Retinal Microvasculature and Cardiovascular Health in Childhood
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abstract

BACKGROUND AND OBJECTIVE: Alterations in retinal microvasculature are associated with increased risk of cardiovascular disease. We examined the associations of retinal vessel caliber with cardiovascular markers in school-age children.

METHODS: Among 4007 school-age children (median age of 6.0 years), we measured cardiovascular markers and retinal vessel calibers from digitized retinal photographs.

RESULTS: Narrower retinal arteriolar caliber was associated with higher systolic and diastolic blood pressure (\(-0.20\) SD score [SDS] \([95\%\text{ CI } -0.24\text{ to } -0.18]\) and \(-0.14\) SDS \([-0.17\text{ to } -0.11]\), respectively, per SDS increase in retinal arteriolar caliber), mean arterial pressure, and pulse pressure, but not with carotid-femoral pulse wave velocity, heart rate, cardiac output, or left ventricular mass. A wider retinal venular caliber was associated with lower systolic blood pressure, mean arterial pressure, and pulse pressure and higher carotid-femoral pulse wave velocity (carotid-femoral pulse wave velocity difference = 0.04 SDS [95\% CI 0.01 to 0.07] per SDS increase in retinal venular caliber). Both narrower retinal arteriolar and venular calibers were associated with higher risk of hypertension at the age of 6 years, with the strongest association for retinal arteriolar caliber (odds ratio 1.35 [95\% CI 1.21 to 1.45] per SDS decrease in arteriolar caliber). Adjustment for parental and infant sociodemographic factors did not influence the observed associations.

CONCLUSIONS: Both retinal arteriolar and venular calibers are associated with blood pressure in school-age children, whereas retinal venular caliber is associated with carotid-femoral pulse wave velocity. Microvascular adaptations in childhood might influence cardiovascular health and disease from childhood onward.

WHAT’S KNOWN ON THIS SUBJECT: Microvasculature alterations are associated with increased risk of hypertension in adults. Not much is known about the association of retinal vessel caliber with cardiovascular risk factors among children.

WHAT THIS STUDY ADDS: Narrower retinal arteriolar caliber is associated with higher blood pressure, mean arterial pressure, and pulse pressure in school-age children, whereas wider retinal venular caliber is associated with higher carotid-femoral pulse wave velocity. Microvascular adaptations might influence cardiovascular health from childhood onward.
It has been suggested that the development of hypertension might originate in early life. Early alterations in microvasculature structure might be part of the underlying mechanism leading to the development of hypertension. Studies in rats have shown that alterations in the microvascular structure, and hence increased peripheral resistance, precede the development of hypertension. Over the last few decades, advances in retinal photography have allowed us to noninvasively assess microvasculature in humans. Several cross-sectional and longitudinal studies among adults have shown that retinal arteriolar narrowing is associated with an increased risk of hypertension, whereas wider retinal venular caliber is associated with an increased risk of metabolic syndrome and inflammation. However, it remains unclear whether microvascular abnormalities also affect cardiovascular risk factors in childhood. The few studies among children that have examined the associations of retinal vessel caliber with cardiovascular risk factors have suggested that retinal arteriolar narrowing correlates with increased blood pressure levels in children at 6 and 12 years old. However, these studies did not have information about other cardiovascular markers. Therefore, in 4007 school-age children, we examined the cross-sectional associations of retinal vessel caliber with detailed cardiovascular markers.

**METHODS**

**Study Design**

This study was embedded in the Generation R Study in Rotterdam, Netherlands. The study protocol was approved by the Medical Ethical Committee of the University Medical Center Rotterdam. In total, 6523 children participated in detailed follow-up measurements at the median age of 6.0 years (95% range 5.7 to 8.0). Retinal vessel calibers were available in 4007 children. Missing retinal vessel measurements were mainly due to the later start of these measurements during the follow-up visits (Supplemental Figure 2). Birth weight was lower in children who were not included in the analyses than in children who were included (Supplemental Table 4).

**Retinal Microvasculature Assessment**

Retinal photographs were taken by well-trained staff in a dedicated research center. Digital retinal photographs centered on the optic disc were taken in 1 eye, at image resolution 4096 by 3072 pixels, using a Topcon digital retinal camera (model TRC-NW300, Oakland, New Jersey). We used the same methods and protocols as described in previous studies among adults to measure retinal vascular caliber from the digitized retinal photographs. Briefly, a semiautomatic computer imaging program was used to measure the caliber of the 6 largest retinal arterioles and venules located 0.5 to 1 disc diameter from the optic disc margin. Using the revised Knudson–Parr–Hubbard formula, absolute arteriolar and venular diameter were estimated in micrometers and were summarized as central retinal arteriolar and venular equivalents, representing the average arteriolar and venular calibers, respectively, of that eye. Graders, masked to birth parameters and other participant characteristics, operated the computer program and monitored all retinal measurements. We constructed grader-specific SD scores for both central retinal and arteriolar equivalents. Interclass correlation coefficients between graders were 0.79 for retinal arteriolar caliber and 0.89 for retinal venular caliber, which suggests adequate reproducibility.

