Relationship of Endothelial Function to Birth Weight in Humans

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OBJECTIVE — Low birth weight has been associated with hyperlipidemia, hypertension, diabetes, and coronary heart disease in adult life, but the precise mechanism is debated. The endothelium is thought to play a pivotal role in each of the above conditions with abnormalities being detectable before the development of overt disease. To investigate the possibility that endothelium has a role in mediating the excessive risk of adult vascular disease associated with low birth weight, endothelial function was assessed in young healthy adults who were either of low or normal birth weight at term.

RESEARCH DESIGN AND METHODS — Twelve low birth weight (2.2 ± 0.05 kg, mean ± SEM) subjects (six men/six women; age 28 ± 0.2 years) and twelve age- and sex-matched normal birth weight (3.3 ± 0.07 kg) control subjects were studied. The L-arginine–nitric oxide pathway was assessed in the forearm vascular bed by using venous occlusion plethysmography during intra-arterial brachial infusion of acetylcholine, sodium nitroprusside, norepinephrine, and N^G-monomethyl-L-arginine (L-NMMA). Von Willebrand factor, a noninvasive marker of endothelial dysfunction, was also measured. Comparisons were made using Student's *t* tests.

RESULTS — Von Willebrand factor was significantly elevated in low birth weight compared with normal birth weight subjects (136.9 ±12.7 vs. 95.6 ± 9.5%; $P = 0.016$). The groups did not differ in the responses to acetylcholine ($P = 0.76$), sodium nitroprusside ($P = 0.84$), norepinephrine ($P = 0.21$), or L-NMMA ($P = 0.35$).

CONCLUSIONS — The finding of elevated von Willebrand factor in low birth weight subjects is suggestive of endothelial cell injury but does not appear to be associated with dysfunction of the L-arginine–nitric oxide pathway.

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Accelerated vascular disease remains the major source of mortality and morbidity in Western society. Over the past decade, there has been a sustained interest in the possible role of intrauterine development in compounding the already well-established risk factors of hypertension, smoking, diabetes, and hyperlipidemia. While an association between birth weight and these conditions has been reported in several populations, the underlying mechanisms remain unclear (1–10).

Over a similar period, increasing evidence has also pointed to the importance of endothelium in atherosclerosis; Furchgott and Zawadzki (11) first postulated the existence of the endothelium-derived relaxing factor in 1980. Since identified as being nitric oxide (NO) (12), this compound contributes to vascular tone, the inhibition of platelet aggregation and adhesion, prevention of leukocyte adhesion, and inhibition of proliferation of smooth muscle cells. NO is produced within the endothelium from L-arginine by the endothelial constitutive isoform of the enzyme NO synthase. Endothelial dysfunction, manifested by impaired basal and stimulated NO production, has been reported in hypertension (13–17), coronary artery disease (18,19), diabetes (20–24), hyperlipidemia (25–28), and smoking (29–31).

It is of interest that both low birth weight and endothelial dysfunction have been independently associated with these same diseases in adult life. Others have sought to explain the association between birth weight and adult disease in various ways. Much attention has focused on the hypothesis by Barker and colleagues (32–34) that this is solely a reflection of fetal undernutrition. Other suggestions include dysfunction of the placental glucocorticoid barrier (35), fewer nephrons at birth (36), or selective survival of those low birth weight infants genetically predisposed to insulin resistance (7).

The aim of the present study was to test the hypothesis that endothelial dysfunction might represent the link between low birth weight and adult disease. To address this hypothesis, we examined both basal and stimulated NO production in a group of healthy young adults who were of low birth weight (<2,500 g), despite normal gestational age (37–40 weeks), and compared them with matched subjects of normal birth weight (>3,000 g). We have also investigated other postulated markers of endothelial dysfunction, such as von Willebrand factor (vWF) and microalbuminuria.

RESEARCH DESIGN AND METHODS

Subjects

The Royal Maternity Hospital, Belfast, has maintained accurate birth records over many decades. We examined the log books in detail for a 3-year period in the late 1960s: 1967–1969. All births of normal gestational age (37–42 weeks' gestation) were identified, and categorized according to birth weight: low (<2,500 g) and normal (3,000–4,000 g). Because there were many more in the latter group, a random number generator was used to select a sample for chart survey. Relevant charts were subse-

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Abbreviations: FBF, forearm blood flow; L-NMMA, N^G-monomethyl-L-arginine; vWF, von Willebrand factor.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Characteristics of study subjects*

