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Impaired Endothelial Function and Increased Carotid Stiffness in 9-Year-Old Children With Low Birthweight

Helena Martin, MD; Jie Hu, MD, PhD; Gerhard Gennser, MD, PhD; Mikael Norman, MD, PhD

Background—Low birthweight (LBW) has been associated with an increased incidence of adult cardiovascular disease. Endothelial dysfunction and loss of arterial elasticity are early markers of hypertension and atherosclerosis. We studied the prevalence of these markers in 44 healthy, prepubertal (age 9 ± 1.3 years) children, 22 with LBW for age.

Methods and Results—Endothelial function in skin was tested with the local application of acetylcholine (inducing endothelium-dependent vasodilation) and nitroglycerin (endothelium-independent vasodilation), and local perfusion changes were measured with the laser Doppler method. The elastic properties of the abdominal aorta and common carotid artery were measured with an ultrasonic vessel-wall tracking system. Endothelium-dependent vasodilation was lower in children with LBW (88 ± 33 perfusion units [PU]) than in normal-birthweight controls (133 ± 34 PU, $P < 0.001$). There was no difference in aortic or carotid elasticity between the 2 groups, but a negative correlation was found between birthweight and stiffness of the carotid artery wall ($r = -0.45$, $P < 0.01$). Endothelium-independent vasodilation and blood pressure were similar in the 2 groups.

Conclusions—Schoolchildren with a history of LBW show impaired endothelial function and a trend toward increased carotid stiffness. These findings may be early expressions of vascular compromise, contributing to susceptibility to disease in adult life. (*Circulation*. 2000;102:2739-2744.)

Key Words: endothelium ■ pediatrics ■ arteries ■ hypertension ■ elasticity

Since the first epidemiological reports a decade ago,^{1,2} the association between low birthweight (LBW) for age and increased incidence of adult cardiovascular disease has been repeatedly confirmed.^{3,4} Poor size at birth also relates to the unfavorable constellation of high blood pressure, glucose intolerance, and hyperlipidemia later in life.⁵⁻⁷ Considering LBW as one expression of a risk syndrome, studies of the functional characteristics of the vascular tree in these children offer an opportunity to further clarify the underlying susceptibility long before structural vascular disease has been established. Endothelial dysfunction and increased arterial stiffness have been reported to be early markers of accelerated vascular aging in prediabetic and prehypertensive states in young adults⁸⁻¹⁰ and have also been found in children with hypercholesterolemia or a family history of hypertension.¹¹⁻¹³ The first studies on the prevalence of these markers in LBW children have recently been presented. The aortic elasticity in prepubertal schoolchildren with intrauterine growth retardation was normal,¹⁴ whereas endothelium-dependent brachial artery dilation in LBW children and young adults^{15,16} and endothelium-dependent microvascular vasodilation in LBW neonates¹⁷ were impaired.

We tested the hypothesis that the impairment of vasodilation in LBW newborn infants persists and can be detected in healthy, prepubertal children. Because there seem to be no

reports in which the vasodilatory capacity was evaluated in small and large arteries of the same LBW children, we studied microvascular vasodilation in the skin and pulse-synchronous vasodilation in segments of 2 large arteries that are commonly affected by later atherosclerosis, ie, the aorta and common carotid artery. The data presented here suggest that LBW schoolchildren have an impaired vasodilatory capacity in small and large arteries and that endothelium-dependent mechanisms and mechanical properties of the large-artery vessel walls are involved.

