Preterm Birth Attenuates Association Between Low Birth Weight and Endothelial Dysfunction

Mikael Norman, MD, PhD; Helena Martin, MD, PhD

**Background**—Low birth weight predisposes to later coronary disease. To further elucidate the mechanisms behind this association and their timing, vascular endothelial function—a key factor in early pathophysiology of atherosclerosis—was studied in 54 infants born either before the third trimester or at term.

**Methods and Results**—All subjects were studied at 3 months of postnatal age. A laser-Doppler technique was used to measure skin perfusion before and after transdermal iontophoresis of acetylcholine (ACh; an endothelium-dependent vasodilator). In infants born at term (n = 19; birth weight range: 2230 to 4205 g), maximum perfusion after ACh was 109±8 perfusion units (PU, mean±SEM) in normal—birth weight controls compared with 56±13 PU among those who had been small for gestational age at birth (P<0.01). In infants born preterm (n = 35; birth weight range, 722 to 1868 g), ACh induced similar perfusion responses among subjects appropriate for gestational age (113±16 PU) and in those small for gestational age at birth (109±19 PU).

**Conclusions**—Impairment in human endothelial function associated with low birth weight occurs or emerges late in life.14,16,17 These factors would be abruptly altered by measures that may contribute to this finding. *(Circulation. 2003;108:996-1001.)*

**Key Words:** acetylcholine ■ coronary disease ■ endothelium ■ vasodilation

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Systemic dysfunction of the vascular endothelium precedes atheroma formation and coronary heart disease.1–4 Already as neonates, subjects with low birth weight (LBW) exhibit endothelial dysfunction that persists into childhood and adult life.5–11 The prevalence of endothelial dysfunction among healthy young subjects may be an expression of early atherogenic susceptibility, and this could be an important link in understanding the global epidemiological associations between LBW and increased risk of coronary heart disease.12–14 The highest risks for endothelial dysfunction and later coronary disease have been reported in thin babies born at term, pointing to the third trimester as a particularly sensitive time period for divergent vascular development and possible adverse effects in the fetus.5,8,14,15 Therefore, studies of subjects born before the third trimester may help in the search for underlying mechanisms and the timing of their emergence. In utero, environmental factors such as fetal malnutrition, elevated maternal blood lipids, and increased oxidative stress may leave a vascular imprint that has been suggested to increase the risk for coronary heart disease later in life.14,16,17 These factors would be abruptly altered by preterm delivery, and this may have long-term significance for the vascular system. A prior study showed normal endothelial function in 15-year-old subjects with LBW attributable to preterm delivery alone, whereas intrauterine growth retardation and preterm delivery was associated with endothelial dysfunction.18 Increased blood pressure, which is another factor associated with endothelial dysfunction and increased cardiovascular risk, has also been found in young adults who had been born preterm.19–22

At this time, there are no studies on endothelial function in preterm infants, ie, at an age at which adult confounding factors cannot affect the results. To test the hypothesis that different growth and environmental factors during the third trimester may affect vascular function already in infancy, we measured endothelium-dependent vasodilation in 3-month-old infants born preterm or at term, appropriate for gestational age (AGA), or small for gestational age (SGA).

**Methods**

**Subjects**

A group of 86 consecutively live-born singletons without chromosomal abnormality or congenital infection and with a gestational age of ≥30 weeks had been treated in our neonatal units in Stockholm. Of these, 12 infants died, 16 were excluded because of ongoing morbidity (chronic lung disease, heart failure, major malformations), and 11 were lost to follow-up. Among the remaining 47 families, 33 agreed to participate in the present study, which was approved by the local ethics committee. One pair of dichorionic same-sex twins discordant in birth weight (one AGA and the other SGA) was included. Gestational age was determined by early routine ultrasound. Infants were characterized according to reference data for the Swedish population23 as AGA (birth weight is mean ±2 SD; n = 17) or SGA (birth weight less than mean ±2 SD; n = 18). Mothers of preterm SGA infants were more often primigravida and had a higher incidence of preeclampsia compared with that of mothers of preterm...
AGA infants (Table 1). There were no statistically significant differences between the 2 groups with regard to use of antenatal steroids for induction of fetal lung maturation. Sex and neonatal morbidity (including low Apgar scores, need for ventilator treatment, systemic infections, patent ductus arteriosus, necrotizing enterocolitis, and retinopathy of prematurity) were equally distributed in the 2 groups of infants. At the time of the present study, all preterm infants had been discharged from the hospitals, and other than taking medications. The majority of infants were exclusively breast-fed (n=18), some infants were partly breast-fed (n=13). As controls, 19 breast-fed singletons (10 AGA and 9 SGA) delivered at term were included. No term infant had neonatal complications, congenital infection, chromosomal abnormality, or malformations. In the term SGA group, 1 mother showed signs of preeclampsia at the time of delivery.

