Effect of Birth Parameters on Retinal Vascular Caliber
The Twins Eye Study in Tasmania

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Abstract—Recent studies reported an association between smaller birth size and narrower retinal vascular caliber, but it remains unclear whether this association is attributed to confounding by shared environment or genetic factors. At a mean age of 9.3 years, 266 twins (49 monzygotic and 84 dizygotic pairs) in the Twins Eye Study in Tasmania underwent an ophthalmic examination including retinal photography. Retinal vascular caliber was measured using a validated protocol. The majority of these twins were also in the Tasmanian Infant Health Study, which prospectively collected data on birth parameters and antenatal maternal factors. We conducted the main analysis using linear mixed models, accounting for birth set clustering. Both the within-pair (−9.73; 95% CI: −14.68 to −4.77 μm per 5-cm decrease in birth length) and between-pair associations (−7.15; 95% CI: −11.54 to −3.01) with retinal arteriolar caliber were significant and of similar magnitude (difference in effect, $P=0.61$), after adjusting for age, sex, maternal smoking, mean arterial blood pressure, and other confounders. These associations remained within dizygotic and monzygotic pairs. Analyses of head circumference and retinal arteriolar caliber were similar to those of birth length (within-pair regression coefficient: −2.41; 95% CI: −5.09 to 0.28; between-pair regression coefficient: −2.60; 95% CI: −5.00 to −0.19). For birth weight, only a between-pair association was evident (−7.28; 95% CI: −13.07 to −1.48). This study demonstrates a consistent association between smaller birth size and narrower retinal arterioles in twins. The independent effect of shorter birth length on retinal arteriolar caliber supports a role for twin-specific supply line factors affecting fetal growth on vascular structure. (Hypertension. 2009;53:487-493.)

Key Words: twins ■ epidemiology ■ blood pressure ■ arterioles ■ birth weight ■ birth length ■ head circumference

Evidence to support the hypothesis of the fetal origins of adult disease has accumulated in the last decade.1,2 Widely reported data from epidemiological studies implicate adverse influence during fetal, infant, and early childhood growth that might ultimately lead to the early onset of cardiovascular diseases, hypertension, and diabetes.3 The specific mechanisms underlying these associations, however, are far from clear. Study of retinal microvascular changes attributed to fetal and perinatal influences offers a unique opportunity to uncover the underlying causal pathways of fetal origins of adulthood vasculopathies. Using new computer-based retinal imaging techniques, we have shown that precisely measured retinal arteriolar narrowing is a strong marker for the development of hypertension and cardiovascular events, including myocardial infarction.4-6 New studies now demonstrate that lower birth weight is associated with narrower retinal arterioles,7,8 narrower bifurcation angle,9 higher retinal vessel tortuosity, and optimality deviance,10 providing initial evidence that fetal origins of cardiovascular disease may partly be mediated by the microcirculation. However, it could be argued that the association between birth weight and microvascular alternation may be confounded by genetic or shared environmental factors (eg, maternal nutrition) that affect both birth size and abnormal microvascular structural development. It is also commonly understood that variation in almost all human traits and diseases has some genetic component, and this is evident for retinal vascular caliber11,12 and size at birth.13

Received October 30, 2008; first decision November 14, 2008; revision accepted December 22, 2008.

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Hypertension is available at http://hyper.ahajournals.org

DOI: 10.1161/HYPERTENSIONAHA.108.125914
Twin studies provide a unique means to distinguish between shared early environment, nonshared environment, and the genetic origins of an association. Twin siblings have half of their genes in common if dizygotic (DZ) and all of their genes in common if monozygotic (MZ). However, regardless of the zygosity, intrauterine environmental factors (which can differ between twins, eg, a differential placental supply), mother-specific pregnancy factors (eg, maternal nutrition and length of gestation), and postnatal environment are usually shared within twins. Interestingly, twins with the same gestational age often differ in size at birth.14 Twin studies can, therefore, provide insights into the importance of confounding by these shared factors; eg, discordance of birth weight and other birth parameters that are evident in a within-twin pair analysis is more likely to reflect differences in placental function or blood and nutrient supply to the individual fetus within the multiple birth set rather than factors constant across the twin pair (eg, gestation).15 In the present study, we investigated the influence of birth parameters (eg, birth weight, birth length, and head circumference) on retinal vascular caliber in 266 twin individuals with known zygosity age ranged 5 to 14 years from the Twins Eye Study in Tasmania (TEST).

