscript. JF: revisions, including final version of the manuscript, statistic (NHST, estimation statistics with permutation), figures, calculation of vascular age, scientific leading, and discussion. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2021.764004/full#supplementary-material

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Exercise and Carotid Properties in the Young–The KiGGS-2 Study

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Background: Carotid intima-media thickness (cIMT) and stiffness (cS) are predictive markers of early vascular aging and atherosclerotic risk. This study assessed, whether exercise has protective effects on carotid structure and function or on vascular risk in the young.

Methods: Volume and change of exercise (recreational and organized sports participation) of German adolescents and young adults was assessed within the prospective population-study KiGGS at KiGGS-Wave-1 (2009–2012) and KiGGS-Wave-2 (2014–2017) using standardized self-reporting questionnaires. CIMT and cS were measured by real-time B-mode ultrasound sequences with semi-automated edge-detection and automatic electrocardiogram-gated quality control in 2,893 participants (14–28 years, 49.6% female). A cumulative index for atherosclerotic risk (CV-R) included z-scores of mean arterial pressure, triglycerides, total/HDL-cholesterol-ratio, body mass index, and HbA1c.

Results: At KiGGS-Wave-2 cross-sectional CV-R but not cS and cIMT was lower in all exercise-groups compared to "no exercise" (B = −0.73, 95%-CI = −1.26 to 0.19, $p = 0.008$). Longitudinal volume of exercise was negatively associated with CV-R (B = −0.37, 95%-CI = −0.74 to 0.00, $p = 0.048$) but not with cS and cIMT. Cross-sectional relative risk of elevated CV-R but not cS and cIMT was lower in all exercise-groups compared to "no exercise" (RR = 0.80, 95%-CI = 0.66 to 0.98, $p = 0.033$). High exercise volumes were associated with lower relative risk of elevated CV-R (RR = 0.80, 95%-CI = 0.65–0.97, $p = 0.021$) and cS in tendency but not with cIMT.

Conclusions: Increased levels of exercise are associated with a better cardiovascular risk profile in young individuals, but not with cS and cIMT. Our study confirms previous recommendations on exercise in this age group without demonstrating a clear benefit on surrogate markers of vascular health.

Keywords: intima-media thickness, arterial stiffness, atherosclerosis, adolescents, exercise, carotid stiffness

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INTRODUCTION

Vascular aging is characterized by endothelial dysfunction, increased arterial stiffness, and structural remodeling of the vascular walls (1). It is a lifelong process, leading to elevated risk of atherosclerotic disease at higher age (2, 3). This process is mainly driven by chronic low-grade inflammatory activity (4, 5) and accelerated by atherosclerotic risk factors (6), i.e., obesity (7), physical inactivity (8), western type diet (9), and elevated blood pressure (10). Pathological stiffening and structural remodeling of the arterial intima-media layer may occur as early as during childhood (11). Ultrasound-based parameters of carotid stiffness (cS) and intima-media thickness (cIMT) are currently the most extensively validated non-invasive biomarkers to visualize these alterations in minors and young adults (12). As far as we know, only studies with confined samples exist, and there is a lack of evidence about the utility of cIMT and cS in epidemiological settings for population based atherosclerotic risk assessment.

Physical activity, and particularly exercise in terms of recreational or organized sports participation, slow down the progression of vascular aging independent from improvements of the before mentioned traditional risk factors (13, 14). Previous studies indicate, that lack of exercise or even inactivity in childhood may translate into increased progression of cIMT and cS in adulthood (15). Regular exercise and vigorous physical activity on the other hand, are favorably associated with lower cS in children (16) and with thinner aortic IMT in a confined sample of adolescents (17) with elevated cardiovascular risk. In healthy individuals, already modest increases of exercise during childhood may sustainably slow down the progression of arterial stiffening and thickening of the arterial intima-media layer in early (18) and even later adulthood (19). However, the latter studies did not adjust for blood pressure, which is a major determinant of cIMT. Whereas, cross-sectional exercise studies frequently reported no effects of exercise on cS and cIMT (20, 21), longitudinal studies found associations of acute exercise at young age with cS and cIMT in middle aged and older adults (22). The few studies that examined the effects of changes in regular exercise during adolescence on carotid wall function and structure in young adulthood deliver inconsistent evidence about short-term effects of exercise interventions on carotid-arterial stiffening and remodeling in the healthy general population with low cardiovascular risk (18, 23). In summary, existing evidence about effects of exercise on progression of cIMT and cS in adolescents and young adults is limited and it remains unclear, whether exercise during adolescence, in terms of recreational or organized sports participation, leads to measurable adaptations in cS and cIMT in early adulthood. Most importantly, however, the associations of exercise with cIMT and cS have not been examined in a representative population based sample. Therefore, this study analyzed these associations in such a sample and assessed the associations of regular exercise and its changes during adolescence on cIMT and cS in young adulthood.

Abbreviations: cIMT, carotid intima-media thickness; cS, carotid stiffness; CV-R, Index of cardiovascular risk; exercise, recreational and organized sports participation.

MATERIALS AND METHODS

This prospective study was approved by the German Federal Commissioner for Data Protection and Freedom of Information. Approval was given by the Ethics committees of Medizinische Hochschule Hannover (No. 2275-2014). Informed consent was obtained from all participants in advance.

Study Sample

The study sample comprised 14–28 year old participants of the second follow-up survey KiGGS-Wave-2 of the "German Health Interview and Examination Survey for Children and Adolescents" (24, 25) (**Figure 1**). Being the first nationwide representative health survey among German children and adolescents, KiGGS was initially conducted in 167 communities between 2003 and 2006. The first follow-up, KiGGS-Wave-1, was conducted between 2009 and 2012 and the second, KiGGS-Wave-2, was conducted from 2014 until 2017. Questionnaire-based assessment of exercise habits was performed at KiGGS-Wave-1 and KiGGS-Wave-2. Measurement of cIMT and cS in B-mode ultrasound sequences was conducted at KiGGS-Wave-2 in 4,798 participants.

Measurement of Carotid Intima-Media Thickness and Stiffness

Seven centrally trained, certified, and repeatedly retrained physicians conducted the ultrasound examinations. Ultrasound examinations and data acquisition were performed semi-automatically using a portable state-of-the-art ultrasound system (UF-760AG, Fukuda Denshi Co. Ltd., Tokyo, Japan). Standard operating procedures, including computation of cIMT and cS parameters, adhered to current guidelines (26, 27) and have been previously described in detail (28). Far wall common carotid IMT and outer lumen diameter were measured bilaterally in two planes (ear-to-ear and horizontal) within a 10 mm segment 10–15 mm proximal to the carotid bifurcation. The measurement was accepted, if cIMT was validly detected in at least one plane over two consecutive heart cycles. All valid values were averaged to a participant-specific cIMT. Computation of cS parameters was possible when blood pressure as well as valid outer lumen diameter at peak systole and end diastole were available (**Figure 1**). The equations used for calculation of distensibility coefficient, Young's elastic modulus, Peterson's elastic modulus and β-stiffness index as well as rates of completeness (89%) are presented in detail elsewhere (28).

General Cardiovascular Risk and Anthropometric Measures

All participants had cardiovascular and metabolic phenotyping based on interviews and medical examinations (25) including metabolic characteristics and risk behavior. Cardiovascular and metabolic profiling included body mass index (by percentile, according to the International Obesity Task Force) (29), resting

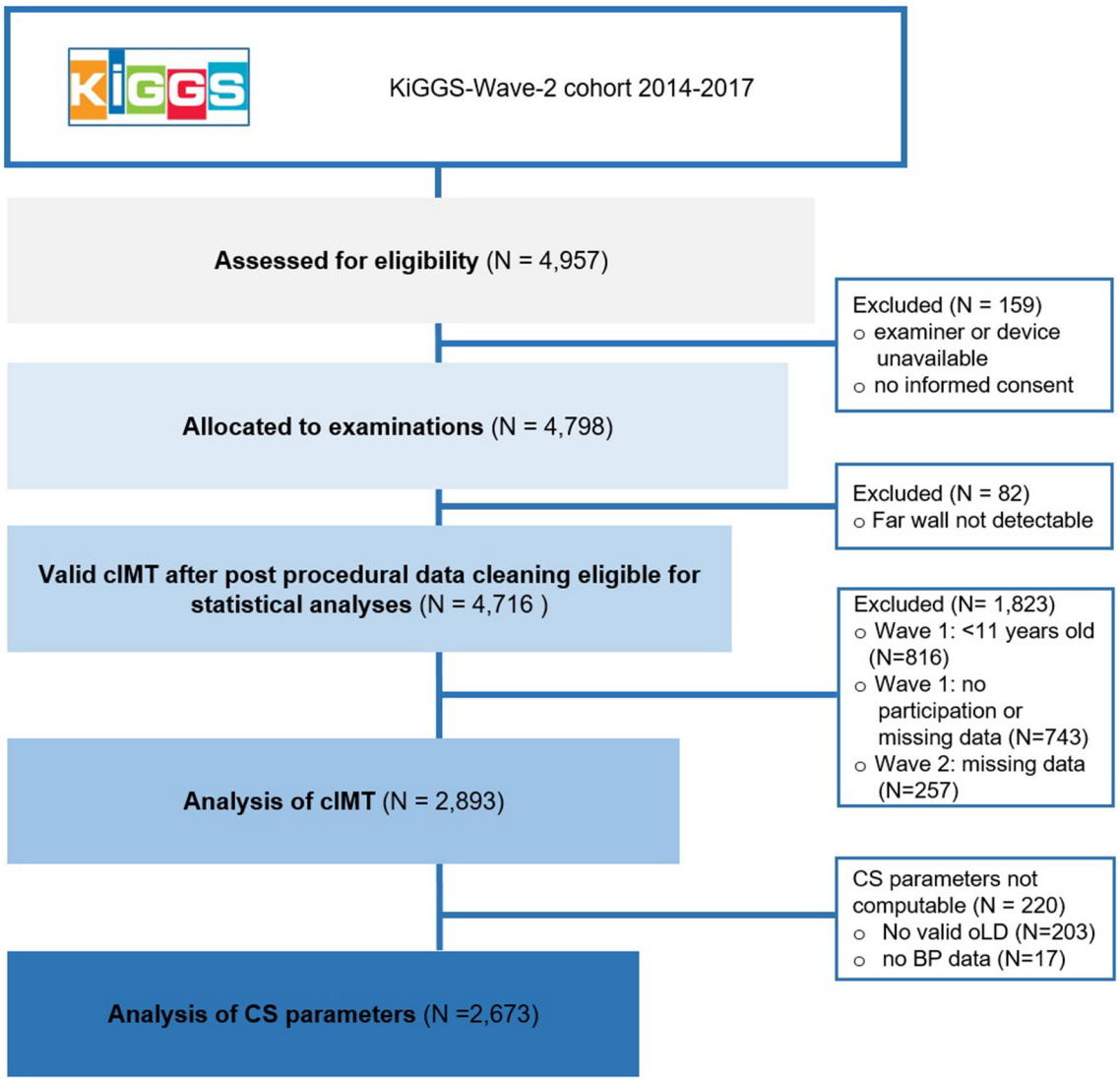

FIGURE 1 | Flow-chart of data collection and cleaning (*n* unweighted).

heart rate (30) and blood pressure (DatascopeAccutorr Plus, Mahwah, NJ) (31), as well as blood analyses of total-, HDL-, and LDL-cholesterol, HbA1c and high-sensitive C-reactive protein. Risk behavior included hazardous drinking, according to the Alcohol Use Disorder Identification Test (32) and current smoking (33). An index of cardiovascular risk (CV-R) was calculated that summarizes z-values based on the KiGGS-Wave-2 study cohort of mean arterial pressure, triglycerides, total/HDL-cholesterol-ratio, body mass index and HbA1c (34).