Methods

Subjects

Forty-four healthy, prepubertal children (21 boys) were studied at a mean age of 9.0 ± 1.3 years. All children were recruited from the same catchment area and had been born at Danderyd Hospital in northern Stockholm. All parents were clinically healthy and non-smokers. Factors in addition to LBW that could have affected fetal-neonatal vascular development, such as a history of multiple pregnancies, maternal diabetes, hypertension, preeclampsia, maternal smoking or medication during the index pregnancy, preterm delivery, neonatal asphyxia, malformations, chromosomal disorders, or congenital infection excluded admission to this study. Moreover, children with present or previous cardiovascular disease and chronic illness or medication were not included. With these exclusion criteria and after examination of the local neonatal database from 1988 to 1991, we found 32 subjects who had been born at term with LBW for

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TABLE 1. Characteristics of the Study Groups

	Low Birthweight (n=22)	Normal Birthweight (n=22)	P
Mothers			
Before pregnancy			
Age, y	30±5	31±3	0.32
Parity	0.5±0.7	0.7±0.7	0.39
Positive family history,* n	7/22	6/22	0.74
Length, cm	166±5.2	167±5.9	0.51
BMI, kg/m ²	21.7±1.6	22.1±1.3	0.42
During pregnancy			
Weight gain, kg	11.8±3.4	12.3±3.0	0.69
SBP, mm Hg			
First trimester	113±11	110±7	0.36
Third trimester	120±10	115±5	0.03
DBP, mm Hg			
First trimester	68±8	65±6	0.21
Third trimester	77±7	69±6	<0.001
Proteinuria or glucosuria, n	0/20	0/20	1.00
Children			
Boys, n	10/22	11/22	0.76
Length of gestation, wk	39±2	39±1	0.18
Birthweight, g	2188±224	3455±458	<0.001
Birthweight deviation, %	-34±5.0	1.7±5.1	<0.001
Birth length, cm	45.6±1.9	50.6±1.7	<0.001
Head circumference, cm	32.8±1.1	34.6±1.1	<0.001
Present age, y	8.9±1.4	9.0±1.2	0.91
Present weight, kg	29.3±5.1	32.3±5.8	0.08
Present length, cm	133±9.0	137±8.9	0.23
BMI, kg/m ²	16.4±1.8	17.1±1.4	0.13

SBP indicates systolic blood pressure; DBP, diastolic blood pressure. Data are mean±SD or number of subjects.

*Positive family history of cardiovascular disorder, diabetes, or hyperlipidemia.

age (<mean -2 SD). In this group, 4 families declined to participate in the study, and 6 had moved from the catchment area. These 10 LBW subjects who were lost to follow-up had birthweights (2260±131 g) that did not differ from the remaining 22 LBW children (2188±224 g) included in the study. Twenty-two normal-birthweight children (3455±458 g, $P<0.001$, Table 1) with similar age and sex distribution were studied as controls. There was no overlapping in birthweight between the 2 groups. In addition to birthweights, we calculated the difference between actual and expected birthweights for the particular gestational age, expressed as percentage of the expected birthweight. The deviations in birthweight were -34±5.0% in LBW subjects and 1.7±5.1% in controls ($P<0.001$, Table 1). To identify leanness at birth (disproportional fetal growth), we studied the relation weight-for-length, both variables given as SD scores and normalized for gestational age and sex.¹⁸ Using a linear regression model for these SD scores (intercept=0, regression coefficient=0.7152) based on data from the Swedish Medical Birth Register, we defined leanness at birth as a weight-for-length relation <-2 SD.¹⁸ Among LBW subjects, leanness at birth was found in 14 of 22, whereas the remaining 8 of 22 LBW children were classified as proportionately small at birth. In the control group, no subject was lean at birth.

There were no differences in age, weight, length, weight gain, or parity between the 2 groups of mothers at the index pregnancy. In the third trimester of the index pregnancy, the mean systolic and

diastolic blood pressures were higher in mothers of LBW infants ($P<0.05$, Table 1). The gestational age had been estimated by early routine ultrasound in all pregnancies. Because of a low symphysis fundic height, a second antenatal ultrasound was performed during the third trimester in 10 of 22 LBW pregnancies, and the fetal growth was impaired in 6 of 10 subjects. Because of maternal concerns, a second antenatal ultrasound was performed during the third trimester in 4 of 22 pregnancies in the control group and was normal in all 4 cases. Four LBW infants were treated for transient neonatal hypoglycemia, but the remaining infants had an uncomplicated neonatal period.