**Methods**

**Study Population**

The TEST is a population-based study designed to investigate the relative influence of genetic and environmental factors on a variety of ocular traits. The study population and methodology have been described in detail elsewhere,16 and our study sample included all of the twins examined between 2000 and 2002 (346 participants). Recruitment of twins was conducted either through the Australian Twin Registry (aged 5 to 16 years) or by local media appeal. Most of these twins (288 participants), predominately white subjects, also participated the Tasmanian Infant Health Study (TIHS), investigating infants at risk of sudden infant death syndrome during 1988 to 1995,17 and their families provided extensive early life information. Among these children, 184 multiplets (born between 1991 and 1993) also additionally participated in a further Childhood Blood Pressure Study examining cardiovascular diseases in 1999,18 which allowed infants at risk of sudden infant death syndrome during 1988 to 1995,17 and their families provided extensive early life information. Among these children, 184 multiplets (born between 1991 and 1993) also additionally participated in a further Childhood Blood Pressure Study examining cardiovascular diseases in 1999,18 which allowed cross-referencing of prospectively collected data relating to individual fetal, infant, and childhood environment.

Of the 346 children, we excluded the following: 9 children without retinal vessel caliber measurement data for the right eye; 12 children (4 triplets) with no zygosity data available; 55 participants with missing birth parameter data; and 4 whose sibling had not been examined, leaving 266 twin children in the current study (49 MZ and 4 triplets) with no zygosity data available; 55 participants with shared early environment, nonshared environment, and the genetic origins of an association. Twin siblings have half of their genes in common if dizygotic (DZ) and all of their genes in common if monozygotic (MZ). However, regardless of the zygosity, intrauterine environmental factors (which can differ between twins, eg, a differential placental supply), mother-specific pregnancy factors (eg, maternal nutrition and length of gestation), and postnatal environment are usually shared within twins. Interestingly, twins with the same gestational age often differ in size at birth.14 Twin studies can, therefore, provide insights into the importance of confounding by these shared factors; eg, discordance of birth weight and other birth parameters that are evident in a within-twin pair analysis is more likely to reflect differences in placental function or blood and nutrient supply to the individual fetus within the multiple birth set rather than factors constant across the twin pair (eg, gestation).15 In the present study, we investigated the influence of birth parameters (eg, birth weight, birth length, and head circumference) on retinal vascular caliber in 266 twin individuals with known zygosity age ranged 5 to 14 years from the Twins Eye Study in Tasmania (TEST).

Of the 346 children, we excluded the following: 9 children without retinal vessel caliber measurement data for the right eye; 12 children (4 triplets) with no zygosity data available; 55 participants with missing birth parameter data; and 4 whose sibling had not been examined, leaving 266 twin children in the current study (49 MZ and 84 DZ (45 opposite-sex and 39 same-sex) twin pairs).

The TEST was approved by the ethics committees of the University of Tasmania, the Royal Hobart Hospital, and the Royal Victorian Eye and Ear Hospital. Research adhered to the tenets of the Declaration of Helsinki, and written informed consent was obtained from all of the participants’ legal guardians before examination.