Physical Activity Questionnaires

Weekly hours of exercise were assessed at KiGGS-Wave-1 *via* a telephone-based interview and at KiGGS-Wave-2 *via* a self-reporting questionnaire based on the MoMo physical activity questionnaire (35). Participants reported regular weekly hours of exercise referring to recreational and organized sports other than school sports. Categories were "none," "<2 h," "2–4 h," or "≥4 h" of exercise per week. Based on these categories, cumulative exercise at both measurement points (KiGGS-Wave-1 and KiGGS-Wave-2) as an estimate for exercise trajectories during adolescence and young adulthood were expressed as "low-low exercise" (always <2 h), "low-high exercise" (<2 h at KiGGS-Wave-1 and ≥2 h at KiGGS-Wave-2), "high-low exercise" (≥2 h at KiGGS-Wave-1 and <2 h at KiGGS-Wave-2), "high-high exercise" (always ≥2 h).

Statistical Analyses

Descriptive characteristics of the sample stratified by weekly hours of exercise at follow-up were presented as either means (± standard deviation) or proportions. To take drop-out and

TABLE 1 | Characteristics of the study sample.

Weekly exercise time at KiGGS-wave-2	All	None	<2 h	2–4 h	≥4 h
KiGGS-Wave-1 (*N*)	2,893	698	620	573	1,002
Age [years]	17.18 ± 3.75	17.74 ± 3.58	17.29 ± 3.66	17.13 ± 0.55	16.69*** ± 0.35
Females (%)	49.64	57.91	60.41	55.14	34.89***
KiGGS-Wave-2 (*N*)	2,893	698	620	573	1,002
Age [years]	22.02 ± 3.75	22.54 ± 3.56	22.13 ± 3.69	21.95 ± 3.84	21.55*** ± 3.84
cIMT [mm]	0.55 ± 0.05	0.54 ± 0.05	0.54 ± 0.05	0.54 ± 0.05	0.55 ± 0.05
Wall-to-lumen-ratio	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.01
β-SI [–]	4.08 ± 0.86	4.07 ± 0.79	4.03 ± 0.88	4.06 ± 0.89	4.14 ± 0.89
DC [10^{-3} kPa]	45.03 ± 11.35	45.02 ± 10.48	45.78 ± 10.87	45.57 ± 12.17	44.22 ± 11.81
EP [kPa]	50.65 ± 12.03	50.55 ± 11.29	49.60 ± 11.55	50.25 ± 12.97	51.64 ± 12.29
YEM [kPA]	0.28 ± 0.08	0.28 ± 0.08	0.28 ± 0.08	0.28 ± 0.08	0.29 ± 0.09
CV-R [–]	0.40 ± 3.25	0.85 ± 3.93	0.36 ± 2.67	0.17 ± 3.10	0.19 ± 2.90
Body mass index [kg/m^2]	24.02 ± 4.59	24.33 ± 4.89	23.97 ± 4.44	24.04 ± 5.01	23.78 ± 4.07
MAP [mmHg]	92.07 ± 8.63	92.17 ± 8.83	91.66 ± 8.67	91.57 ± 8.87	92.55 ± 8.19
HbA1c [mmol/mol]	32.66 ± 4.89	32.97 ± 6.85	32.50 ± 2.75	32.30 ± 3.17	32.72 ± 4.52
Triglycerides [mg/dL]	1.30 ± 0.95	1.40 ± 1.19	1.32 ± 0.74	1.30 ± 0.87	1.21* ± 0.85
Total-/HDL-chol. ratio [–]	3.35 ± 0.88	3.49 ± 0.98	3.38 ± 0.84	3.29* ± 0.82	3.25*** ± 0.81
LDL-cholesterol	2.60 ± 0.77	2.72 ± 0.80	2.66 ± 0.74	2.56* ± 0.76	2.49** ± 0.73
Resting heart rate [bpm]	75.10 ± 11.65	78.32 ± 10.75	76.81 ± 11.20	73.98*** ± 12.06	72.01*** ± 11.53
hsCrP [mg/dL]	2.35 ± 4.20	2.44 ± 4.22	2.64 ± 4.39	2.63 ± 5.22	1.93 ± 3.22
Overweight or Obesity[&] (%)	14.36	17.82	13.12	14.90	12.03*
Current smoking (%)	30.94	40.40	24.58***	28.05**	28.98**
Hazardous drinking[#] (%)	28.01	21.94	26.87	29.20	33.08***

Data presented as weighted mean ± standard deviation (SD), unless specified otherwise. Significance codes: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

[&] Based on KiGGS (40).

[#] Hazardous drinking (32): AUDIT-C >5 (male)/4 (female).

cIMT, carotid intima-media thickness; β-SI, ß stiffness index; DC, distensibility coefficient; EP, Peterson's elastic modulus; YEM, Young's elastic modulus; CV-R, Index of cardiovascular risk (sum of z-scores of mean arterial pressure, triglycerides, total/HDL-cholesterol-ratio, body mass index and HbA1c). MAP, mean arterial pressure; HbA1c, glycated hemoglobin; hsCrP, high-sensitive C-reactive protein; bpm, beats per minute.

selective re-participation as well as population characteristics into account, weighting factors were used as described elsewhere in detail (25). Both, elevated cIMT and cS, were defined as values ≥90th centile. These are higher than the centiles issued by other recent studies on risk factors of subclinical atherosclerosis in adolescence (12, 36), which have more selective populations. Linear and log-binomial regressions were performed with Stata SE 14.2 (Stata Corp., College Station, TX, US, 2015). Log-binomial regression was performed instead of logistic regression as the odds ratios obtained by the latter overestimate the relative risk when outcomes are common (37). Because elevated cIMT and arterial stiffness as outcomes are derived from sex- and age-specific centiles, the relative risks are all adjusted for sex and age. In addition, log-binomial and logistic regressions were adjusted for height and mean arterial pressure. This decision was based on evidence suggesting that age, sex and height are major determinants of cIMT and cS (38), whereas systolic and diastolic blood pressure seem to be strong confounders of associations involving these vascular biomarkers (39). 95%–confidence intervals are reported to assess the precision of our estimates.

RESULTS

General Population Characteristics

General population characteristics are presented in **Table 1**. Participants' age ranged from 11 to 24 years at KiGGS-Wave-1 and 14–28 years at KiGGS-Wave-2 and was equally distributed. Biomarkers of vascular function and structure as well as general cardiovascular and metabolic factors were not significantly different between groups of exercise, only LDL-cholesterol levels and resting heart rate were lower in the highly active groups ($p < 0.001$). The proportion of active smokers was significantly higher in the "no exercise" group, whereas the proportion of hazardous drinking was highest in the most active group. Mean exercise time decreased from KiGGS-Wave-1 to KiGGS-Wave-2, as indicated by a higher proportion of participants reporting "no exercise" at KiGGS-Wave-2 compared to KiGGS-Wave-1 (26.9 vs. 14.2%), and by a higher proportion of participants reporting less exercise than reporting more exercise at KiGGS-Wave-2 compared to KiGGS-Wave-1 (36.7 vs. 24.9%). The proportion of participants reporting high volumes of exercise ("≥4 h") was similar in KiGGS-Wave-2 compared to KiGGS-Wave-1 (33.0%). Details about proportions of changes in exercise

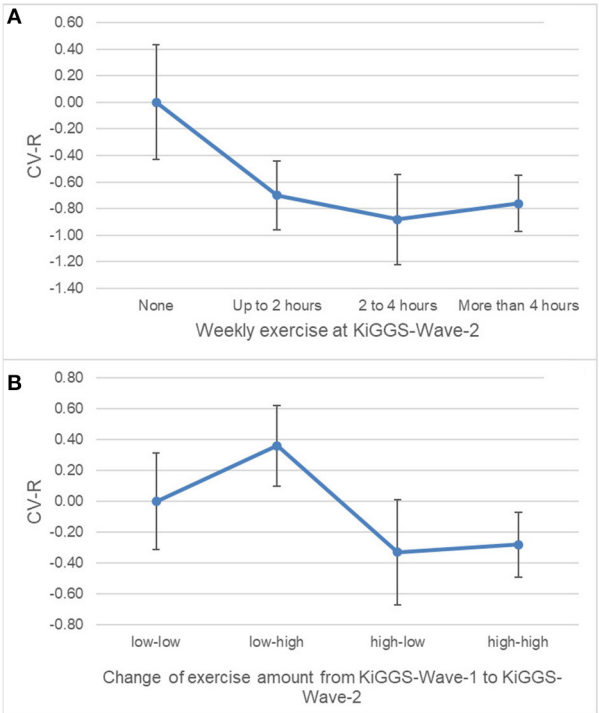

FIGURE 2 | Margin plots demonstrating associations of the cardiovascular risk index (CV-R) with cross-sectional **(A)** and longitudinal **(B)** volumes of exercise. CV-R is significantly lower with higher cross-sectional and longitudinal volumes of exercise. "None" set as reference. Weighted estimations from linear regression; $n = 2{,}893$. Vertical bars = 95%–confidence intervals. Models adjusted for sex and age.

habits from KiGGS-Wave-1 to KiGGS-Waver-2 are provided in the **Supplementary Table 1** in **Supplementary Material**.