	Low birth weight	Normal birth weight	*P*
Sex (M/F)	6/6	6/6	—
Age (years)	28.0 ± 0.21	27.9 ± 0.29	NS
BMI (kg/m^2)	23.8 ± 0.97	24.9 ± 0.90	NS
Fasting plasma glucose (mmol/l)	4.2 ± 0.11	4.3 ± 0.17	NS
HbA_{1c} (%)	4.3 ± 0.11	4.1 ± 0.09	NS
Fasting serum insulin (mU/l)	7.8 ± 1.5	6.3 ± 0.82	NS
Serum total cholesterol (mmol/l)	4.8 ± 0.26	4.5 ± 0.25	NS
Serum LDL cholesterol (mmol/l)	2.9 ± 0.26	2.8 ± 0.26	NS
Serum HDL cholesterol (mmol/l)	1.2 ± 0.09	1.2 ± 0.11	NS
Serum triglycerides	1.4 ± 0.26	1.2 ± 0.31	NS
Fibrinogen (g/l)	3.04 ± 0.03	2.68 ± 0.26	NS
Factor VII (%)	114.7 ± 6.5	117.3 ± 11.0	NS
Serum creatinine (μmol/l)	77.9 ± 5.4	74.0 ± 5.1	NS
Serum cortisol (nmol/l)	388.1 ± 77.9	256.1 ± 27.7	NS

Data are means ± SEM.

quently retrieved from hospital archives. After explanation of the research protocol and screening for exclusion criteria, 24 healthy Caucasian volunteers (aged 27–29 years) were studied, equal numbers being of low (2.23 ± 0.05 kg; mean ± SEM) and normal (3.33 ± 0.07 kg) birth weights. None had any significant past medical history nor were they receiving medication before or during the study. There was a parental history of diabetes in one subject in each group, a history of hypertension in three parents of the low birth weight subjects and four of the normal birth weight subjects, and one subject in each group smoked ~15 cigarettes per day. The clinical characteristics of the two groups are summarized in Table 1. The study was approved by the Research Ethical Committee of the Queen's University of Belfast, and all subjects gave written informed consent.

Experimental protocol

Biochemical results shown were determined in fasting serum samples. "Office" systolic and diastolic blood pressures represent the average of three readings, taken with subjects relaxed and supine, by an automatic oscillometric digital blood pressure monitor (Omron HEM-705CP, Tokyo). Ambulatory blood pressure data was the average over a 24-h period, with monitoring every 30 min (QuietTrak; Welch Allyn, Skaneateles Falls, NY).

All studies were performed in the morning. Subjects fasted and abstained from alcohol, caffeine, and nicotine from 10:00 P.M. They lay comfortably supine in a temperature-controlled environment (22–24°C, within ± 0.5°C during each study), with both forearms supported above the level of the right atrium. A 27-gauge unmounted needle (Cooper's Needle Works, Birmingham, U.K.) sealed with dental wax to an 18-gauge epidural catheter (Portex, Hythe, Kent, U.K.) was inserted into the brachial artery of the nondominant side (usually the left) after local anesthesia with 1% lidocaine hydrochloride. A rest period of at least 30 min was then allowed before infusion of vasoactive substances to ensure stabilization of forearm blood flow.

Forearm blood flow (FBF) was measured in both arms simultaneously by strain gauge (mercury-in-silastic) venous occlusion plethysmography (37) for 10 out of every 15 s during the last 90 s of each infusion period. The strain gauges were electrically calibrated to measure the percentage change in forearm volume and were connected to a plethysmograph (Vasculab SPG16; Medasonics, Mountain View, CA), which in turn was connected to a computerized chart recording system (Maclab, AD Instruments, Castle Hill, NSW, Australia). The hands were excluded from the circulation by wrist cuffs at suprasystolic pressure for 1 min before and during each measurement period, and upper arm cuffs were inflated to 40 mmHg by a rapid cuff inflator (model E-20; Hokanson, Bellevue, WA).

Four incremental doses of acetylcholine (25, 50, 100, and 200 nmol/min) and sodium nitroprusside (2.5, 5, 10, and 20 nmol/min) were infused for 4 min each in all subjects. The order of these agents was randomized. These infusions were followed by norepinephrine (100, 200, and 400 pmol/min) in 22 individuals (11 in each group), and finally, N^G-monomethyl-L-arginine (L-NMMA) (2, 4, and 8 μmol/min; Calbiochem-Novabiochem, U.K.) in all subjects, each dose for 5 min. There was a 20- to 30-min washout period between infusion of the various agents to allow forearm blood flow to return to baseline. Pulse and blood