**Birth Parameters Assessment**

Medical birth records, including mode of delivery, birth weight, gestational age, birth length, and head circumference, as well as Apgar scores at 1 and 5 minutes, were obtained from the TIHS. Information on birth order was identified by research staff who had access to the birth notification forms in the TIHS, and parents also gave details of birth order. Low birth weight was defined as <2500 g. The date of the last menstrual period was obtained from the mothers when they first presented at antenatal care. Last menstrual period was used to estimate gestational duration for mothers with the data available, otherwise gestation may have been estimated early in pregnancy with level of β-human chorionic gonadotropin or by ultrasound or clinical examination. Prematurity was defined as <37 weeks’ gestation duration.19

**Retinal Photography and Retinal Vascular Caliber Measurements**

All of the twins and non-twin siblings had 10° stereoscopic optic disc-centered photographs taken (Nidek fundus camera 3-Dx/F, Nidek) after dilatation of the pupils with cyclopentolate 1%. All of the fundus photographs were digitalized at high resolution (2102×1435 pixels, 2900 dots per inch, 36 bit color) using a Nikon CoolScan scanner (Nikon Corp.).

Retinal vascular caliber was measured at the Retinal Vascular Imaging Centre (University of Melbourne) with computer-assisted software (IVAN, University of Wisconsin) used in previous population-based studies.20,21 Two trained graders, masked to participant characteristics, performed the vessel measurements on the optic disc–centered image of both eyes for all of the participants in this study. The largest 6 arterioles and venules coursing through a zone between 0.5 and 1.0 disc diameters from the optic disc margin were measured. Images were considered ungradable if the largest 6 arterioles and venules could not be measured. Estimates were summarized as central retinal arteriolar equivalent and central retinal venular equivalent, representing the average diameter of arterioles and venules of the eye, respectively, using a modification of the Parr-Hubbard formulae as described by Knudtson et al.22

Remeasurement of 67 randomly selected retinal photos 3 months apart showed high intragrader reproducibility, with intraclass correlation coefficients (95% CI) of 0.95 (0.92 to 0.97) for central retinal arteriolar equivalent and 0.99 (0.98 to 0.99) for central retinal venular equivalent. The intergrader reliability was assessed in 52 randomly selected retinal images, and interclass correlation coefficient (95% CI) was 0.93 (0.88 to 0.96) for central retinal arteriolar equivalent and 0.98 (0.97 to 0.99) for central retinal venular equivalent, respectively.

**Zygosity Testing**

Blood or mouth swab samples were collected and DNA extracted in the TEST. Twin pair zygosity was confirmed by ≤12 highly polymorphic short tandem-repeats, with an accuracy of >99%.23

**Measurement of Other Variables**

In the TIHS, a standardized interview was conducted with parent(s) when the infant was 4 days old to collect information on sociodemographic factors, maternal smoking during pregnancy, antenatal alcohol consumption, and other antenatal factors.16 Home ownership and maternal and paternal education by time of birth were used as markers of socioeconomic status.

Blood pressure, childhood weight, and childhood height were obtained in the Childhood Blood Pressure Study. The blood pressure measurement has been described in detail previously.24 After ≥10 minutes of rest, each child’s blood pressure was measured 3 times in a seated position with an adult and pediatric vital signs monitor (Dinamap, Critikon). The mean of 3 measurements was computed for analyses. Mean arterial blood pressure was calculated as two thirds of the diastolic blood pressure plus one third of the systolic value. Early childhood body mass index (BMI) was calculated as kilograms per meter squared. Of the 266 participants included in our analysis, only 169 of them had blood pressure and 170 had childhood height and weight data available. Maternal pregnancy complications such as preeclampsia, gestational hypertension, and gestational diabetes were also obtained from the Childhood Blood Pressure Study. In the TEST, we assessed the optic disc area of each digitalized retinal stereophotograph using a standardized grading program.25

**Statistical Analysis**

Retinal arteriolar and venular calibers were the primary outcomes of interest and were approximately normally distributed. Both birth
parameters and retinal vascular caliber were analyzed as continuous measures.