Association of Exercise With Cardiovascular Risk

CV-R was significantly lower with higher cross-sectional (B = −0.73, 95%-CI = −1.26 to 0.19, $p = 0.008$) and longitudinal (B = −0.37, 95%-CI = −0.74 to 0.00, $p = 0.048$) exercise (**Figure 2**; **Supplementary Tables 2**, **3** in **Supplementary Material**). Relative risk for elevated CV-R was significantly lower in all exercise-groups compared to "no exercise" (RR = 0.80, 95%-CI = 0.66–0.98, $p = 0.033$) (**Figure 3**). High volumes of exercise at both measurement points were associated with lower relative risk of elevated CV-R (RR = 0.80, 95%-CI = 0.65–0.97, $p = 0.021$) (**Figure 4**).

Association of Exercise With Vascular Structure and Function

There were no between-group differences of cIMT and cS for cross-sectional and also longitudinal exercise (**Supplementary Tables 2**, **3** in **Supplementary Material**). There were also no between-group differences of relative risk for elevated cIMT and cS for cross-sectional exercise. Relative risk for elevated cS was lower in tendency with high volumes of exercise at both measurement points, whereas relative risk for elevated cIMT showed no between-group differences (**Figures 3**, **4**).

DISCUSSION

In summary, the study results of the analysis of a representative, so far largest cohort of adolescents and young adults demonstrate a more favorable constellation of classical atherosclerotic risk factors as well as a lower risk for an early increase of cS in healthy individuals who engage in regular exercise. However, biomarkers of vascular function and structure measured with state-of-the-art ultrasound equipment and vascular analysis software were not significantly associated with regular short- (cross-sectional) and long-term (longitudinal) exercise, contradicting previous findings (18). This might raise doubts for the utility of cS and cIMT to monitor effects of exercise on vascular atherosclerotic changes at such a young age.

On the other hand, our results confirm existing evidence (41, 42) demonstrating that physical activity, particularly exercise at higher intensities, may favorably influence atherosclerotic risk. However, the extent to which exercise can decelerate the progression of atherosclerotic changes may depend on the chronic cumulative exposure toward exercise rather than on high volumes in the short-term (43). Whereas, short-term exercise interventions lasting several months found only little or no effects of resistance training on carotid wall structure in young (21) and of aerobic training in older subjects (20), long-term interventions lasting at least 4 years demonstrated a slower progression of functional and structural vascular decline in regularly active, older individuals (44, 45). Yet again, only one small short-time exercise study was found that assessed the effects of 1 year recreational or organized sports participation on cIMT, but not cS, in adolescents (46). Accordingly, the current study adds valuable evidence.

The assessment of vascular protective effects of exercise on the in the general healthy population may have to take the chronological course of vascular aging into account. Results from our study agree with those from a smaller cohort in the European Youth Heart Study indicating, that parameters of vascular function and structure in children might remain independent from a child's activity level, whereas atherosclerotic risk factors may be favorably altered in those being exposed to regular exercise (47). That does not implicate the ineffectiveness of regular exercise in terms of protecting vascular health, but rather might reflect the compensatory capacities of a juvenile organism toward an unhealthy lifestyle. Results from the Amsterdam Growth and Health Longitudinal Study (22) and the Cardiovascular Risk in Young Fins Study (19) indicate that in middle-aged adults, functional biomarkers, such as cS, are significantly different in those with a lifelong high exercise-level compared to low-active peers. Thus, cIMT and cS might be indicators of a slower progression of vascular aging due to long-term exposure to regular exercise in adults rather than an accelerated progression of vascular aging in sedentary adolescents.

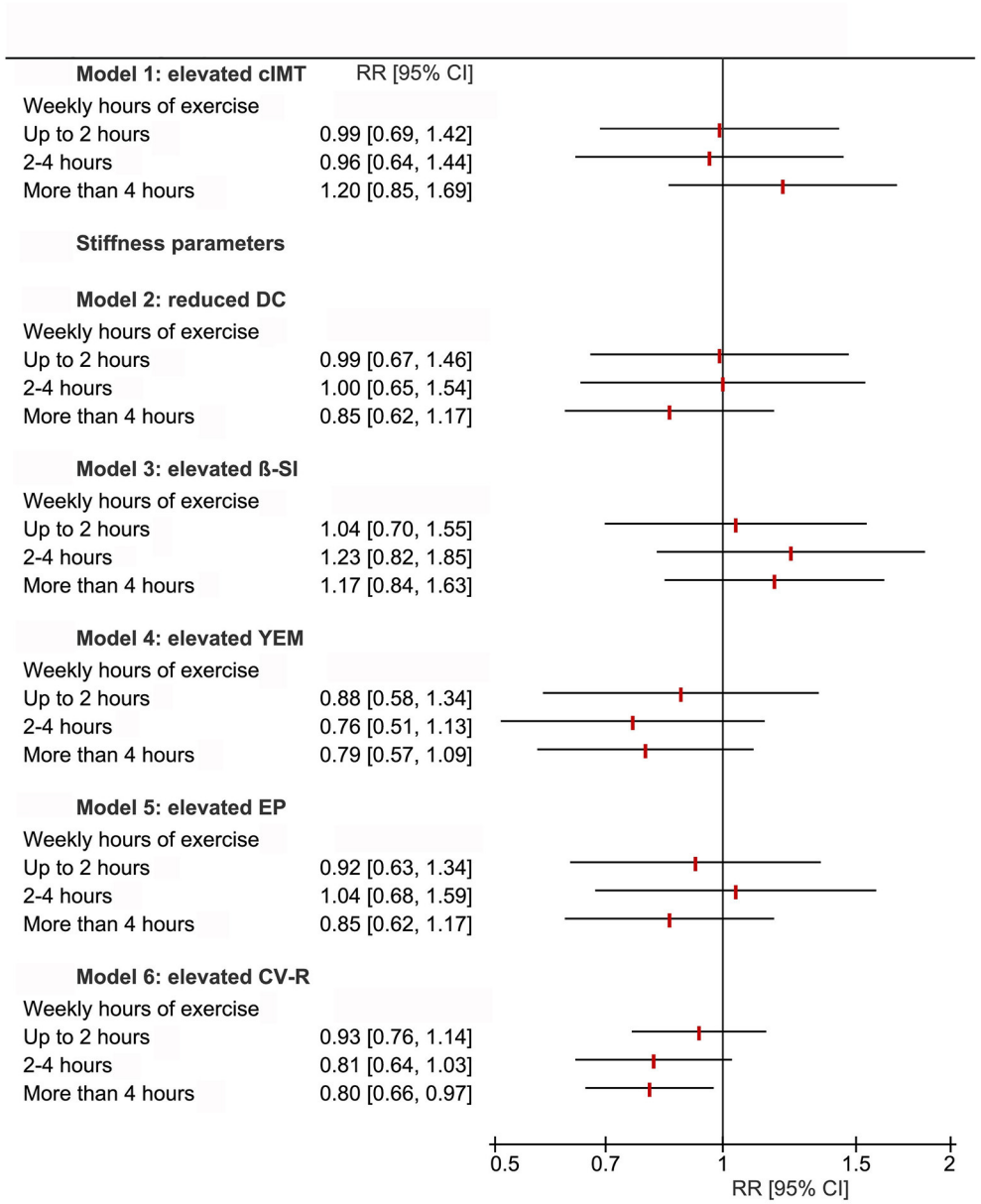

FIGURE 3 | Forest plot demonstrating relative risk for intima-media thickness (cIMT ≥ 90th percentile), parameters of carotid stiffness (cS ≥ 90th percentile) and elevated cardiovascular risk (CV-R ≥ 1 standard deviation) stratified by cross-sectional exercise at KiGGS-Wave-2. Relative risk for elevated CV-R is significantly lower with higher volumes of cross-sectional exercise. No between-group differences were observed for relative risk of elevated cS parameters and cIMT. Results from log binomial regression models. If the 95% confidence interval does not include the null value (RR = 1), the finding is statistically significant. Reference level of exposure: no regular exercise; weighted analyses. DC, distensibility coefficient; β-SI, ß stiffness index; YEM, Young's elastic modulus; EP, Peterson's elastic modulus; CV-R, Index of cardiovascular risk (sum of *z*-scores of mean arterial pressure, triglycerides, total/HDL-cholesterol-ratio, body mass index, and HbA1c); RR [95%-CI], Relative risk [95%–confidence interval].

The lack of association between cIMT and exercise in our study stands in contrast to another study (17) that found a slower progression of aortic IMT with higher volumes of intensive physical activity already during adolescence. However, peripheral arteries, such as the carotid artery, are inherently stiffer than the aortic artery and their age-related stiffening may be less marked (48). Thus, measurement of central arterial stiffness and wall thickness might be more indicative for exercise-related vascular effects than cS and cIMT in adolescents and young adults.

Practical Considerations

Our study results on a population-based cohort confirm data from previous studies on selected samples, that a high volumes of regular exercise (>4 h/week) during adolescence