**Cardiovascular Markers**

We measured blood pressure at the right brachial artery with the child lying quietly in supine position, 4 times at 1-minute intervals, using a validated automatic sphygmomanometer (Datascope Accutor Plus, Paramus, New Jersey). A cuff was selected with a width ~40% of the arm circumference and long enough to cover 90% of the arm circumference. We calculated the mean value for systolic and diastolic blood pressure using the last 3 blood pressure measurements of each participant. We subsequently calculated the mean arterial pressure (mean arterial pressure = [systolic blood pressure + 2 × blood pressure]/3) and pulse pressure (pulse pressure = [systolic blood pressure – diastolic blood pressure]). We defined hypertension as systolic and diastolic blood pressure above the 95th percentile, using age- and height-specific cut points defined by the National High Blood Pressure Education Program Working Group on High Blood Pressure. Carotid-femoral pulse wave velocity was assessed by using the automatic Complior SP device (Artech Medical, Pantin, France) with participants in supine position, as previously described in detail. The distance between the recording sites at the carotid (proximal) and femoral (distal) artery was measured over the surface of the body to the nearest centimeter. Carotid-femoral pulse wave velocity was calculated as the ratio of the distance traveled by the pulse wave and the time delay between the waveforms, as expressed in meters per second. Carotid-radial pulse wave velocity can be measured reliably in large pediatric population-based cohorts, with interclass correlation coefficients within and between technicians higher than 0.96. Two-dimensional echocardiography was used to measure the interventricular end-diastolic septal thickness, left ventricular end-diastolic diameter, left ventricular end-diastolic posterior wall thickness, heart rate, and cardiac output. These
measurements were performed using methods recommended by the American Society of Echocardiography and were used to calculate the left ventricular mass and left ventricular relative wall thickness (2 × [diastolic posterior wall thickness ÷ left ventricular end-diastolic diameter]).18,19  

Covariates  
Using questionnaires, we collected information about maternal age, parity, educational level, prepregnancy BMI, smoking during pregnancy, and folic acid supplement use. Maternal and paternal blood pressure were assessed at enrollment. Information on ethnicity was obtained from the first questionnaire at enrollment in the study.9 Gestational age at birth was based on fetal ultrasound examination during the first ultrasound visit. Birth weight was obtained from medical records. Information on breastfeeding and average TV watching time were assessed by questionnaire. At the child’s age of 6 years, we measured height and weight and calculated BMI (kg/m²).  

Statistical Analyses  
First, we examined the correlations between all retinal vessel calibers and cardiovascular markers using Pearson correlation coefficients. Second, we used multiple linear regression models to assess the associations of retinal vessel calibers with vascular outcomes (systolic and diastolic blood pressure, mean arterial pressure, pulse pressure, and carotid-femoral pulse wave velocity) and cardiac outcomes (heart rate, cardiac output, left ventricular mass, and left ventricular relative wall thickness). For these analyses, we constructed SD scores (SDSs) ([observed value − mean]/SD) for all variables to enable comparison in effect size of different outcome measures. Because the outcomes were highly correlated, we divided them into 3 groups and applied Bonferroni–Holm correction, taking into account 3 groups of outcomes. Third, we used logistic regression models to examine the association of retinal vessel caliber with the risk of hypertension in childhood. All analyses were adjusted for image grader and child age, gender, and ethnicity. We additionally adjusted these models for parental and infant sociodemographic factors, birth outcomes, and childhood BMI. We tested for interaction terms between gender and BMI with retinal vessel caliber in relation to cardiovascular risk factors in childhood. Because no significant interaction terms were present, no further stratified analyses were performed. Analyses were performed by using the Statistical Package of Social Sciences version 21.0 (SPSS, Chicago, Illinois).  

RESULTS  
Subject Characteristics  
Table 1 shows characteristics of the study population. The mean (SD) arteriolar and venular calibers were 159.0 (14.9) and 219.0 (20.2) μm, respectively. Supplemental Table 5 gives the correlation coefficients between the retina vessel calibers and cardiovascular markers.  