We first performed standard linear regression with ‘twins as individuals’ initially controlling for age at examination and sex. Two multivariable linear regression models were then constructed. Model 1 adjusted for age, sex, maternal smoking, antenatal alcohol consumption, prematurity, and optic disc area measurement. Model 2 adjusted the variables included in model 1 and further controlled for mean arterial blood pressure and BMI. We also performed additional adjustment for child height. Factors related to parents’ socioeconomic status were also examined in the multivariable linear models in a stepwise backward method.

The main form of analysis consisted of linear mixed regression models, treating each twin pair as a cluster, which correct for correlated data. This model provided estimates of both within-pair ($\beta_w$) and between-pair ($\beta_b$) associations. Stratified analysis by zygosity was then conducted using linear mixed models. Covariates included in the model were based on models 1 and 2 and are detailed in the Table 3 footnotes.

We also used the national birth weight percentiles by gestational age for twins born in Australia to estimate birth weight for gestational age, which may allow an assessment of the relationship between birth weight and retinal vascular caliber that is independent of gestational age. Birth weight for gestational age was categorized into 8 different scores ($<5$th, $>5$th to 10th, $>10$th to 25th, $>25$th to 50th, $>50$th to 75th, $>75$th to 90th, $>90$th to 95th, and $>95$th percentile). Birth weight for gestational score under the 10th percentile was used as a proxy for fetal growth restriction.

Finally, we conducted analysis in the whole sample and separately by zygosity after excluding 45 opposite-sex DZ twin pairs to exclude a potential gender effect on birth size and cardiovascular risk factors. All of the $P$ values are 2 sided, and the above statistical analyses were performed for the right eye using Stata 10.0 (Stata Corp).

### Results

The mean birth weight of the study population was 2.52 (±0.56 SD) kg, mean birth length was 46.16 (±3.10 SD) cm, and mean head circumference at birth was 32.55 (±2.08 SD) cm. The mean retinal arteriolar and venular calibers were 168.41 (±14.82 SD) μm and 247.48 (±18.99 SD) μm, respectively. The demographic information, parental socioeconomic status, birth parameters, antenatal risk factors, maternal pregnancy complications, and childhood characteristics by zygosity are described in Table 1. In general, parents of MZ twins were more likely to own their home and less likely to be smokers during pregnancy as compared with parents of DZ twins. MZ twins were more likely to be of low socioeconomic status, birth parameters, antenatal risk factors, maternal pregnancy complications, and childhood characteristics by zygosity after excluding 45 opposite-sex DZ twin pairs to exclude a potential gender effect on birth size and cardiovascular risk factors. All of the $P$ values are 2 sided, and the above statistical analyses were performed for the right eye using Stata 10.0 (Stata Corp).

Table 1. Characteristics of MZ and DZ Twin Pairs

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>MZ Twins (n=98, 49 Pairs)</th>
<th>DZ Twins (n=168, 84 Pairs)</th>
<th>$P$†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographic factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>9.2</td>
<td>9.3</td>
<td>0.77</td>
</tr>
<tr>
<td>Male gender, %</td>
<td>43.0</td>
<td>53.3</td>
<td>0.10</td>
</tr>
<tr>
<td>Socioeconomic status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Home ownership, %</td>
<td>85.7</td>
<td>73.5</td>
<td>0.02</td>
</tr>
<tr>
<td>Maternal high school graduate, %</td>
<td>48.5</td>
<td>47.1</td>
<td>0.80</td>
</tr>
<tr>
<td>Paternal high school graduate, %</td>
<td>39.4</td>
<td>39.0</td>
<td>0.99</td>
</tr>
<tr>
<td>Birth parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low birth weight, %</td>
<td>52.0</td>
<td>34.7</td>
<td>0.006</td>
</tr>
<tr>
<td>Prematurity, %</td>
<td>47.0</td>
<td>37.1</td>
<td>0.12</td>
</tr>
<tr>
<td>Antenatal risk factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal smoking, %</td>
<td>18.0</td>
<td>29.4</td>
<td>0.04</td>
</tr>
<tr>
<td>Alcohol consumption, %</td>
<td>45.6</td>
<td>40.6</td>
<td>0.50</td>
</tr>
<tr>
<td>Maternal pregnancy complications</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preeclampsia, %</td>
<td>10.7</td>
<td>7.1</td>
<td>0.76</td>
</tr>
<tr>
<td>Birth weight for gestational score &lt;10th percentile, %</td>
<td>12.2</td>
<td>8.9</td>
<td>0.28</td>
</tr>
<tr>
<td>Caesarean section, %</td>
<td>32.7</td>
<td>36.3</td>
<td>0.46</td>
</tr>
<tr>
<td>Gestational hypertension, %</td>
<td>28.6</td>
<td>26.8</td>
<td>0.12</td>
</tr>
<tr>
<td>Childhood parameters*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI, kg/ m²</td>
<td>16.6</td>
<td>17.4</td>
<td>0.11</td>
</tr>
<tr>
<td>Mean arterial blood pressure, mm Hg</td>
<td>66.0</td>
<td>68.2</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Data show crude means or percentage.