Before pregnancy, all mothers-to-be were healthy nonsmokers who were not taking any medications or using special diets. No woman showed signs of glucose intolerance during pregnancy. All parents were interviewed to obtain a family history of non–insulin-dependent diabetes mellitus, myocardial infarction, stroke, hypertension, and hyperlipidemia among their first-degree relatives (Table 1).

Vascular Studies
Vascular measurements were performed once at a postnatal age of 3 months. For preterm infants, this corresponded to a postconceptional age of 40±0.2 weeks. A laser-Doppler (LD) instrument (Periflux 4001, Perimed AB) and a micropharmacology system were used to measure skin perfusion before and after transdermal delivery of acetylcholine (ACh), an endothelium-dependent vasodilator. The LD signal is proportional to the number and velocity of moving blood cells in the illuminated superficial skin microvessels and is expressed in perfusion units (PU) of output voltage (1 PU=10 mV). The temperature of the LD probe facing the skin was standardized to 32°C. After adjusting to the room temperature (22°C) for 20 minutes, the vascular studies were performed with the infant lying down in a cot. The combined drug-delivery and LD probe was fixed to the dorsalis aspect of the hand by double-adhesive tape. Basal skin perfusion was recorded for 2 minutes, after which 2% ACh (acetylcholine chloride, Sigma-Aldrich) was transferred across the skin by iontophoresis (anodal current of 0.1 mA for 20 seconds, repeated 5 times). The combined drug-delivery and LD probe was fixed to the illuminated superficial skin microvessels and is expressed in perfusion units (PU) of output voltage (1 PU=10 mV). The temperature of the LD probe facing the skin was standardized to 32°C. After adjusting to the room temperature (22°C) for 20 minutes, the vascular studies were performed with the infant lying down in a cot. The combined drug-delivery and LD probe was fixed to the dorsalis aspect of the hand by double-adhesive tape. Basal skin perfusion was recorded for 2 minutes, after which 2% ACh (acetylcholine chloride, Sigma-Aldrich) was transferred across the skin by iontophoresis (anodal current of 0.1 mA for 20 seconds, repeated 5 times at 60-second intervals). Basal perfusion and changes in response to ACh were measured as area under the curve. Details of the methodology have been reported and discussed previously. The arterial blood pressure was measured with a standard oscillographic sphygmomanometer. The given blood pressures represent the mean of 3 measurements.

**Statistical Analyses**
Results are presented as mean±SEM or proportions. Student’s t test, χ², and ANOVA were used to test for group differences. Correlation coefficients were calculated to detect possible associations, and regression analyses (linear and stepwise) were used to describe relations between variables. A 2-factor ANOVA model for repeated measurements was used to assess perfusion responses to ACh in SGA and AGA infants. A probability value of 0.05 was considered significant, and the sample size allowed for detection of a mean difference of ≥1 SD between preterm groups (power=0.80).

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**TABLE 1. Subject Data**

<table>
<thead>
<tr>
<th></th>
<th>3-Month-Old Infants</th>
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<td></td>
<td>Preterm at Birth</td>
<td>Term at Birth</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SGA (n=18)</td>
<td>AGA (n=17)</td>
<td>SGA (n=9)</td>
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<td>Age, y</td>
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<td>33±0.9</td>
<td>32±1.2</td>
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<td>Antenatal steroid treatment</td>
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<td>14</td>
<td>...</td>
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<td>Data on infants at birth</td>
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<td>Girls/boys, n</td>
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<td>8/9</td>
<td>6/3</td>
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<td>28.5±0.3</td>
<td>39.6±0.4</td>
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<tr>
<td>Birth weight, g</td>
<td>966±48‡</td>
<td>1346±56</td>
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<td>Birth length, cm</td>
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<tr>
<td>Weight, g</td>
<td>2866±172</td>
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<tr>
<td>Feeding: breast milk/mixed/formula, n</td>
<td>9/3/6</td>
<td>8/2/7</td>
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</tr>
</tbody>
</table>

Data are given as mean±SEM or number of subjects. NIDDM indicates non–insulin-dependent diabetes mellitus.