Retinal Vessel Calibers and Cardiovascular Markers in Childhood  
Table 2 shows that in the models adjusted for image grader and child age, gender, and ethnicity, narrower retinal arteriolar caliber was associated with higher systolic and diastolic blood pressure (−0.20 SDS [95% confidence interval (CI) −0.24 to −0.18] and −0.14 SDS [−0.17 to −0.11], respectively), mean arterial pressure (−0.18 SDS [−0.21 to −0.15]), and pulse pressure (−0.10 SDS [−0.13 to −0.06]) per SDS increase in retinal arteriolar caliber. No association was present for retinal arteriolar caliber with carotid-femoral pulse wave velocity. Narrower retinal venular caliber was associated with higher systolic blood pressure (−0.05 SDS [95% CI −0.08 to −0.02] per SDS increase in retinal venular caliber), mean arterial pressure, and pulse pressure, but not with diastolic blood pressure in childhood. Wider retinal venular caliber was associated with higher carotid-femoral pulse wave velocity (0.04 SDS [95% CI 0.01 to 0.07] per SDS increase in retinal venular caliber). Additional adjustment for parental and infant sociodemographic factors and birth outcomes did not materially affect the observed associations. These associations remained significant even after taking multiple testing into account.  

Table 3 shows that no associations were present for retinal arteriolar and venular calibers with heart rate, cardiac output, left ventricular mass, and left ventricular relative wall thickness. A sensitivity analysis performed among preterm-born children and children born with a low birth weight showed results similar to those of the total group (data not shown).  

Retinal Vessel Caliber and Risk of Hypertension  
Fig 1 shows that narrower retinal arteriolar and venular calibers were associated with higher risk of hypertension at the age of 6 years, with the strongest associations for retinal arteriolar caliber (odds ratio 1.35 [95% CI 1.21 to 1.45] and 1.19 [1.00 to 1.32] per SDS decrease in arteriolar and venular caliber, respectively). Additional adjustment for parental and infant sociodemographic factors and birth outcomes did not influence the observed associations.  

DISCUSSION  
We observed, in a low-risk community-based population of school-age children, that narrower retinal arteriolar caliber was associated with higher systolic and
diastolic blood pressure, mean arterial blood pressure, and pulse pressure, whereas wider retinal venular caliber was associated with lower systolic blood pressure and higher carotid-femoral pulse wave velocity. Children with narrower retinal arteriolar and venular calibers were more likely to have an increased risk of elevated blood pressure.

**Methodological Considerations**

We used a population-based cohort study design with a large number of subjects. Of all children participating in follow-up measurements at the age of 6 years, 64% participated in the retinal vessel and cardiovascular follow-up studies. Nonresponse could lead to biased effect estimates if the associations of different obesity measures and cardiovascular risk factors with retinal vessel calibers were different between children included and not included in the analyses. Birth weight was lower in children who were not included in the current analyses compared with those who were included ($P < .01$).

We used validated techniques to measure retinal vessel caliber. We did not take into account other ocular factors, such as axial length and refractive error, that might affect retinal vessel measurement. However, it has been previously shown among adults that these factors have only a small impact on the measurement of retinal vessel caliber. Because of the cross-sectional nature of the analyses, we were not able to explore directions and causality of the observed associations.

**Interpretation of Main Findings**

Cardiovascular risk factors at younger ages are associated with higher risk of disease and premature death in adulthood. Previous studies have hypothesized that microvasculature alterations might be part of early underlying mechanisms in the development of cardiovascular...
disease. These studies were mostly conducted among adults and focused on the association of retinal vessel caliber with blood pressure. Among adults, retinal arteriolar narrowing is strongly associated with higher pressure and independently predicted the risk of stroke, whereas wider retinal venular caliber was associated with increased risk of metabolic syndrome and measures of inflammation. It remains unclear whether microvascular abnormalities are a result of cardiovascular disease or are part of the factors that relate to the development of these diseases. Thus, examining the associations among children without clinical cardiovascular disease may provide further insight into the pathways underlying these associations.