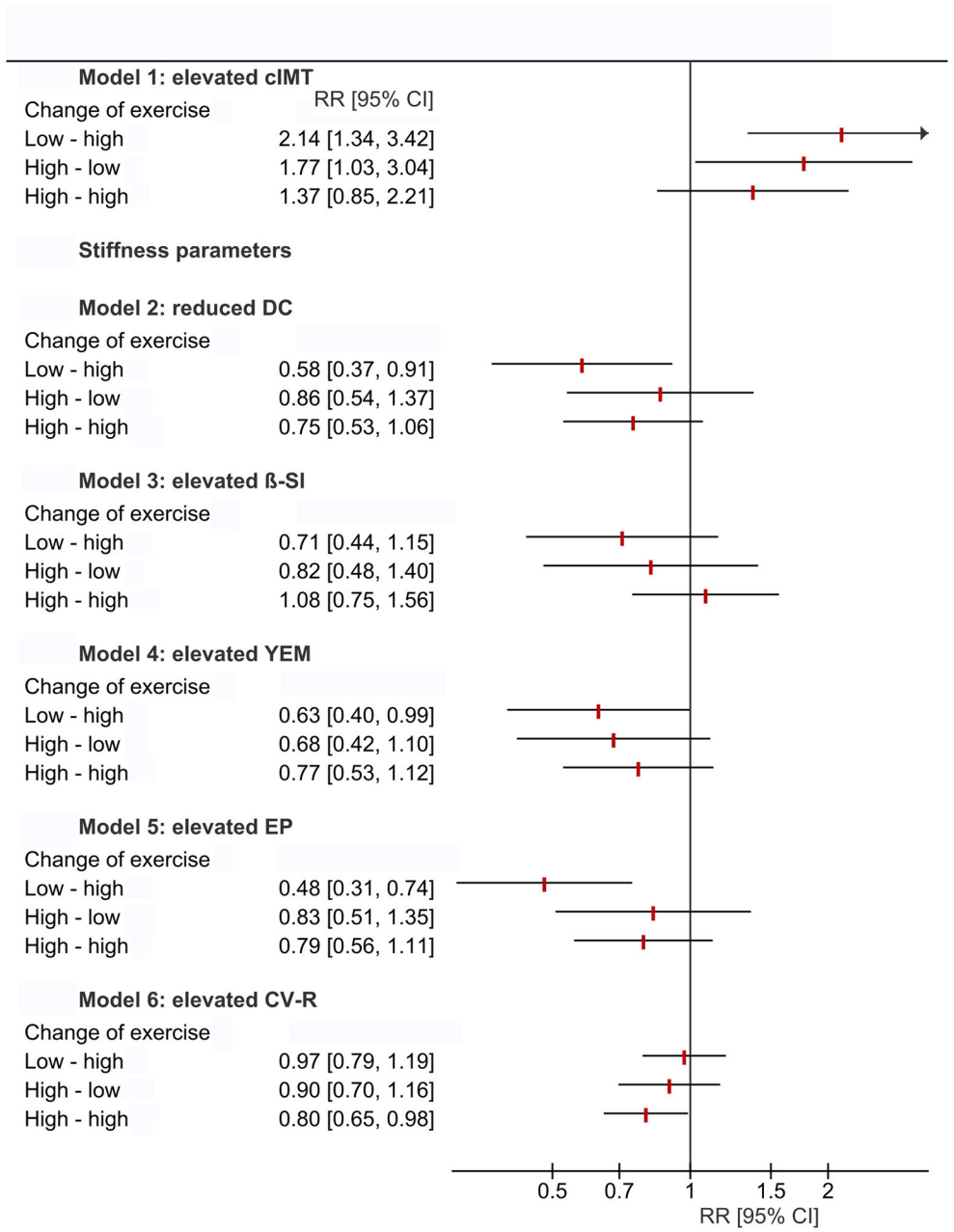

FIGURE 4 | Forest plot demonstrating relative risk for intima-media thickness (cIMT ≥ 90th percentile), parameters of carotid stiffness (cS ≥ 90th percentile) and elevated cardiovascular risk (CV-R ≥ 1 standard deviation) stratified by longitudinal changes of exercise between KiGGS-Wave-1 and KiGGS-Wave-2. Relative risk for elevated CV-R is significantly lower with regularly higher volumes of exercise, as is the relative risk for elevated cS parameters in tendency. No between-group differences were observed for relative risk of elevated cIMT. Results from log binomial regression models. If the 95% confidence interval does not include the null value (RR = 1), the finding is statistically significant. Changes of exercise from KiGGS-Wave-1 to KiGGS-Wave-2: "low-low" (always <2 h), "low-high" (<2 h at KiGGS-Wave-1 and ≥2 h at KiGGS-Wave-2), "high-low" (≥2 h at KiGGS-Wave-1 and <2 h at KiGGS-Wave-2), "high-high" (always ≥2 h); Reference level of exposure: "low-low"; weighted analyses; DC, distensibility coefficient; β-SI, ß stiffness index; YEM, Young's elastic modulus; EP, Peterson's elastic modulus; CV-R, Index of cardiovascular risk (sum of *z*-scores of mean arterial pressure, triglycerides, total/HDL-cholesterol-ratio, body mass index and HbA1c); RR [95%-CI], Relative risk [95%–confidence interval].

and young adulthood, play a vital part in the maintenance of low atherosclerotic risk in the healthy general population. This effect most likely translates into lower cardiovascular morbidity and mortality later in life (2). In addition, the comparison of interventional studies in young (49, 50) vs. older (20, 51) individuals suggests higher cardiovascular adaptive

responsiveness toward exercise programs in the young when as much as 95% of cardiovascular risk appear modifiable (17, 52). Furthermore, patterns of physical activity during childhood and adolescence track into adulthood remaining more or less stable along the life course (53). Pediatricians, but also other clinicians and health care providers, should thus be encouraged to promote the participation in regular recreational and organized sports of $\geq$4 h per week at the youngest possible age.

Methodological Considerations

A clear strength of this study is the assessment of multiple biomarkers of carotid wall structure and function. However, we only measured cIMT and cS of the common but not of the internal carotid artery or the carotid bulb, because of its higher visibility, completeness, procedural standardization and predictive value (54–56). Screening of the carotid tree for atherosclerotic plaques was not conducted because of an extremely low prevalence in adolescents and young adults (57). Furthermore, it has to be kept in mind, that exercise-related vascular adaptations may show some degree of local variation, due to distinct shear patterns induced by the type of exercise and the area of exposure (58). Especially in individuals engaged in sports predominantly involving the lower extremities favorable local vascular effects might not be detected by carotid arterial ultrasound. The application of a third-generation cIMT detection software, which includes automatic quality control already during image acquisition, promotes the thorough adjustment of the measurement plane by the sonographer. Together with the extensive post-procedural quality control and the rigorous adherence to current guidelines (12), this implies the currently highest quality of cIMT and cS data in a large representative population sample. We did not adjust for the ethnic background of study participants, as the sample was very homogeneous, with more than 95% Caucasians and because the relevance of ethnicity and geographical factors, such as latitude, for cIMT-related atherosclerotic risk assessment is likely to be rather low (59).

The assessment of physical activity based on self-reported questionnaires is a limitation. For validation purposes, volume of exercise in terms of moderate-to-vigorous physical activity was assessed via accelerometer in a subsample of our study cohort at KiGGS-Wave-2, showing significant correlations with questionnaire-based exercise. Furthermore, due to feasibility reasons, exercise habits could be assessed only twice during the study period. However, as exercise habits that have been developed during childhood tend to remain stable until adulthood (53), we assume that this sufficiently characterizes participants according to their long-term exercise habits. This is indirectly supported by lower levels of LDL-cholesterol and resting heart rate in the highly active groups with 2–4 and more than 4 h of weekly exercise. In addition, assessment of physical activity in this study was based on the MoMo physical activity questionnaire (35), which show good validity and reliability specifically for physical activity at higher intensities, such as sport exercise.

We did not adjust our models for maturation status, as there is currently no evidence supporting a relevant effect on cIMT and cS in models accounting for the strongest confounders (age, sex, height, carotid lumen diameter, and blood pressure) (60, 61).

Conclusions and Perspectives

Increasing and maintaining high levels of physical activity are associated with a better cardiovascular risk profile in adolescents and young adults. However, data available so far including this first study based on a representative national cohort show no association with common carotid structural and functional biomarkers. This study demonstrates, that on a population level exercise may contribute to an overall favorable constellation of atherosclerotic risk factors already during adolescence and young adulthood, if applied regularly over several years. The measurement of cS and cIMT for monitoring needs and success of preventive measures at such a young age and in a population sample with low atherosclerotic risk may be questioned. Despite great improvements in measuring techniques for these vascular biomarkers, exercise-related changes in the young and healthy individuals are subtle. Studies with longer follow-up periods, repeated measurements and more granular risk factor trajectories including lifestyle behavior are needed to further investigate the variable utility of vascular biomarkers throughout the life course. Yet, from a practical point of view, adolescents and young adults should be encouraged to engage in regular exercise according to the WHO guidelines on physical activity and sedentary behavior of at least 2 h or more per week and avoid periods of inactivity in order to maintain optimal cardiovascular health (62).

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because of restrictions due to data protection policies. Requests to access the datasets should be directed to NeuhauserH@rki.de.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Medizinische Hochschule Hannover (No. 2275-2014). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

KK wrote the manuscript, conducted data analysis, and participated in the conception and conduction of the study. JB participated in the conception of the study, data analysis, and revision of the manuscript drafts. GS and SK participated in the conduction of the study, extensively engaged in preparation, and revision of the manuscript drafts. HN and AS-T participated in the conception of the study, supervised the conduction and data analysis, extensively engaged in preparation, and revision of the manuscript drafts. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.767025/full#supplementary-material

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Sedentary Behavior in Childhood, Lower Arterial Compliance and Decreased Endothelial Function-Cross Sectional Data From a German School Cohort

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Background: Endothelial function by flow-mediated dilatation assesses early markers of atherosclerotic progression. Greater amounts of physical activity and physical fitness in children are associated with cardiovascular health benefits. We aimed to explore factors, influencing endothelial function and arterial compliance in a cohort of healthy school children.

Methods: The 94 participants (41 girls, 53 boys) in the study were young, healthy children from a German school cohort. Anthropometric data, body composition and blood pressure were assessed. Blood was drawn (8 h overnight fast), assessing total cholesterol, high density lipoprotein and low density lipoprotein and triglycerides. Endothelial function was diagnosed by flow-mediated dilatation with ultrasonography (ALOKA/Hitachi, Prosound alpha 6). Tracking gates were set on the intima in B-mode. The waveform of diameter changes over the cardiac cycle was displayed in real time using the FMD-mode of the eTRACKING system. Changes in arterial diameter at baseline, ischaemia and vasodilatation were measured. A symptom limited pulmonary exercise test on a bicycle ergometer was performed to test cardiorespiratory fitness. Physical activity was assessed using GT3x accelerometers (Actigraph, USA), over 4 days (including 1 week-end day), with a minimum wear-time duration of 10 h.

Results: The median age was 12.2 years (11.8–12.8). Children were normal weight, blood lipid profiles (cholesterol, high-density lipoprotein, low-density lipoprotein, triglyceride) were in normal range. Baseline measurements during the diagnostics of endothelial function revealed higher arterial compliance of the brachial artery in boys. Boys' cardiorespiratory fitness was higher than compared to girls. Boys met the recommendations of 60 min moderate to vigorous activity, whereas girls were significantly less active and did not meet current recommendations. More time spent in sedentary activity was the main predictor for lower arterial compliance (adjusted for age and sex), accounting for 14% of the variance. No significant model revealed, analyzing the influencing factors such as anthropometric data, blood lipids, physical activity and fitness on endothelial function.

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Conclusion: This is the first study on endothelial function in association to objectively measured physical activity and cardiorespiratory fitness in healthy school children in Germany. The study highlights the importance of reducing time spent being sedentary to maintain endothelial health.

Keywords: endothelial (dys) function, arterial compliance, physical activity, physical fitness, children, sedentariness

INTRODUCTION

Cardiovascular (CV) dysfunction, contributing to myocardial infarction, heart failure and stroke is one of the major cause of death in today's society worldwide (1). Increased arterial compliance and impairment of arterial endothelial function play an important role in the development of atherosclerosis and are strong predictors of CV events independent of traditional risk factors in adulthood (2, 3). Several pediatric studies have already reported reduced endothelial function (FMD) in children and adolescents at risk for atherosclerotic CV disease (4, 5).