*Data were available for only 28 MZ and 56 DZ twin pairs.

† $P<0.05$ represents a statistical difference in means or proportions, adjusted for age and sex, except for age and male sex.

### Study

The mean birth weight of the study population was 2.52 (±0.56 SD) kg, mean birth length was 46.16 (±3.10 SD) cm, and mean head circumference at birth was 32.55 (±2.08 SD) cm. The mean retinal arteriolar and venular calibers were 168.41 (±14.82 SD) μm and 247.48 (±18.99 SD) μm, respectively. The demographic information, parental socioeconomic status, birth parameters, antenatal risk factors, maternal pregnancy complications, and childhood characteristics by zygosity are described in Table 1. In general, parents of MZ twins were more likely to own their home and less likely to be smokers during pregnancy as compared with parents of DZ twins. MZ twins were more likely to be of low socioeconomic status, birth parameters, antenatal risk factors, maternal pregnancy complications, and childhood characteristics by zygosity after excluding 45 opposite-sex DZ twin pairs to exclude a potential gender effect on birth size and cardiovascular risk factors. All of the $P$ values are 2 sided, and the above statistical analyses were performed for the right eye using Stata 10.0 (Stata Corp).

Table 2. Overall Associations of Birth Weight, Birth Length, Head Circumference, and Gestation With Retinal Arteriolar Caliber in Childhood in the Twins Cohort

<table>
<thead>
<tr>
<th>Birth Parameters</th>
<th>$\beta$, (95% CI)</th>
<th>$P$*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight (per kg decrease)</td>
<td>$-4.53$ (−7.72 to $-1.33$)</td>
<td>0.006</td>
</tr>
<tr>
<td>Birth length (per 5-cm decrease)</td>
<td>$-4.79$ (−7.77 to $-1.81$)</td>
<td>0.002</td>
</tr>
<tr>
<td>Head circumference (per 2-cm decrease)</td>
<td>$-2.68$ (−4.43 to $-0.94$)</td>
<td>0.003</td>
</tr>
<tr>
<td>Gestation (per wk decrease)</td>
<td>$-1.02$ (−1.69 to $-0.36$)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

$\beta$ indicates common (twins as individuals) regression coefficient.

*Data are adjusted for age and sex.
Shorter birth length was associated with narrower retinal arteriolar caliber ($P$ for trend $= 0.004$). Birth length was not associated with retinal venular caliber ($P$ for trend $= 0.88$).