Informed consent was obtained from the children and their parents before the investigation, and the study protocol was approved by the local Ethics Committee at Karolinska Hospital. Both parents were interviewed about a possible family history of diabetes, myocardial infarction, stroke, hypertension, and hyperlipidemia among their first-degree relatives (Table 1).

Vascular Studies

A laser Doppler (LD) instrument (Periflux 4001, wavelength 780 nm) and a micropharmacology system were used to measure perfusion changes during vascular provocations in the dorsal hand skin (Perimed AB). The LD signal is proportional to the number and velocity of moving blood cells in illuminated superficial skin microvessels. The LD output is semiquantitative and expressed in

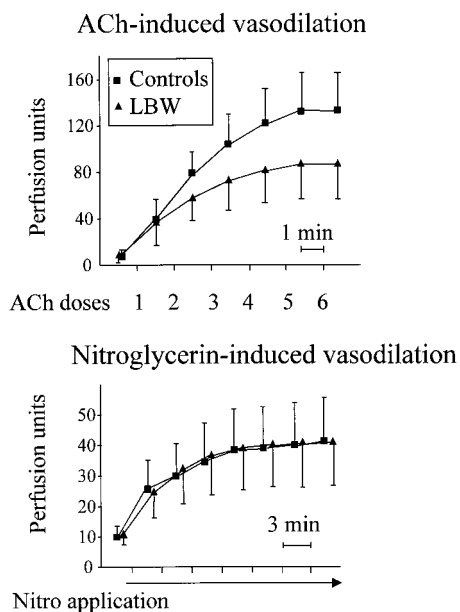


Figure 1. Skin perfusion in response to ACh (endothelium-dependent vasodilation) or nitroglycerin (endothelium-independent vasodilation) in schoolchildren with low ($n=22$) and normal birthweight ($n=22$).

perfusion units of output voltage (1 perfusion unit [PU]=10 mV). The temperature of the LD probe facing the skin could be changed and was standardized to 32°C. After adjustment to room temperature ($23\pm 1^\circ\text{C}$) for 20 minutes, the vascular studies were performed with the child lying supine with both arms beside the body.

To study endothelium-dependent vasodilation, basal perfusion was recorded for ≥ 2 minutes, after which 2% acetylcholine (ACh, acetylcholine chloride, Sigma-Aldrich AB) was transferred across the skin by iontophoresis (anodal current of 0.1 mA for 20 seconds). A stimulus-response curve was produced by repeating iontophoretic stimulation 6 times at 60-second intervals (Figure 1). The anodal current alone does not elicit vascular responses, and the CV was 18% for repeated measurements of maximum perfusion change after ACh stimulation.⁹

To study endothelium-independent vasodilation, an exogenous NO donor was applied to the contralateral dorsal hand skin. Nitroglycerin cream (glyceryl nitrate 1% in Essex cream, 0.07 mL) was placed in the drug-delivery chamber in the head of the LD probe and attached to the skin for 30 minutes. Unlike ACh provocations, nitroglycerin was allowed to diffuse through the skin without the use of an electrical charge (Figure 1). In a pilot study of 10 healthy volunteers, we found a significant increase in local blood perfusion but no systemic cardiovascular reactions in response to the same small amounts of nitroglycerin as used in the present study. Thus, blood pressure, heart rate, and skin perfusion in the contralateral hand did not change during the 30 minutes of drug application (ANOVA: $P=0.72$ to 0.75). The CV was 9.2% ($n=8$) for repeated measurements of maximum local perfusion change after nitroglycerin stimulation.

The dynamic properties of the large arteries were studied by measuring the abdominal aorta (3 to 4 cm above its bifurcation) and the left common carotid artery (1 to 2 cm proximal to its bifurcation). The methodological details have been described elsewhere.¹⁹ Briefly, a high-fidelity system comprising a real-time B-mode ultrasonic scanner (Hitachi EUB 240), 2 pairs of electronic echo-trackers ([TM]Diamove/[TM], Teltec), and a desktop computer was used to detect and measure the pulsatile movements of the vessel walls. From these waveforms, the following variables were measured: the end-diastolic diameter (mm), the pulse amplitude of the diameter (mm), and the maximum incremental velocity of the diameter during the systolic phase (MIV, mm/s). These data and those of simulta-

neously measured blood pressures in the brachial artery were computed to yield the stiffness index (β)²⁰ of the arterial wall at the selected sites. The mean value of 3 recordings, each comprising 6 to 10 consecutive heart cycles, was taken as the subject's reading. The repeatability of the method has been shown to be satisfactory.⁹

The heart rate and brachial blood pressure were recorded intermittently with a sphygmomanometer of oscillometric type, and the values are presented as the mean of 3 repeated recordings.