High-frequency ultrasound is considered the gold standard for assessment of endothelial function in both adults (6, 7) and children (6).

Several studies have shown a relationship between cardiorespiratory fitness (CRF) and arterial compliance (8, 9). Veijalainen et al., for example, have given evidence that limited CRF is related to lower arterial compliance and higher arterial stiffness (10) measured by pulse wave velocity between carotid and femoral arteries. Equally important, it has been proven that physical activity may have a positive effect on reducing the blood pressure and the arterial stiffness in older adults (11, 12). It reduces the risk of cardiovascular diseases and has potential benefit on improved endothelial function (9).

Also, in obese children, it has been shown that the endothelial cell function can be improved significantly through a 12-week after school activity program. The school exercise intervention program leads to an improvement of vascular repair and endothelial cell function, leading toward an improved cardiovascular health (13). Similar relationships between exercise and vascular function in overweight children and adolescents are stated in the studies of Watts et al. and Woo et al. (14, 15).

The underlying mechanisms behind arterial remodeling seem to be complex. Factors such as shear stress, inflammation, sympathetic drive and oxidative stress lead to changes in cell signaling (16, 17). Especially the increased bioavailability of nitric oxide activated by signaling pathways after physical activity plays an important role in improving arterial compliance (18). The impact of exercise may be associated with the balance between reactive oxygen species (ROS) and the antioxidant defenses which influence the availability of nitric oxide (19).

Although the relationship between physical activity and arterial stiffness is complicated, the results of the studies mentioned above have given reasonable evidence regarding the positive effects of exercise training on arterial compliance. Reasoning based on these outcomes (20), reducing the cardiovascular risk factors may also be beneficial for children and prevent them from developing atherosclerosis.

However, studies investigating the associations between physical fitness, physical activity and arterial compliance and endothelial function in healthy children are limited. Currently no data in endothelial function in a German school children cohort exist.

Moreover, little is known of the effects of time spent being sedentary on arterial compliance and endothelial function in healthy children.

Therefore, our primary objective was to explore the factors influencing endothelial function, arterial compliance in a cohort of healthy school children and to quantify associations between physical fitness, physical activity, arterial compliance and endothelial function.

Our secondary objective was to examine possible sex differences on cardiorespiratory fitness and the amount of physical activity in different intensity levels in boys and girls in Germany. Girls are known to be less physically active than boys (21, 22) and spend less time in moderate to vigorous physical activity (23). A German cohort study further described lower physical fitness of girls compared to boys (24, 25). Earlier research of our study group demonstrated sex differences on A. carotid structure in healthy children (26). Further associations between physical activity intensity levels and arterial stiffness measured by pulse wave velocity have been described (27). It is, therefore, possible that endothelial function and arterial compliance may differ by childhood sex.

MATERIALS AND METHODS

Study Design and Study Population

Data collection was part of the "Get fit-stay healthy" project funded by the German Heart Foundation, a prospective study conducted in Bavaria, Germany that already started in 2013. The study focused on cardiovascular risk screening in healthy children and adolescents with the main emphasis on arterial structure and function as well as endothelial function. Ethic approval was obtained from the Ethics Committee of the Faculty of Medicine, Technical University Munich (project number: 4027/11). All data were assessed prior to the Covid-19 pandemic, with lock down restrictions in Germany from March 2020 onwards. Data analysis took part between 2017 and 2019. All pupils (n =1 54) children from sixth grade of the participating school were asked to participate in the study. N = 105 volunteered to participate in the study. Written informed consent was given by the participating children and their guardians.

Examination Process at the School

The study was performed under laboratory conditions in the medical room of the school. The room was quiet and temperature controlled. Temperature during diagnostics were 22°C (range 21–25°C, with a little ventilation if needed).

On the day of the examination the children were asked to come to school in a fasting state (8–10 h overnight fasting). The diagnostic routine started with the assessment of body composition, followed by blood collection and the diagnostic of endothelial function. The time of examination for body composition, blood collection and endothelial function was in the mornings between 7:30 and 11 am. After that the children had a light breakfast consistent of a banana and cereal, apple or orange juice and mineral water. The cardiovascular fitness test was performed later on the same day, in the same room with at least 1 h rest after breakfast. Finally, children were handed out the accelerometer which they wore for 7 days to objectively assess their physical activity.

Assessment of Body Composition and Blood Pressure

Anthropometric measurements were assessed by trained staff according to standardized guidelines (28). Portable scales and stadiometers (Seca 799, MedicallLine, Hamburg, Germany) were used for quantifying body weight to the nearest 0.1 kg (in light sports clothing) and height to the nearest 0.1 cm. A non-flexible tape (Seca 201, Hamburg, Germany) was used for the measurement of waist circumference at the middle between last rib and the anterior superior iliac spine at the midclavicular line and of hip circumference at the anterior superior iliac spine. The body mass index (BMI) was calculated as weight in kilograms divided by height in meters-squared and converted into z-scores using the reference values of a German cohort (29). According to the German Obesity Association childhood overweight was defined as a BMI between 90^{th} and 97^{th} percentile, obesity was defined as a BMI greater than the 97^{th} percentile for children with the same age and sex. Underweight was defined as a BMI $<10^{th}$ percentile (29).

Peripheral systolic and diastolic blood pressure were measured non-invasively at rest with a Mobil-O-Graph (IEM, Healthcare, Stolberg, Germany). Blood pressure measurements were performed on the left arm with the children in supine position. In order to select the appropriate arm cuff, subjects' arm circumferences were assessed before starting the tests.

Cuffs were chosen according to the measured left upper arm circumference (five different cuff sizes were used: 14–20 cm/20–24 cm/24–32 cm/32–38 cm/38–55 cm). The Mobil-O-Graph has already been validated for measurement of peripheral blood pressure (30, 31) and 24h ABPM (ambulatory blood pressure measurement) according to the BHS and ESH criteria (32, 33). Values were classified according to German age and sex specific norm values (20, 34).

Laboratory Analysis

Blood was collected after at least an 8 h overnight fast in different tubes (Sarstedt, Nümbrecht, Germany) for preparation of serum or EDTA-plasma samples. All samples were processed within 2 h after sampling. If tests were not performed within the same day, the samples were stored frozen at −40°C. A complete blood count including reticulocytes was measured in EDTA-anticoagulated whole blood (Sysmex XE-5000). Total cholesterol, HDL-C, and triglycerides were assessed using routine methods on a Cobas Integra 800 analyzer (Roche Diagnostics, Mannheim, Germany). LDL-C was calculated according to Friedewald's formula (35).

Measurements of Endothelial Function

Before initiating the diagnostic of endothelial function by flow-mediated dilatation (FMD) the examination was explained to the children. Children laid down in a supine position and rested for at least 10 min to guarantee stable conditions during measurement. The right arm was extended and immobilized with foam supports to guarantee a comfortable position. Since the A. brachialis is located medially, the arm is slightly turned outside (supination) to allow consistent imaging of the brachial artery.

Vascular endothelial function of the right brachial artery (BA) was studied with ultrasonography (Aloka/Hitachi, Prosound alpha 6) with a high-frequency (5–13 MHz) linear-array transducer. Mean arterial pressure was determined from the Mobil-O-Graph on the contralateral arm. The transducer was placed in the distal third of the upper arm to image the brachial artery. Tracking gates were set on the intima in B-mode, the artery resulting tracking lines, indicating the tracking position, are presented on the monitor in M-mode. The waveform of diameter changes over the cardiac cycle was displayed in real time using the FMD-mode of the eTRACKING system.

Changes in arterial diameter at baseline (1 min), ischaemia (5 min) and vasodilatation (2 min) were measured. Ischemia was developed by a blood pressure cuff placed around the forearm inflated to a pressure of 220 mmHg. After 5 min the cuff was deflated, causing increased flow-mediated vasodilatation. Peak artery diameter and the time taken to reach the maximal diameter after the release of the cuff, were recorded. From these data, FMD%, an index indicating the percentage dilated at the maximum vessel diameter in peak vasodilatation after cuff deflation, relative to maximum vessel diameter at baseline, was calculated. BA distensibility was defined by arterial compliance (AC), pressure strain elastic modulus (Ep), and PWV ß according to the following formula.

AC describes the ability of an artery to change its volume due to a given change in arterial blood pressure. The compliance is calculated from the diameter of the blood vessel (D) and BP.

$$AC = \pi(D_{max2}\text{-}D_{min2})/[4(BP_{max}\text{-}BP_{min})]$$

Pressure strain elastic modulus (Ep) is the ratio of stress and strain on the arterial wall and measures the intrinsic stiffness (Moo). An increase in stiffness leads to a higher Ep value.

$$Ep = (BP_{max}\text{-}BP_{min})/[D_{max}\text{-}D_{min})/D_{min}]$$

Beta-Index (ß) is another parameter to depict arterial stiffness. The higher the ß-Index, the lower is the arterial elasticity.

$$B = \ln(BP_{max}/BP_{min})/[D_{max}\text{-}D_{min})/D_{min}]$$

PWV is the velocity of the pressure wave transmitted between two portions of the arterial tree (36). PWV β is measured as the local pulse wave velocity of BA, calculated from β.

$$PWV\ \beta = \sqrt{((\beta * BP_{min})/(2p))}$$

TABLE 1 | Characteristics of the study population.