The results from the mixed linear regression models for the association between birth length and retinal arteriolar caliber fitted for the whole sample of twins (MZ+DZ) and separately by zygosity (MZ or DZ) are displayed in Table 3. Factors related to parents’ socioeconomic status were not included in the final model based on stepwise backward method. Both the within-pair ($-6.11; 95\% CI: -10.02 to -2.21$) and between-pair ($-4.48; 95\% CI: -8.06 to -0.91$) associations of birth length with retinal arteriolar caliber were significant and of similar magnitude, after adjusting for age, sex, maternal alcohol consumption and smoking, prematurity, and optic disc area. Both within-pair ($-9.73; 95\% CI: -14.68 to -4.47$) and between-pair ($-7.15; 95\% CI: -11.37 to -2.94$) effect strengthened after further adjustment for BMI and mean arterial blood pressure. Similarly, these associations persisted after adjustment for the factors listed in model 2 and child height ($\beta_w: -9.40; 95\% CI: -14.43 to -4.37; \beta_m: -7.11; 95\% CI: -11.44 to -2.78$), indicating that the association between birth length and retinal arteriole caliber was not merely because children with short birth length grew up to be shorter, and child height was not associated with retinal arteriolar caliber.

The within-pair association was of similar magnitude in DZ twins ($-8.99; 95\% CI: -15.19 to -2.79$) and MZ twins ($\beta_w: -9.35; 95\% CI: -19.15 to 0.46$), although the CIs for the latter were wider, reflecting that only 49 MZ pairs were available for analyses. No significantly different within-pair and between-pair effects were observed in the overall cohort or MZ and DZ twins, as indicated by the likelihood ratio test. Table 4 shows the results from the mixed linear regression models for the associations between birth weight or head circumference and retinal arteriolar caliber in the whole sample of twins and separately by zygosity. Birth weight was associated with retinal arteriolar caliber overall, but no association was evident in within-pair analyses. There was a strong between-pair association between birth weight and arteriolar caliber in the cohort (MZ+DZ) and among MZ twins. Analyses of the association with retinal arteriolar vasculature and head circumference (Table 4) were similar to those of birth length (Table 3), although the associations were of lower magnitude ($\beta_w: -2.41, 95\% CI: -5.09 to 0.28; \beta_m: -2.60, 95\% CI: -5.00 to -0.19$).

In this twin sample, we could not adequately analyze birth weight relative to gestational age, because few twins had low birth weight for gestational age (27 had birth weight for

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**Table 3. Associations Between Birth Length and Retinal Arteriolar Caliber in the Entire Sample of Twins and by Zygosity**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MZ+DZ (n=266)</th>
<th>MZ (n=98; 49 Pairs)</th>
<th>DZ (n=168; 84 Pairs)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta_m$</td>
<td>$-4.79 (-8.37$ to $-1.20)^*$</td>
<td>0.009</td>
<td>$-5.86 (-11.00$ to $-0.72)^*$</td>
<td>0.03</td>
</tr>
<tr>
<td>$\beta_w$</td>
<td>$-7.27 (-11.54$ to $-3.01)^*$</td>
<td>$&lt;0.001$</td>
<td>$-9.70 (-15.89$ to $-3.51)^*$</td>
<td>0.002</td>
</tr>
<tr>
<td>$\beta_m$</td>
<td>$-6.11 (-10.02$ to $-2.21)^*$</td>
<td>0.002</td>
<td>$-7.22 (-13.04$ to $-1.40)^*$</td>
<td>0.02</td>
</tr>
<tr>
<td>$\beta_w$</td>
<td>$-9.73 (-14.68$ to $-4.77)^*$</td>
<td>$&lt;0.001$</td>
<td>$-9.35 (-19.15$ to $0.46)^*$</td>
<td>0.06</td>
</tr>
<tr>
<td>$\beta_m$</td>
<td>$-4.48 (-8.06$ to $-0.91)^*$</td>
<td>0.01</td>
<td>$-5.98 (-11.06$ to $-0.90)^*$</td>
<td>0.02</td>
</tr>
<tr>
<td>$\beta_w$</td>
<td>$-7.15 (-11.37$ to $-2.94)^*$</td>
<td>$&lt;0.001$</td>
<td>$-9.92 (-17.75$ to $-2.08)^*$</td>
<td>0.01</td>
</tr>
<tr>
<td>Test for difference $^\ddagger$</td>
<td>0.09</td>
<td>0.43</td>
<td>0.61</td>
<td>0.93</td>
</tr>
</tbody>
</table>

Linear mixed regression models were used. Twins were treated as individuals, but the models accounted for clustering within a pair and allowed for different correlations in MZ and DZ pairs. $\beta_c$ indicates common (twins as individuals) regression coefficient.