*P<0.05 vs mothers of preterm infants with appropriate birth weight.
†P<0.05 vs all mothers.
‡P<0.05 vs infants with appropriate birth weight and similar gestational age.
Results

Endothelial Function, Preterm Delivery, and Birth Weight

From an overall mean SEM basal skin perfusion of 17±2 PU, ACh-induced vasodilation resulted in a peak perfusion value of 102±9 PU. Basal skin perfusion did not differ significantly between preterm and term infants, SGA or AGA at birth. In infants born at term, endothelium-dependent vasodilation developed significantly more slowly and the magnitude of the response was lower in SGA than in AGA controls (P<0.01) (Figure). After ACh administration, the peak perfusion was 56±13 in term SGA compared with 109±9 PU in term AGA infants (P<0.05). By contrast, endothelium-dependent vasodilation in preterm infants did not differ in relation to AGA or SGA. The peak perfusion was 109±19 in preterm SGA and 113±16 PU in preterm AGA infants (Figure and Table 2). According to stepwise regression, there were no significant relationships between gestational age or weight at study and endothelium-dependent vasodilation.

Blood Pressure and Anthropometric Data

The blood pressure was within normal limits in all infants, defined as systolic blood pressure <105 mm Hg. The mean systolic blood pressure at 3 months of postnatal age was 82±1; mean diastolic pressure, 43±1 mm Hg. Entering gestational age, SGA or AGA, and present weight as independent variables in a stepwise regression model, systolic and diastolic blood pressures were found to be associated with body weight at the time of the study (1.9 mm Hg in systolic pressure and 1.6 mm Hg in diastolic pressure per 1-kg increase in body weight, r=0.39 and 0.48, respectively; P<0.01) (Table 2).

Influence of Family History, Preeclampsia, and Sex

Endothelium-dependent vasodilation did not differ significantly in relation to family history of cardiovascular disease/non–insulin-dependent diabetes mellitus or sex. The Ach-induced peak perfusion was 105±16 PU in preterm infants of preeclamptic mothers and 117±22 PU in those without a history of preeclampsia (P=0.65).

In infants born at term, but not preterm, systolic blood pressure was on average 2.3 mm Hg higher in infants with positive family history of cardiovascular disease than in those without such family history (P<0.05). Infant blood pressures did not vary in relation to maternal preeclampsia, and apparent sex differences disappeared once body weight at the time of the study was added as an independent variable.

Influence of Postnatal Growth and Diet in Preterm Infants

There was a weak inverse correlation between weight gain in preterm infants and endothelium-dependent vasodilation (r=-0.37, P<0.05), whereas endothelial function did not differ between those fed breast milk and formula. Systolic blood pressure also correlated to weekly weight gain in preterm infants (r=0.39, P<0.05) but not to feeding practices.

There were no other statistically significant associations between endothelial function, blood pressures, and independent variables.

Discussion

The present study confirms that healthy SGA subjects born at term show impaired vascular endothelial function already in infancy. Several previous studies, in which vasodilation was measured both in small resistance vessels and in larger conduit arteries in term neonates, children, and adults, show similar findings. Lower endothelium-dependent vasodilatory capacity has not been confined only to those unusually small at birth, as in the present study with birth weights below −2 SD. In both pediatric and adult studies with large numbers and sufficient power, a gradual increase in large-artery endothelium-dependent vasodilation has been found over the whole range, from low to high birth weight. The magnitude of this effect has been reported to be in the same order as that caused by smoking.
Different explanations for the association between LBW, endothelial dysfunction, and increased risk for later cardiovascular disease have been proposed. The fetal origins of adult disease hypothesis suggests that adverse intrauterine environmental influences during pregnancy cause long-lasting alterations in vascular function and structure, which may account for some of the morbidity and mortality in coronary heart disease in the later adult population. The exact mechanisms for such fetal programming are still largely unknown with regard to endothelial dysfunction, but potentially modifiable factors—such as alterations in maternal—fetal nutrition and lipid profile, fetal oxidative stress, and immune status—may be involved. Ex utero life offers an escape from such proposed adverse influences in utero. This may have contributed to our findings in very preterm infants in whom endothelial function, as opposed to the situation in full-term infants, did not vary in relation to birth weight.