	Girls			Boys						*p*-Values
Anthropometric data	***n***	**Median**	**IQR**	***n***	**Median**	**IQR**	***n***	**Median**	**IQR**	
Age [years]	41	12.4	(11.9–12.8)	53	12.2	(11.8–12.9)	94	12.2	(11.8–12.9)	0.625
Height [cm]	41	156.0	(149.0–162.0)	53	155.0	(148.5–162.5)	94	156.0	(149.0–162.0)	0.994
Weight [kg]	41	44.1	(38.9–55.7)	53	45.0	(38.9–50.2)	94	44.6	(38.9–51.6)	0.661
BMI [kg/m^2]	41	18.70	(16.05–22.25)	53	18.10	(16.15–19.95)	94	18.30	(16.10–20.80)	0.519
BMI z-score	41	0.08	(−1.11–1.17)	53	−0.17	(−0.81–0.64)	94	−0.820	(−0.854–0.730)	0.254
Hip circumference [cm]	41	76.0	(69.3–81.0)	53	74.0	(67.0–78.5)	94	75.5	(69.0–80.0)	0.153
Waist circumference [cm]	41	66.0	(60.3–74.0)	53	67.0	(62.0–72.8)	94	66.0	(62.0–73.8)	0.892
Hip to waist ratio	41	1.13	(1.08–1.16)	53	1.07	(1.05–1.12)	94	1.09	(1.05–1.14)	0.002*
Blood lipids										
Cholesterol [mg/dl]	31	175.00	(146.00–195.00)	41	166.00	(139.00–190.50)	72	167.00	(140.75–191.25)	0.400
HDL [mg/dl]	31	59.20	(46.80–76.30)	41	66.20	(57.45–76.40)	72	64.50	(53.10–76.30)	0.136
LDL [mg/dl]	31	95.30	(71.70–109.00)	41	88.60	(71.85–106.50)	72	91.50	(71.80–107.00)	0.336
Triglyceride [mg/dl]	31	57.40	(49.60–77.40)	41	47.30	(39.35–59.80)	72	53.35	(43.40–66.38)	0.015*
LDL_HDL_ratio	31	1.51	(1.14–2.24)	41	1.34	(1.05–1.73)	72	1.40	(1.08–1.87)	0.071
Cardiovascular parameters and endothelial function										
Heart rate [bpm]	40	79	(69–85)	52	73	(65–80)	92	74	(67–83)	0.017*
Ep [kPa]	40	307.03	(196.15–379.08)	52	249.78	(185.47–339.93)	92	276.25	(189.20–357.62)	0.127
ß-Index	40	27.54	(17.14–36.31)	52	22.45	(17.00–30.08)	92	23.98	(17.14–31.14)	0.119
Systolic blood pressure[mmHg]	40	108.00	(104.50–114.00)	52	110.50	(106.00–113.00)	92	109.50	(106.00–114.00)	0.761
Diastolic blood pressure [mmHg]	40	62.00	(56.25–67.00)	52	65.00	(58.50–68.00)	92	64.00	(57.25–68.00)	0.567
Systolic blood pressure z-score	40	0.05	(−0.21–0.27)	52	0.24	(−0.61–0.61)	92	0.108	(−0.275–0.367)	0.254
Diastolic blood pressure z-score	40	−0.46	(−1.37–0.15)	52	−0.15	(−1.15–0.44)	92	−0.302	(−1.293–0.44)	0.434
PWVß_BA [m/s]	40	10.41	(8.24–11.80)	52	9.46	(8.27–11.28)	92	10.03	(8.27–11.48)	0.234
Arterial compliance [mm^2/kPa]	40	0.06	(0.04–0.08)	52	0.08	(0.04–0.10)	92	0.07	(0.04–0.10)	0.046*
Baseline diameter in systole [mm]	40	3.22	(2.94–3.84)	52	3.39	(3.01–3.98)	92	3.36	(2.98–3.94)	0.252
Peak diameter in systole [mm]	40	3.85	(3.36–4.67)	52	3.89	(3.47–4.75)	92	3.86	(3.43–4.67)	0.611
FMD_Systole [%]	40	10.72	(8.47–14.56)	52	12.47	(6.38–14.56)	92	11.23	(7.97–14.56)	0.631
Time to peak in systole[s]	40	59.00	(53.25–70.00)	52	55.00	(52.00–62.75)	92	58.00	(52.25–65.00)	0.969
Physical activity and fitness										
Sedentary activity [min/day]	41	337.57	(268.14–414.14)	53	330.14	(243.14–387.07)	94	333.29	(250.57–390.21)	0.556
Light activity [min/day]	41	148.00	(105.93–181.86)	53	151.07	(94.79–186.54)	94	150.71	(101.00–183.14)	0.739
Moderate activity [min/day]	41	42.57	(32.36–52.93)	53	57.07	(43.18–79.18)	94	49.00	(37.07–68.79)	0.005*
Vigorous activity [min/day]	41	3.14	(1.71–6.29)	53	4.64	(3.04–9.68)	94	4.00	(2.29–8.36)	0.032*
Very vigorous activity [min/day]	41	0.14	(0.00–0.43)	53	0.29	(0.04–1.57)	94	0.14	(0.00–0.93)	0.016*
MET rate	41	1.62	(1.49–1.77)	53	1.78	(1.57–1.98)	94	1.67	(1.51–1.90)	0.017*
Peak oxygen uptake [ml kg^{-1} min^{-1}]	41	39.20	(33.30–41.75)	53	45.75	(40.48–51.50)	94	41.80	(37.25–47.15)	<0.001**
Peak workload [W]	41	145.0	(127.0–165.0)	53	165.5	(141.5–193.5)	94	152.0	(136.5–184.0)	0.011*
Maximal heart rate [bpm]	41	188	(182–196)	53	187	(180–195)	94	187	(181–196)	0.674

*Data are presented as median (interquartile range). Differences between boys and girls were analyzed by Mann Whitney U test, *p < 0.05; **p < 0.01.*
BMI, body mass index; HDL, high density lipoprotein; LDL, low density lipoprotein; Ep, Pressure strain elastic modulus; ß-Index, ß stiffness index; PWVß, local pulse wave velocity measured at the brachial artery; FMD_systole, flow-mediated dilatation measured in systole; MET, metabolic equivalent of task.

The echo-tracking system implemented in the ultrasound machine allows accurate measurements of diameter changes, based on radio frequencies (RF) signals, able to detect variations of the arterial diameters with a strictness of 0.01 mm (37).

Assessment of Cardiorespiratory Fitness

Physical fitness was tested *via* a symptom limited pulmonary exercise test on a bicycle ergometer (Geratherm Respiratory, Ganshorn Medical, Germany). Baseline values were established during 2 min of rest followed by 2 min of unloaded pedaling. Afterwards, an increase in load was achieved *via* a ramp-wise protocol of 30 watts per minute. Criteria for ending the test was the maximum exhaustion of the subject and a drop of cadence below 60/min. The test featured a breath to breath gas exchange using a metabolic chart (Vmax Encore 229, SensorMedics, Viasys Healthcare, Yorba Linda, California). All exercise tests with a

respiratory exchange ratio of >1.0 were discharged because of insufficient effort. Peak oxygen uptake was defined as the highest mean uptake of any 30 s time interval during exercise. Reference values for age, body mass, body height and sex, expressed in "% predicted" were calculated as previously described (38).

Assessment of Physical Activity

Physical activity was assessed using GT3x accelerometers (Actigraph, USA), over 4 days (including 1 week-end day), with a minimum wear-time duration of 10 h. The accelerometer was positioned with an adjustable belt on the right iliac crest. Executive zeros over a period of 20 min were deleted. The time spent in inactivity as well as in light, moderate and vigorous PA was categorized using the cut-points per minutes (cpm) suggested by Evenson (39). Threshold counts < 100 cpm indicated physical inactivity, 101–2,295 cpm light PA, 2,296–4,011 cpm moderate PA and >4,012 cpm vigorous PA. Sedentary time was calculated as *wear time–(time spent in light PA + moderate PA + vigorous PA)* and total inactivity was defined as the sum of non-wear time and sedentary time. The time spent in different PA intensities was adapted for the number of days when the accelerometer was worn and expressed as minutes per day. All activity data measured by the ActiGraph GT3X+ was processed with the data analyzes software ActiLife, version 6.11.4 (ActiGraph, Pensacola, Florida, USA.

Data Analysis

Statistical analyses were conducted using IBM SPSS Statistics (Version 23). The study cohort was characterized by standard descriptive statistics. Data for boys and girls were compared using the Mann-Whitney test. Data distribution was initially examined for normality using the Kolmogorov-Smirnov test. Due to non-normal distributed variables descriptive statistics were presented as median and interquartile range.

Due to significant differences in cardiorespiratory fitness and physical activity levels we calculated potential relations between anthropometric data, blood lipids physical fitness and activity and endothelial function separately for boys and girls. Univariate analysis between the variables was calculated with the Spearman's correlation coefficients. We further performed linear regression analysis to determine the main predictors for endothelial function and arterial compliance. Independent variables of the vascular function, were FMD (%, in systole) and arterial compliance (AC). FMD was adjusted for baseline diameter in systole, age and sex, whereas arterial compliance was adjusted for age and sex. As dependent variables BMI z-score, Hip to waist ratio, systolic and diastolic blood pressure z-scores, cholesterol, HDL-LDL ratio, daily physical activity, time spent in sedentary, light moderate and vigorous activity levels, VO_{2max} as well as $Watt_{max}$ were integrated in the model as dependent variables. Criteria of normal distribution of the residuals were fulfilled so that logarithmic transformation was not necessary. Covariates were tested for collinearity. Correction for collinearity was not required as variance inflation factor (VIF) was <15. A value of $p < 0.05$ was considered to be statistically significant. *Post hoc*, compute achieved power was calculated; effect size $d = 0.05$; alpha error probability of 0.05; sample size of group 1 ($n = 40$) compared to sample size group 2 ($n = 52$). The outcome revealed the power (1-ß error prob = 0.745). Non-centrality parameter delta $\delta = 2.323$, critical $t = 1.662$, Df = 85.85.

RESULTS

Descriptive Characteristics in Boys and Girls

$N = 105$ participated in the study. Seven children were obese ($n = 3$ boys and $n = 4$ girls). Four boys demonstrated hypertensive blood pressure values. The data of these children were excluded from the analysis since they did not meet the criteria of being normal weight and normotensive. Overall, data of 94 healthy children (41 girls, 53 boys) were analyzed. Twelve children refused to have blood drawn. Two endothelial diagnostics had to be excluded due to artifacts during flow-mediated dilatation. All children did not take any medication, and did not suffer from known cardiovascular or metabolic disorder. The median age was 12.2 years (11.8–12.8). The characteristics of the sample population are displayed in **Table 1**.

Boys and girls of the studies population did not differ in height, weight and BMI. Comparison of anthropometric differences between boys and girls only revealed a higher hip to waist ratio in girls 1.13 (1.08–1.16) compared to a 1.07 (1.05–1.17) ($p = 0.002$). Blood lipid profiles (cholesterol, high-density lipoprotein, low-density lipoprotein and triglyceride) were in normal range. Baseline measurements during the diagnostics of endothelial function revealed higher arterial compliance of the brachial artery in boys ($p = 0.046$) combined with a significantly lower heart rate at rest ($p = 0.017$), before cuff inflation. Boys also demonstrated higher $VO2_{max}$ ($p < 0.001$) and $Watt_{max}$ values ($p = 0.011$) during the cardiopulmonary exercise test than girls. All children reached their age and sex-specific norm values during the symptom limited pulmonary exercise test on a bicycle ergometer (38). There was no difference between maximum heart rate in boys compared to girls.