*Model 1 was adjusted for age, sex, alcohol consumption, maternal smoking, and optic disc area.

†Model 2 was further adjusted for BMI and mean arterial blood pressure.

$^\ddagger$Data show the likelihood ratio test for the heterogeneity of the between- and within-pair effects.
gestational score under the 10th percentile). In addition, the majority of the twin pairs (89 of 133 pairs [67%]) were concordant for birth weight by gestational age scores (either within the same score or different by 1 score). Similar significant associations of shorter birth length with narrower retinal arteriolar caliber were shown in the analyses of the total cohort (MZ+DZ) and the DZ twin subgroups after excluding the opposite-sex DZ twins (data not shown).

### Discussion

In this population-based study consisting of Australian twin pairs, we found a consistent association between shorter birth length and narrower retinal arteriolar caliber. This remained evident even in a within-pair assessment of MZ twins. The between-pair effect of the association between shorter birth length and narrower retinal arteriolar caliber was also significant and of similar magnitude to the within-pair effect. Our study thus supports the hypothesis that microvascular arteriolar structure changes are associated with impaired fetal development and are likely related to individual specific factors (eg, different fetal nutrient supply lines), independent of possible vasculature determinants that would be constant across twin pairs (eg, maternal nutrition, smoking, gestational diabetes, and any unmeasured maternal factors). The within-pair association between birth length and arteriolar caliber was evident even among MZ twins alone; therefore, genetic differences in the inherited genome are unlikely to explain this association. With regard to birth anthropometry, head circumference, birth length, and birth weight are often the set for primary exposure measures. Both the between-pair and within-pair associations of head circumference with arteriolar caliber were of borderline significance, and although an overall and between-pair association between birth weight and retinal arteriolar caliber was evident, a within-pair association between birth weight and retinal arteriolar caliber was not detected in this cohort, which was not expected. We also found no associations between birth parameters and retinal venular caliber.

To our knowledge, this is the first twin study to investigate the association of birth parameters with retinal vascular caliber. Twin studies provide insights into whether the association between birth size and retinal vascular structure reported in previous studies in children and adults is because of shared environment (eg, maternal factors), common genetic factors, or other pathways associated with common diseases in later life. Both the within-cohort and between-pair effect of the association between shorter birth length and narrower arteriolar caliber. This remained evident with increased magnitude in models with further adjusting for mean arterial blood pressure, BMI, and child height, reflecting that it was not the individual-specific differences in these later child factors that contributed to the association between shorter birth length and narrower arteriolar caliber.

Controlling for optic disc area in our analysis did not alter the positive association between birth length and retinal arterioles, suggesting that image magnification–related factors are unlikely to confound the associations. Our study may also provide an explanation for recent findings from a large twin study (16 265 same-sex twins), which identified an association of decreased birth weight and an increased risk of hypertension that was not confounded by common genetic factors, familial environment, or common risk factors of hypertension in adulthood (eg, BMI). Our findings suggest that this association may be mediated by the microcirculation.