Increased blood pressure is the leading factor for cardiovascular disease, and pulse pressure has been recognized as a risk factor for stroke. Previously, a study among 5628 adults showed that narrower retinal arteriolar caliber is associated with increased blood pressure, independent of baseline blood pressure and BMI. Studies among children also observed significant inverse associations between retinal arteriolar caliber and blood pressure. Two cross-sectional studies among 1953 children age 6 to 8 years and 587 children age 11 years found significant inverse associations between retinal arteriolar caliber and systolic and diastolic blood pressure levels and mean arterial pressure. We also observed that narrower retinal arteriolar caliber was associated with higher systolic and diastolic blood pressure, mean arterial pressure, and pulse pressure, independent of parental and infant sociodemographic factors and childhood BMI. Results from previous studies on the association of retinal venular caliber with blood pressure are inconsistent. A study among 578 children at the age of 11 years observed that narrower retinal venular caliber was associated with higher diastolic, but not systolic, blood pressure. Another cross-sectional study among children age 4 to 5 years showed that increased systolic blood pressure was associated with wider venular caliber. In our study, we observed that narrower retinal venular caliber was associated with higher systolic blood pressure and pulse pressure, but not with diastolic blood pressure. Retinal arteriolar and venular calibers were positively correlated. Therefore, the observed associations of retinal venular caliber with systolic blood pressure might be due to confounding by retinal arteriolar caliber.

A meta-analysis showed that retinal arteriolar narrowing, likely indicative of increased peripheral vascular resistance, leads to subsequent development of hypertension in adults. Also, cross-sectional studies among children age 6 to 8 and 12.7 years have shown that narrower retinal arterioles were significantly correlated with increased risk of...
hypothesis.8,31 In line with these studies, we also found significant associations of narrower retinal arteriolar and venular calibers with increased risks of elevated blood pressure in school-age children.

Carotid-femoral pulse wave velocity is a marker of arterial stiffness and is associated with cardiovascular disease in adults.32 Among adults, a cross-sectional study among 135 individuals showed that arterial stiffness measured by central pulse pressure was inversely correlated with wall-to-lumen ratio of retinal arterioles.33 Another study among 197 adults found that retinal arteriolar narrowing was associated with higher carotid-femoral pulse wave velocity.34 No previous study in children examined the associations of retinal vessel calibers with carotid-femoral pulse wave velocity. In our study, we observed that wider retinal venular caliber was associated with a higher carotid-femoral pulse wave velocity, suggesting that microvasculature alterations might affect carotid-femoral pulse wave velocity already from childhood onward.

Increased left ventricular mass is an early pathogenic process in the development of heart failure.35 In children, increased left ventricular mass is correlated with higher adiposity and hypertension.36,37 A longitudinal study among 132 children showed that increased left ventricular mass tends to track with age, between 13 and 27 years.36 The few studies that examined associations of retinal microvasculature with left ventricular mass were all conducted among adults. A study among 4593 adults showed that narrower retinal arteriolar caliber was associated with left ventricular mass remodeling, independent from traditional risk factors for cardiovascular diseases.38 In contrast, in our population of school-age children, we did not find significant associations of retinal vessel caliber with left ventricular mass. These associations may become more apparent at older ages. We also did not find significant associations of retinal vessel caliber with cardiac output and heart rate, which are important cardiac measures and are shown to contribute to cardiovascular disease development. To the best of our knowledge, no previous study examined the association of retinal vessel caliber with these cardiac measures.

Our findings suggest that microvasculature alterations might be involved in the early mechanisms leading to cardiovascular disease in later life. The retinal vessels share similar anatomic and physiological properties with coronary and cerebral microcirculation and have been suggested as biomarkers to estimate systemic vascular health.6,39 Eutrophic remodeling40 and anatomic alterations such as intimal thickness and hyalinization may be mechanisms leading to narrowing retinal arteriolar vessels, which as a consequence might lead to an increase in peripheral resistance and higher blood pressure in later life.2 Although the observed effect estimates for the associations of retinal vessel caliber with different cardiovascular markers were small, these findings are important from an etiological perspective. Previous studies have shown that cardiovascular risk factors tend to track with age from childhood into adulthood.1,41 A large meta-analysis showed that blood pressure tracks from childhood into adulthood, and early-life blood pressure is associated with increased cardiovascular risk in later life.42 Thus, these studies suggest that subclinical differences in risk factors for cardiovascular disease in childhood are related to the development of cardiovascular disease in later life. Further studies are needed to examine the long-term consequences of the observed differences in cardiovascular indices throughout the life course.

CONCLUSIONS
We observed that narrower retinal arteriolar caliber was associated with higher systolic and diastolic blood pressure, mean arterial blood pressure, and pulse pressure, whereas wider retinal venular caliber was associated with lower systolic blood pressure and higher carotid-femoral pulse wave velocity. These results suggest that retinal arteriolar and venular adaptations are markers of microvascular pathways that ultimately lead to the development of cardiovascular risk factors in later life. Further studies are needed to examine the predictive value of alterations in the retinal microvasculature in the development of clinical cardiovascular diseases throughout the life course.

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