Regarding the physical activity level of the children, boys spent significantly more time doing moderate-intensity ($p = 0.005$), vigorous-intensity ($p = 0.032$) and very vigorous-intensity physical activity ($p = 0.016$), respectively. The median time spent in moderate- to vigorous-intensity physical activity was 61.71 min a day (IQR, 46.21–88.86) in boys, whereas the median time spent in moderate to vigorous activity of girls was only 45.71 min a day (IQR, 34.07–59.21).

No differences between the sex sub-groups existed in time spent being sedentary and the amount of time spent in light-intensity physical activity level. Overall, boys had a higher MET rate ($p = 0.017$).

Relationships Between Anthropometric Data, Blood Lipids Physical Fitness, Physical Activity and Endothelial Function

Associations between anthropometric parameters, blood lipids physical fitness, physical activity, arterial compliance and endothelial function were analyzed for boys and girls as

TABLE 2 | Relationships between anthropometric parameters, blood lipids, physical activity and fitness and arterial compliance (AC) and flow-mediated dilatation at time of systole (FMD_Systole_BA).

	AC [l/kPa]									FMD_Systole_BA [%]								
	Girls			Boys			Total			Girls			Boys			Total		
	r	*p*	*N*	*r*	*p*	*N*	*r*	*p*	*N*	*r*	*p*	*N*	*r*	*p*	*N*	*r*	*p*	*N*
Anthropometric parameters																		
Age [years]	0.016	0.923	40	0.181	0.200	52	0.119	0.257	92	0.047	0.774	40	0.024	0.868	52	0.024	0.823	92
Height [cm]	0.230	0.153	40	0.337*	0.015	52	0.329**	0.001	92	−0.051	0.753	40	0.037	0.794	52	−0.012	0.909	92
Weight [kg]	0.172	0.289	40	0.279*	0.045	52	0.240*	0.021	92	0.176	0.276	40	0.039	0.786	52	0.076	0.472	92
BMI [kg/m^2]	0.151	0.351	40	0.066	0.640	52	0.081	0.442	92	0.284	0.076	40	0.022	0.88	52	0.120	0.255	92
Hip circumference [cm]	0.191	0.245	40	0.210	0.135	52	0.168	0.111	92	0.158	0.338	40	−0.098	0.488	52	0.007	0.948	92
Waist circumference [cm]	0.147	0.372	40	0.184	0.192	52	0.173	0.101	92	0.166	0.313	40	−0.053	0.71	52	0.036	0.733	92
Hip to waist ratio	0.018	0.911	40	0.092	0.515	52	−0.036	0.732	92	−0.036	0.827	40	−0.069	0.627	52	−0.004	0.973	92
Heart rate [bpm]	0.046	0.780	40	−0.009	0.951	52	−0.050	0.634	92	0.006	0.972	40	0.005	0.974	52	0.001	0.990	92
Blood lipids																		
Cholesterin [mg/dl]	−0.051	0.789	31	−0.078	0.632	41	−0.115	0.342	72	−0.003	0.988	31	−0.121	0.459	41	−0.071	0.559	72
HDL [mg/dl]	−0.121	0.525	31	−0.011	0.945	41	−0.030	0.807	72	−0.16	0.4	31	−0.031	0.853	41	−0.090	0.461	72
LDL [mg/dl]	−0.075	0.694	31	−0.028	0.864	41	−0.095	0.436	72	0.143	0.451	31	−0.246	0.131	41	−0.073	0.553	72
Triglyceride [mg/dl]	−0.006	0.977	31	0.023	0.886	41	−0.063	0.602	72	−0.017	0.929	31	0.13	0.424	41	0.086	0.481	72
LDL_HDL_ratio	0.082	0.667	31	−0.008	0.960	41	−0.034	0.78	72	0.158	0.404	31	−0.162	0.325	41	0.010	0.937	72
Physical activity and fitness																		
Daily activity [min]	−0.084	0.604	40	−0.169	0.231	52	−0.134	0.204	92	−0.083	0.611	40	0.074	0.6	52	0.028	0.790	92
Sedentary activity [min]	−0.222	0.168	40	−0.213	0.133	52	−0.235*	0.025	92	−0.241	0.135	40	0.221	0.118	52	0.032	0.762	92
Light activity [min]	0.058	0.721	40	−0.372**	0.007	52	−0.186	0.078	92	−0.256	0.111	40	0.047	0.744	52	−0.07	0.509	92
Moderate activity [min]	0.104	0.524	40	−0.142	0.320	52	−0.024	0.818	92	−0.097	0.551	40	−0.122	0.394	52	−0.105	0.320	92
Vigorous activity [min]	0.049	0.766	40	0.107	0.456	52	0.113	0.288	92	0.129	0.427	40	−0.153	0.285	52	−0.035	0.744	92
Very vigorous activity [min]	−0.101	0.536	40	0.092	0.521	52	0.079	0.454	92	−0.046	0.777	40	−0.084	0.558	52	−0.064	0.547	92
MET_rate	0.110	0.501	40	0.034	0.815	52	0.111	0.297	92	0.091	0.577	40	−0.259	0.067	52	−0.094	0.377	92
Peak oxygen uptake [ml/kg^{-1}/min^{-1}]	0.114	0.484	40	0.065	0.652	52	0.192	0.068	92	−0.086	0.598	40	−0.125	0.381	52	0.17	0.104	92
Peak workload [W]	0.348*	0.028	40	0.304*	0.030	52	0.380**	0.000	92	0.156	0.337	40	−0.058	0.685	52	0.358**	0.000	92
Maximal heart rate [bpm]	−0.166	0.306	40	0.105	0.467	52	−0.013	0.901	92	0.087	0.594	40	−0.304*	0.032	52	−0.04	0.713	92

*BMI, Body mass index; HDL, high density lipoprotein; LDL, low density lipoprotein; AC, arterial compliance; FMD_Systole_BA, Flow-mediated dilatation in systole measured at the A. Brachialis; MET, metabolic equivalent of task. *p < 0.05; **p < 0.01.*

well as for the total study group. Results are displayed in **Table 2**.

In boys, height was positively associated to AC ($r = 0.337$, $p = 0.015$), and weight ($r = 0.279$, $p = 0.045$) demonstrated a positive relation to AC. Better physical fitness, especially the maximal workload was positively correlated to AC ($r = 0.304$, $p = 0.030$). Regarding the amount physical activity, more time spent in light activity was negatively correlated to AC ($r = -0.372$, $p = 0.007$). A negative correlation was observed between FMD% and the maximum heart rate in boys ($r = -0.304$, $p = 0.032$).

An indicator for physical strengths and fitness is the performance measured in Watt during the cardiorespiratory fitness test. In girls a positive correlation was observed between the maximum workload (Watt) and AC ($r = 0.348$, $p = 0.028$).

No associations revealed between parameters of arterial stiffness and endothelial function with the blood lipid profiles.

Predictors for Arterial Compliance and Endothelial Function

Next to the calculation of associations, we further assessed the main influencing factors for arterial compliance in children. After adjustment for age and sex (model 1), the linear regression analysis revealed that more time spent in sedentary activity (ß $= -0.280$, $p = 0.037$) was the main predictor for lower arterial compliance, accounting for 14% of the variance [$R^2 = 0.014$, $F_{(13,57)} = 0.767$]. Taking BMI and systolic blood pressure also into account the model still revealed significant ($R^2 = 0.0122$, $F_{(10,60)} = 0.744$, $p = 0.027$) (**Table 3**).

To determine the influencing factors for endothelial function in the total study group, FMD % in systole was adjusted for baseline diameter, age and sex (model 1) $R^2 = 0.098$, $F_{(13,57)} = 0.479$, $p = 0.928$ and further also adjusted for BMI and systolic blood pressure. The regression also did not analysis reveal a significant prediction model $R^2 = 0.077$, $F_{(11,59)} = 0.449$, $p = 0.926$ (**Table 4**).

TABLE 3 | Multivariable correlates of arterial compliance.

	Model 1 AC				Model 2 AC			
	ß	**SE**	***P***	**95.0% CI**	**ß**	**SE**	***P***	**95.0% CI**
BMI (z-score)	0.253	0.253	0.260	[−0.184, 0.667]	-	-	-	
Hip to waist ratio	0.082	0.082	0.559	[−3.375, 6.174]	0.076	2.333	0.570	[−3,194,5.743]
Systolic blood pressure (z-score)	−0.159	−0.159	0.302	[−0.780, 0.246]	-	-	-	
Diastolic blood pressure (z-score)	0.114	0.114	0.459	[−0.212, 0.464]	0.048	0.149	0.728	[−0.246, 0.350]
Cholesterol [mmol/dl]	0.125	0.125	0.478	[−0.008, 0.017]	0.064	0.006	0.701	[−0.009, 0.014]
LDL/HDL ratio	−0.116	−0.116	0.500	[−0.871, 0.430]	−0.047	0.297	0.767	[−0.682, 0.506]
Daily physical activity [min/day]	0.052	0.052	0.697	[−0.009, 0.013]	0.081	0.005	0.538	[−0.007, 0.014]
Sedentary activity [min/day]	−0.280	−0.280	0.037	[−0.001, 0.000]	−0.295	0.000	0.027	[−0.001, 0.000]
Light activity [min/day]	−0.047	−0.047	0.776	[−0.001, 0.001]	−0.092	0.000	0.569	[−0.001, 0.001]
Moderate activity [min/day]	0.004	0.004	0.988	[−0.003, 0.003]	0.059	0.001	0.791	[−0.002, 0.003]
Vigorous activity [min/day]	−0.023	−0.023	0.901	[−0.010, 0.009]	−0.027	0.005	0.886	[−0.010, 0.009]
Peak oxygen uptake [ml/kg^{-1}/min^{-1}]	0.107	0.107	0.625	[−0.042, 0.069]	−0.024	0.021	0.885	[−0.044, 0.038]
Peak workload [W]	0.073	0.073	0.701	[−0.009, 0.013]	0.165	0.005	0.320	[−0.005, 0.014]

ß, Standardized coefficient beta; CI, Confidence interval; BMI, Body mass index; LDL, low density lipoprotein; HDL, high density lipoprotein.
Model 1: AC, Arterial compliance adjusted for age and sex as corvariates; n = 70, $R^2 = 0.014$, $F_{(13,57)} = 0.767$, $p = 0.037$.
Model 2: AC, Arterial compliance adjusted for age, sex, BMI and systolic blood pressure as covariates; n = 70, $R^2 = 0.0122$, $F_{(10,60)} = 0.744$, $p = 0.027$.