Statistical Analyses

Values are given as mean \pm SD or as the number of subjects and proportions. Student's *t* test, ANOVA, or χ^2 test was used to compare groups of data. Correlation coefficients were used to calculate possible associations between variables, and multiple regression analysis was used to detect any relationships between the variables studied. Assessments of perfusion responses to drug provocations in the 2 groups were made by use of 2-factor ANOVA for repeated measurements. A value of $P<0.05$ was considered significant.

Results

Influence of Family History

Microvascular endothelial function, large-artery dynamics, and blood pressures were similar in children with a positive family history of cardiovascular disease among second-degree relatives (13 of 44) and in those without such a history.

Influence of Age, Sex, and Body Mass Index

The distributions of age and sex were the same in both study groups, but a trend toward a lower current body mass index (BMI) in the LBW group was found (Table 1). The variations in microvascular perfusion induced by endothelium-dependent and -independent mechanisms were not associated with age, sex, or current BMI. The arterial diameter correlated with age ($r=0.56$, $P<0.001$) and BMI ($r=0.42$, $P<0.01$) in the aorta and with BMI ($r=0.44$, $P<0.01$) in the carotid artery. The stiffness index of the carotid artery correlated with age ($r=0.44$, $P<0.01$) but not with sex or current BMI. The stiffness index of the aorta did not correlate with age, sex, or current BMI. No other associations were found between large-artery dynamics and age, sex, or BMI.

Blood Pressure Data

The blood pressures were within normal limits in all children, and no sex differences were found. The LBW group had almost significantly lower systolic (106 ± 10 mm Hg) and diastolic (57 ± 6 mm Hg) blood pressures than the control children (systolic 111 ± 6 and diastolic 61 ± 4 mm Hg). Lower systolic blood pressures were found only in proportionately small children (102 ± 8 mm Hg), whereas systolic blood pressures were similar in LBW children lean at birth (110 ± 12 mm Hg) and control children. Diastolic blood pressures were about the same in proportionately small (57 ± 6 mm Hg) and lean (58 ± 7 mm Hg) LBW children. In the multiple regression analysis, the apparent blood pressure differences between the groups disappeared when BMI was added as an independent variable. No difference in heart rate was found between the 2 groups of children.

LBW and Microvascular Endothelial Function

Basal skin perfusion values were similar in the 2 groups (8.3 ± 4.6 PU in LBW and 7.1 ± 2.8 PU in control children).

TABLE 2. Comparison of Elastic Properties of the Aorta and Common Carotid Artery in Children Born With Low or Normal Birthweight

	Low Birthweight (n=22)	Normal Birthweight (n=22)	P
Aorta			
Dd, mm	9.3±0.7	9.6±0.9	0.13
ΔD, mm	1.8±0.4	1.9±0.4	0.43
β	3.4±0.8	3.1±0.6	0.33
MIV, mm/s	40.4±9.7	45.2±9.1	0.09
Carotid artery			
Dd, mm	6.0±0.4	6.1±0.3	0.31
ΔD, mm	0.81±0.1	0.83±0.1	0.67
β	4.7±1.0	4.2±0.8	0.15
MIV, mm/s	21.3±2.3	21.9±5.7	0.68

Dd indicates end-diastolic diameter; ΔD, pulse amplitude (systolic–diastolic diameter); and β, stiffness index. Data are mean±SD.