We have shown in previous studies that retinal arteriolar narrowing is a preclinical marker of hypertension and predicts future blood pressure elevation, subsequent coronary heart disease, and stroke. Our finding of shorter birth length with narrower retinal arteriolar caliber adds further support to the fetal origins of cardiovascular disease, suggesting an adverse effect of fetal growth on microvascular structure, which may ultimately lead to increased risk of common diseases in later life. Both the within-cohort and
within-pair associations between birth length and retinal arteriolar caliber were significant and were of similar magnitude, indicating that these associations were, at least in part, independent of genetic and shared environmental factors and that factors specific to the individual fetus, such as individual fetal nutrition and related growth in utero, must be involved. Although the nature of our study does not confirm a causal relationship, it seems reasonable to infer from these data that differences in the fetoplacental unit may contribute to the relation of fetal growth with later microvascular abnormalities. Although the sample size was not large enough to allow a detailed examination of birth weight by gestational age, the finding of within-pair associations of birth length and head circumference with retinal arteriolar caliber indicates that there is an effect of birth size independent of gestation duration. That is, within twin pairs (which is standardized for gestation), the effect of birth size on retinal caliber remained evident. This is consistent with other research on larger samples of mainly singletons that indicates that the effect of birth weight on subsequent ischemic heart disease risk is also largely attributed to birth weight and not gestational age. The relatively small sample size and the issue of statistical power in this study highlight the need for larger twin studies of more precise analysis in this area. Because our cohort was not large, some of the null findings reported may partially reflect inadequate statistical power, although the lack of influence of birth parameters on retinal venular caliber is consistent across all twin subtypes and is in agreement with estimates from singletons and with previous observations of no association of venular caliber with blood pressure. The association between gestational duration and retinal arteriolar caliber in this study was not independent of birth weight. This is of interest because twins have a higher prevalence of prematurity, and some studies of singletons reported an inverse association between prematurity and blood pressure. The mechanisms underlying prematurity are still unclear; it has been suggested that elevated maternal corticotropin-releasing hormone and cortisol, maternal prenatal anxiety and depression, and biochemical and psychosocial variables may be potential predictors. Our finding provides further evidence that, although maternal factors associated with gestation length may differ between twins and singletons, it is likely that these findings, particularly for the within-pair assessments, which have standardized for gestation, are applicable beyond twins with the gestation profile of this cohort.

Strengths of the study include validated documentation of birth parameters, standardized measurement of retinal vascular caliber, and detailed information on a variety of confounders. The conclusions regarding associations or lack of them must be drawn with caution for several reasons. First, residual confounding may partly explain some of the associations, particularly for the between-pair assessments. Our analyses also could not rule out the possibility that the within-pair birth length and retinal arteriolar caliber association observed may also reflect later individual-specific epigenetic change or postnatal environmental influences. Second, regression analysis does not provide full information as compared with a full genetic analysis, and the number of children with complete measurements of all of the potential confounders in our study is small and does not allow further meaningful genetic analysis. We have examined additional twins who were not in the TIHS and, thus, do not have the same data on birth parameters readily available; however, the total set of 1000 twins should allow further genetic analysis in the future. Third, proper information on chorion type was not available to assist our interpretation. Finally, information regarding some of the maternal pregnancy complications, such as oligohydramnios and intrahepatic cholestasis, was not available in the study. However, this point highlights the strength of our approach, ie, the within-pair association of birth length with retinal arteriolar caliber that is clearly evident cannot be attributed possibly to measured or unmeasured factors acting at the maternal level.

**Perspectives**

The findings here support that shorter birth size, such as birth length, a consequence of adverse fetal development, may be associated with narrower retinal arterioles and, thus, altered childhood microvasculature. The strength of this study is the ability to determine that this association cannot be explained by confounders at the level of the family (eg, socioeconomic status), mother (eg, maternal nutrition), or innate genotype. This report adds to the growing literature that suggests that fetal programming in utero may be a long-term determinant of human vasculature development, with implications for later cardiovascular disease risk.

**Sources of Funding**

The research reported in this article was supported by Ophthalmic Research Institute of Australia, Foundation for Children Australia, National Health and Medical Research Council, Canberra Australia (project grant 350415), and the Clifford Craig Medical Research Trust. C.S. is the recipient of a National Health and Medical Research Council postgraduate scholarship. D.A.M. is the recipient of the Pfizer Australia Senior Research Fellowship.

**Disclosures**

None.

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Hypertension. 2009;53:487-493; originally published online January 12, 2009;
doi: 10.1161/HYPERTENSIONAHA.108.125914

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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