TABLE 4 | Multivariable correlates of endothelial function measured by flow-mediated dilatation.

	Model 1 FMDsys [%]				Model 2 FMDsys [%]			
	ß	**SE**	***P***	**95.0% CI**	ß	**SE**	***P***	**95.0% CI**
BMI (z-score)	0.122	0.213	0.595	[−0.312, 0.540]	-	-	-	
Hip to Waist ratio	0.125	2.386	0.389	[−2.708, 6.849]	0.094	2.211	0.490	[−2.888, 5.960]
Systolic blood pressure (z-score)	0.097	0.256	0.541	[−0.356, 0.671]	-	-	-	
Diastolic blood pressure (z-score)	0.074	0.169	0.640	[−0.259, 0.418]	0.088	0.147	0.532	[−202, 0.388]
Cholesterol [mmol/dl]	−0.044	0.006	0.810	[−0.014, 0.011]	−0.070	0.006	0.682	[−0.014, 0.009]
LDL/HDL ratio	−0.078	0.325	0.661	[−0.794, 0.507]	−0.051	0.294	0.756	[−0.680, 0.496]
Daily physical activity [min/day]	−0.110	0.006	0.430	[−0.016, 0.007]	−0.115	0.005	0.396	[−0.015, 0.006]
Sedentary activity [min/day]	−0.195	0.000	0.155	[−0.001, 0.000]	−0.210	0.000	0.119	[−0.001, 0.000]
Light activity [min/day]	−0.054	0.000	0.753	[−0.001, 001]	−0.055	0.000	0.742	[−0.001, 0.001]
Moderate activity [min/day]	−0.184	0.001	0.434	[−0.004, 0.002]	−0.169	0.001	0.458	[−0.004, 0.002]
Vigorous activity [min/day]	0.113	0.005	0.557	[−0.007, 0.012]	0.560	0.005	0.578	[−0.007, 0.012]
Peak oxygen uptake [ml/kg^{-1}/min^{-1}]	−0.011	0.028	0.962	[−0.057, 0.054]	−0.058	0.020	0.734	[−0.048, 0.034]
Peak workload [W]	−0.005	0.006	0.982	[−0.011, 0.011]	0.007	0.005	0.967	[−0.009, 0.010]

ß, Standardized coefficient beta; CI, Confidence interval, FMD_{sys} [%], flow-mediated dilatation in systole adjusted for age, sex and baseline diameter at systole; BMI, Body mass index; LDL, low density lipoprotein; HDL, high density lipoprotein.
Model 1: FMDsys, Flow-mediated dilatation adjusted for baseline diameter at systole, age and sex as corvariates; n = 70, $R^2 = 0.098$, $F_{(13,57)} = 0.479$, $p = 0.928$.
Model 2: FMDsys, Flow-mediated dilatation adjusted for baseline diameter at systole, age sex, systolic blood pressure and BMI as covariates; n = 70, $R^2 = 0.077$, $F_{(11,59)} = 0.449$, $p = 0.926$.

DISCUSSION

The purpose of this study was to assess factors influencing the arterial compliance and endothelial function in healthy children, taking possible sex differences into account. Our data yielded two important findings:

Firstly, after adjustment for age and sex, the main predictor the amount of time spent in sedentary behavior was the main predictor for lower arterial compliance. Secondly, sex differences exist regarding physical activity levels, cardiorespiratory fitness and arterial compliance.

The Impact of Physical Activity on Arterial Compliance and Endothelial Function

Sedentary lifestyle increases the risk of prematurely developing of atherosclerosis. Regular physical activity affects endothelial function (40). The results of our study emphasize the importance of reducing and limiting the amount of time spent being

sedentary, particularly the amount of recreational screen time which is recommended in the current guidelines of the World Health Organization (41). Physical activity can be described by duration of the activity and level of intensity (light, moderate, vigorous and very vigorous activity). Abbott et al. (42) described a significant relationship between physical activity and FMD%, even among a group of moderately active children. This gives credence to the importance of physical activity in early childhood. However, the underlying mechanisms behind arterial remodeling are complex. Training induces a direct impact on the vasculature (43). The pattern of blood flow and the amount of shear stress that occur during exercise may be related to the specific training characteristics, including training intensities (44). Next to moderate training intensity, high-intensity interval training is relationed to vascular function for improving the range of physiological, functional and clinical parameters, including endothelial function (45).

Children's general play and physical activity is characterized by changes in intensity levels and repeated sprint running is a natural exercise for children. A classical analysis reported that children have a preference for intermittent, explosive and intense activities of very short duration (< 15 s), which causes partly anaerobic-lactic metabolic conditions (46).

In the present study, girl's physical activity was lower than in boys. Further our data demonstrated that time spent in vigorous and very vigorous activities was significantly lower in girls than in boys. Girls only spent 45.71 min a day (IQR, 34.07–59.21) in moderate to vigorous activity and did not meet the recommendations of 60 min per day, which would confer to multiple beneficial health outcomes, such as cardiorespiratory fitness (47) cardiometabolic health (48) and cardiovascular health (49).

There is a need to reduce sedentary time and especially motivate girls to more intensive activities or to design sex-specific interventions with special attention to vigorous and very vigorous physical activity intensities in girls (e.g., ball games, robe skipping, running activities).

Favorite sports in boys (e.g., ball games and team sports such as soccer and basketball) can be defined as activities and sports with changing intensity levels similar to interval training (50).

Lower levels of physical activity have been related to stiffer arteries measured by pulse wave velocity between the femoral artery and low vascular function (51, 52).

Our findings are further in line with Veijalainen et al. (10) who observed that children with less stiff arteries have higher levels of unstructured physical activity and cardiorespiratory fitness. This was especially the case in boys whereas girls were in the opposite halve of these variables (10). However, though the authors found no associations between time spent in sedentary behavior and arterial stiffness (10), we found that time in sedentary behavior was the main predictor of lower arterial compliance, describing 14% of the evidence.

In the present study we could not demonstrate relationships between FMD and fitness after adjustment for age and sex. It has to be kept in mind that in the present population the most established risk factors for endothelial dysfunction such as smoking or hypercholesterolaemia are lacking (4). Previous studies reported data on the beneficial effect of exercise on endothelial health are most consistent in subjects with impaired endothelial function (53). One of the suggested new training types could be aerobic interval training, since data indicate that this type of training is superior in reversing endothelial dysfuction in children (54, 55).

Endothelial maximum dilatation is primarily due to endothelial cells releasing relaxing factors. To find out whether the FMD is solely dependent on the endothelium, a vasodilation through the direct action of the smooth muscles must be investigated. In adults, this is achieved by measuring vasodilation in response to glyceryl trinitrate (GTN) (42). However, it was ethically not possible to administer GTN to young healthy children in this voluntary prevention study.

In this context the time to peak is an indicator for endothelial health. Data in the present study showed that the time to peak dilation was 58 s. (sys, median) and the IQ range was 52.25–65.00 s. Our results are in line with results of Hopkins et al. who described a mean time of 60.7 (95%CI 59.0–62.5) s. to peak dilatation (56). The results are further comparable to the data of Järvisalo et al. They reported the highest mean dilation at 70.00 s. after cuff release in healthy children and adolescents (9–16 years). The majority of the subjects obtained their dilation peak diameter between 40 and 120 s, presenting a large range in response. Järvisalo et al. concluded that for true FMD peak response brachial artery diameter measurement is necessary up to 120 s. after cuff release as there is a certain variety in healthy children and adolescents (57).

Measurement of endothelial function by FMD is non-invasive, reproducible and non-painful (6), so it is particularly suitable for the investigation of young adults and children with the earliest stages of atherosclerosis, hence, providing the best opportunity for prevention (7).

Our data showed no sex differences in baseline diameter and also endothelial function, assessed by FMD was similar in both sexes. Our finding goes in line with Pahkala et al. (58). The group studied over 500 healthy boys and girls (13 years old) and reported no significant difference in endothelial function (FMD). In contrast, Hopkins et al. (56) described significant difference between boys and girls.

In this study we focused on an apparently healthy sample of school children.

Currently no reference values have been published for endothelial function assessed by FMD in children in a German cohort.

Strengths and Limitations of the Study

The major strengths of the study are that we used well established diagnostic measures for the assessment of physical examination, laboratory, ultrasound data as well as objective measurement of physical activity and cardiorespiratory fitness.

We also used established ultrasound diagnostics to assess the flow-mediated dilatation of the brachial artery as a response to a physiological stimulus (shear stress). Since we performed our diagnostics in healthy children we did not measure smooth

muscle dependent vasodilatation as a response to exogenous sources of NO such as nitroglycerin due to ethical considerations. Another limitation of the study is that we did not have data on the puberty status of the children. From the possible $n = 154$ pupils only 68% volunteered to participate in the study. This might be due to the fact that the examinations took place in the mornings during school hours.

CONCLUSION

This is the first study on endothelial function in association to objectively measured physical activity and cardiorespiratory fitness in healthy school children in Germany.

Time spent in sedentary behavior was the main predictor for lower arterial compliance in healthy children. Girls were less active than boys and did not meet current WHO guidelines for moderate-intensity activity.

This highlights the importance of maintaining or making every effort to promote physical activity to an average of 60 min per day per week and to reduce time being sedentary in childhood (41). Among the lifestyle-related factors measured in the present study, time spent in sedentary behavior has a stronger effect than BMI or blood lipids or cardiovascular fitness, on lower arterial compliance in healthy children.

Future studies should consider behavior approaches to further investigate differences and preferences of physical activity in boys and girls, resulting in possible sex-specific activity interventions. Prospective studies should further deepen the knowledge of vascular adaptations with regard to different activity intensities in healthy children, resulting in a better understanding of the promotion of an active healthy lifestyle to prevent cardiovascular risk factors and endothelial dysfunction.

The promotion of an active and healthy lifestyle becomes even more important, considering the fact that the present data was collected before the corona pandemic. Due to an increased sedentary behavior and an increased lack of exercise during the pandemic, even more far-reaching effects on the health of children must be feared.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the Faculty of Medicine, Technical University of Munich (project number: 4027/11). Written informed consent to participate in this study was provided by the participants' legal guardian.

AUTHOR CONTRIBUTIONS

RO-F, BB, JE, and JM contributed to the conception and design of the study. BB was main responsible for data collection as project coordinator and drafted the manuscript. BB, JM, and JE were involved in the practical work. BB, JE, JM, and HK contributed to the statistical analysis and interpretation of the data. HK assisted drafting the manuscript and literature research. RO-F, JM, and HK critically revised the manuscript. All gave final approval and agreed to submit the work.

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