Endothelium-dependent vasodilation was significantly lower in LBW children than in controls. The peak perfusion induced by ACh was 88±33 PU in LBW children and 133±34 PU in controls ($P<0.001$, Figure 1). Endothelium-dependent vasodilation correlated with birthweight ($r=0.54$, $P<0.001$) and with relative birthweight deviation ($r=0.58$, $P<0.001$).

Endothelium-independent vasodilation values were similar in the 2 groups of children. The basal perfusion was 10.6±4.3 PU in LBW and 10.0±4.7 PU in control children, and the peak response to nitroglycerin was 40±16 PU in LBW children and 41±16 PU in controls ($P=0.82$, Figure 1).

In the LBW group, children lean at birth ($n=14$) had lower endothelium-dependent vasodilation than children born proportionately small ($n=8$, peak perfusion 68±5 versus 107±36 PU, $P<0.01$). No such difference was found regarding endothelium-independent vasodilation.

LBW and Dynamic Properties of the Large Arteries

Carotid Measurements

Comparison between children of low and normal birthweight showed no significant differences in arterial diameter, pulse amplitude of the diameter, stiffness index (β), and MIV of the carotid artery (Table 2). The carotid stiffness index correlated with birthweight ($r=-0.45$, $P<0.01$, Figure 2) and with relative birthweight deviation ($r=-0.46$, $P<0.01$). LBW children lean at birth had a higher stiffness index ($5.0±1.0$) than those born proportionately small ($4.2±0.6$, $P<0.05$) and control children ($4.2±0.8$, $P<0.05$). In LBW children lean at birth, the carotid stiffness index correlated with birthweight ($r=-0.76$, $P<0.01$, Figure 2) and with relative birthweight deviation ($r=-0.73$, $P<0.01$).

Aortic Measurements

The aortic diameter, pulse amplitude of the diameter, stiffness index, and MIV were similar in the 2 groups (Table 2). Unlike the findings in the carotid artery, aortic measurements showed no correlation with birthweight or relative birthweight deviation, and the stiffness indices were similar in

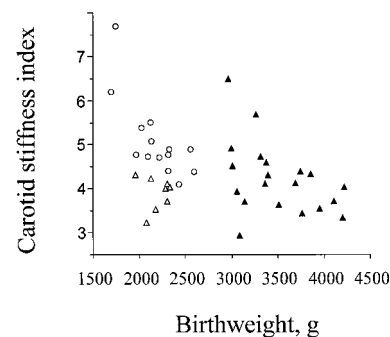


Figure 2. Carotid stiffness index (β) in healthy schoolchildren with normal ($n=22$, solid symbols) and low ($n=22$, open symbols) birthweight (LBW). A correlation between birthweight and carotid stiffness index was found ($r=-0.45$, $P<0.01$). After subgrouping LBW children into lean ($n=14$, open circles) and proportionately small at birth ($n=8$, open triangles), the correlation between birthweight and carotid stiffness index was restricted to lean LBW children ($r=-0.76$, $P<0.01$).

lean and proportionately small LBW children ($3.5±0.9$ versus $3.2±0.6$) and in controls ($3.1±0.6$).

Relation Between Microvascular Endothelial Function and Large-Artery Dynamics

A weak negative correlation was found between ACh-induced peak perfusion in the skin of the hand and carotid stiffness ($r=-0.33$, $P<0.05$). No other relations were found between microvascular endothelial function and large-artery dynamics.

Discussion

This study showed that LBW correlated with disturbed vasoregulation at school age in terms of limited vasodilatory reserve in the peripheral circulation and premature stiffening of the carotid artery. Impaired capacity for endothelium-dependent vasodilation and loss of large-artery elasticity are thought to be related to adult hypertension, diabetes, and atherosclerosis, including coronary disease.^{8,12,21} The prevalence of these markers in a group of healthy children may therefore be expressions of early atherogenetic susceptibility linked to LBW and a potential risk of clinical vascular complications later in life. In this pediatric study, other known causes of vascular compromise presumably did not affect the findings. For example, adult lifestyle factors, such as smoking, could not have confounded the results. Our findings are in line with those of other studies in which conventional cardiovascular risk factors, including blood lipids, showed little if any relationship with endothelium-dependent vasodilation in 9- to 11-year-old children¹⁵ and fit young adults,¹⁶ whereas LBW did. We have previously reported impaired microvascular endothelium-dependent vasodilation in LBW infants, even at birth.¹⁷ Although longitudinal studies are needed to pursue endothelial function over time, present and previous studies point to the possibility that endothelial dysfunction may be an inborn characteristic of subjects with LBW that persists throughout childhood into adult life.