The fetal insulin hypothesis, on the other hand, suggests that a genetic predisposition of insulin resistance explains LBW, limited vasodilatory function in infancy, and later increased risk of diabetes and cardiovascular disease in adults. Insulin is a major fetal growth factor, and monogenic defects causing insulin resistance or lowered insulin secretion have been shown to result in smaller babies. Abnormal vascular development in fetal life and childhood is postulated to occur on a basis of genetically determined insulin-resistant endothelium, incapable of normal NO formation and vasodilatory capacity. Consistent with this concept, the present study and a previous study showed that term SGA babies have little if any response to ACh. However, in postconception same-age infants who had been living outside the womb 3 months before term, endothelial function did not vary in relation to birth weight. It is possible that a genetically determined endothelial dysfunction may emerge later in SGA preterm babies than in those born small at term. The diversities between term and preterm infants could also reflect differences in vascular programming related to intra- or extrauterine life during the third trimester. Programming does not exclude genetic contributions; in fact, it may represent different environmental interactions with genes regulating early vascular differentiation and signaling. In this context, early dietary factors may play a role after preterm delivery. Recently published data showed that variations in infant feeding practices influence later arterial function and blood pressure. In adolescence, ex-preterm subjects who in infancy had been randomized to receive banked breast milk in the nursery had lower systolic blood pressure than did those who had been provided with formula.

The association between birth weight and endothelial function has previously been studied in ex-preterm adolescents. In that study, brachial artery flow-mediated dilation (FMD) was used, a method that correlate to ACh-induced small-vessel perfusion increase in the hand (M. Norman, MD, PhD, unpublished data, 2003). Ex-preterm subjects who had been SGA at birth had, on average, 0.03 mm lower FMD (mean arterial diameter, 3.5 mm), but similar reactive hyperemia, compared with that for adolescents born preterm with appropriate birth weights. The present study was not powered to detect such differences. It is also important, however, that those ex-preterm adolescents had been born significantly closer to term than were the infants included in our study. If adverse fetal influences during the third trimester contribute to later vascular dysfunction, SGA subjects born moderately preterm, but well within the third trimester, would be expected to show some degree of vascular dysfunction at later follow-up.

In developed countries, preeclampsia dominates as a cause of early fetal growth retardation and preterm delivery. This was reflected by a high proportion (9/18) of preeclamptic mothers to preterm SGA infants. As there is evidence that preeclampsia is a vascular disorder involving endothelial dysfunction, SGA infants of preeclamptic mothers may represent a different subgroup from the others studied. However,
in the present study the endothelial function was similar in preterm SGA infants with preeclamptic mothers and in those without such history.

Vasodilatation induced by ACh is a well-established method to evaluate endothelial function. The vascular response to ACh is impaired or lost once the endothelium is damaged or removed. Among noninvasive techniques for vascular studies, the LD method used herein provides to date the only alternative for pharmacological challenges in human infants. We have previously ruled out the possibility that nonspecific neurogenic skin vasodilation occurs because of iontophoretic current alone. In addition, LD output among infants does not vary in relation to skin thickness, and there is no reason to believe that the ACh doses varied among the groups of infants in the present study. Moreover, at 3 months after birth, the normal postnatal rearrangement of skin microvasculature has been reported to be completed and the skin capillary density to be unrelated to birth weight.

Therefore, our measurements in infants born at term most likely reflect variations in microvascular function, involving one or several of the endothelial systems releasing vasodilators such as NO, endothelial-derived hyperpolarizing factor, and/or prostanoids. Early endothelial dysfunction may precede disturbances in capillary recruitment: In prepubertal schoolchildren, low skin capillary recruitment during postocclusive reactive hyperemia has been reported to be associated with LBW.

So far, there is no information on the structure of the cutaneous microvascular bed in very preterm infants. These babies are at risk for microangiopathy in another vascular bed, ie, in the retina (retinopathy of prematurity). Abnormal retinal vascularization in preterm infants may be a general vascular phenomenon, and associations between preterm delivery, microvascular abnormalities in the eye, and elevated blood pressure have been reported in adults.

Although we found no association between retinopathy of prematurity and vascular responses, it cannot be excluded that postnatal microvascular proliferation or rarefaction in the skin of preterm infants could have contributed to larger variations and attenuation of the relation between ACh response and birth weight.

Our observations provide further evidence for a link between intrauterine growth restriction and endothelial dysfunction. Birth before the third trimester attenuated this functionality than those occurring in utero. Although this must not be misunderstood as an argument for promoting therapeutic preterm delivery, it adds new aspects to the discussion on the optimal time for delivery of a growth-retarded fetus. Our findings also have implications for further research on perinatal strategies to prevent increased public health risks in humans.

Acknowledgments

This study was supported by the Swedish Research Council (project No. 71P-14158), Swedish Heart Lung foundation, Swedish Society of Medicine, Karolinska Institute research foundations, and Sällskapet Barnavård, Karolinska Hospital.

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Circulation. 2003;108:996-1001; originally published online August 18, 2003; doi: 10.1161/01.CIR.0000085069.09770.3D
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

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