A growing literature indicates that leanness at birth, as opposed to LBW, is associated with metabolic disturbances

such as glucose intolerance and insulin resistance.^{7,22} In our study, leanness at birth correlated with the lowest endothelium-dependent microvascular responses and the highest carotid stiffness indices measured in healthy schoolchildren. The small number of subjects who had been proportionately small at birth (n=8) limits the conclusions that can be drawn from our measurements in this subgroup of LBW children. However, they had vascular functions that did not differ greatly from those in the control group. Our observations support the hypothesis that disproportionate intrauterine growth, whether a direct effect of poor nutrition or secondary to genetic factors influencing fetal insulin secretion,²³ may have large and lasting effects on angiogenesis and vascular function in the developing fetus.

The structure and mechanical properties of large arteries can be permanently affected by altered hemodynamic stress in fetal life. For example, subjects born with a single umbilical artery exhibit a significant difference in vessel wall compliance between the 2 iliac arteries, and in the iliac artery accommodating increased blood flow in utero, established atherosclerotic changes have been described already in childhood.^{24,25} Moreover, selective atherosclerosis of the carotid arteries in elderly people has been reported to be more prevalent and severe in subjects with LBW.²⁶ As an interesting explanation for this association, it has been suggested that the preferential perfusion of the fetal head and the altered blood flow velocity waveforms that can be seen in intrauterine growth retardation may selectively accelerate degenerative processes in the carotid arteries.²⁶ Our study adds further knowledge to the association between LBW and carotid atherosclerosis, ie, the carotid artery was found to be less elastic and prone to premature stiffening already at school age in LBW subjects lean at birth.

The elastic properties of the large arteries are not uniformly distributed in the vascular tree, and they exhibit differential changes over time. As confirmed by our study, the carotid artery is normally stiffer than the aorta in children and young adults.^{9,27} During aging, however, stiffness increases to a greater extent in the aorta, and from middle age onward, the aorta is stiffer than the carotid artery vessel wall.²⁷

Rapid vasodilation 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.²⁸ We have previously ruled out the possibility that iontophoresis causes nonspecific neurogenic skin vasodilation due to current alone.¹⁷ In addition, considering the similar basal perfusion values and the stimulus-response curves, there is no reason to believe that the 2 groups received different doses of the drug in the skin. The vascular response to ACh involves endothelial release of NO, endothelium-derived hyperpolarizing factor, and/or prostanoids.^{29,30} Compared with children of normal birthweight, a dysfunction in one or several of these endothelial systems probably contributed to the low microvascular responses induced by ACh in LBW children. A selective endothelial dysfunction in these children was confirmed by transdermal introduction of an exogenous NO donor, resulting in similar skin microvascular responses in children of low and normal birthweight.

Impaired endothelium may be involved in both microvascular and large arterial dysfunction found in LBW children. In addition to its key role in vascular reactivity, experimental studies have shown that the endothelium may also be important for long-term arterial remodeling and in the control of elastic properties of the arterial wall.^{31,32} Damage to the endothelium in large arteries has been shown not only to impair active diameter changes³³ but also to increase the viscosity of the arterial wall.³²

A total of 10 of 32 eligible LBW subjects were lost to follow-up. Because the birthweights of these children did not differ from those in the study cohort, we do not believe that the selection biased the results in this study.

In summary, schoolchildren who had been small and lean at birth exhibited impaired endothelial function and increased carotid stiffness. These results contribute to a better understanding of the link between LBW and adult cardiovascular disease, and they have implications for lifestyle and health risks in children and adults.

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