Table 2—*Maternal details and offspring characteristics at birth*

	Low birth weight	Normal birth weight	*P*
Maternal			
Age (years)	28.8 ± 2.1	30.5 ± 1.8	NS
Parity	2.2 ± 0.9	2.6 ± 0.5	NS
Systolic blood pressure (mmHg)			
Booking	121.2 ± 3.1	120.9 ± 4.0	NS
Admission	126.3 ± 3.2	127.3 ± 4.8	NS
Discharge	115.7 ± 3.6	115.4 ± 4.0	NS
Diastolic blood pressure (mmHg)			
Booking	77.1 ± 2.3	75.6 ± 2.3	NS
Admission	81.0 ± 2.7	78.0 ± 2.7	NS
Discharge	70.5 ± 2.7	74.1 ± 3.8	NS
Gestational age at booking (weeks)	17.9 ± 2.1	13.9 ± 1.2	NS
Offspring			
Birth weight (kg)	2.23 ± 0.05	3.33 ± 0.07	<0.001
Head circumference (cm)	31.9 ± 0.31	34.4 ± 0.46	<0.001
Length (m)	0.47 ± 0.01	0.51 ± 0.01	<0.001
Ponderal index (kg/m^3)	21.2 ± 0.59	25.6 ± 0.97	0.001
Gestational age (weeks)	39.2 ± 0.34	39.9 ± 0.36	NS
Placental weight (kg)	0.49 ± 0.05	0.61 ± 0.03	NS

Data are means ± SEM.

Table 3—*Baseline clinical characteristics*

	Low birth weight	Normal birth weight	*P*
Systolic blood pressure (mmHg)			
Office	115.9 ± 3.6	117.5 ± 4.1	NS
Ambulatory	117.1 ± 3.3	116.5 ± 3.1	NS
Diastolic blood pressure (mmHg)			
Office	71.3 ± 2.5	71.3 ± 2.4	NS
Ambulatory	66.7 ± 2.2	67.7 ± 1.9	NS
vWF (%)	136.9 ± 12.7	95.6 ± 9.5	0.016
Urinary albumin/creatinine ratio (random)	0.60 ± 0.17	0.44 ± 0.07	NS
Overnight albumin excretion rate (μg/min)	3.99 ± 0.94	2.32 ± 0.41	NS
Resting forearm blood flow (ml · 100 ml^{-1} · min^{-1})	2.95 ± 0.29	2.59 ± 0.14	NS

Data are means ± SEM.

pressure were recorded in the noninfused arm between each of the four infusion periods by oscillometric means (Omron HEM-705CP, Tokyo).

Statistical analysis

Forearm blood flow is expressed as milliliter per 100 ml forearm volume per minute (37), and the average of five recordings at each infusion step was calculated for both the infused and noninfused (control) arms. The percentage change in the ratio of blood flow in the infused arm to that in the control arm (infused:control ratio) was calculated at baseline and with each drug dosage, thus controlling for any effects of unavoidable external and systemic factors, which should affect blood flow in both arms similarly (38). A dose-response curve was constructed for each drug in each subject, with the area under these dose-response curves providing a summary measure for the overall response to each drug in each individual (39). The Student's *t* test for independent samples was used to allow comparison of individual responses at each dose between groups. Statistical significance was taken as $P < 0.05$. Between-day mean coefficient of variation for forearm blood flow is 10.5% (40). Thus, the number of subjects required in each group to give 90% power (at $P < 0.05$) to detect a 20% change is 6 and to detect a 15% change is 10.

RESULTS — The two groups differed significantly in regard to birth weight ($P < 0.001$), head circumference ($P < 0.001$), length ($P < 0.001$), and ponderal index ($P = 0.001$). There were no differences in gestational age, maternal age, parity, or maternal blood pressure, whether at booking, admission, or discharge (Table 2).

The routine biochemical and hematological parameters were similar in the two groups (Table 1). Although fasting insulin was greater in low birth weight subjects (7.8 ±1.5 vs. 6.3 ± 0.8 mU/l), as was fasting morning serum cortisol (388.1 ± 77.9 vs. 256.1 ± 27.7 nmol/l), this was not statistically significant. Office blood pressure and ambulatory blood pressure monitoring profiles did not differ between the groups (Table 3).

In contrast, vWF was significantly elevated in low birth weight compared with normal birth weight subjects: 136.9 ± 12.7 vs. 95.6 ± 9.5%; $P = 0.016$ (Table 3). There was an overall negative correlation between birth weight and vWF ($r = -0.413$, $P = 0.04$).

Effect on forearm blood flow of acetylcholine and sodium nitroprusside

The infusions of acetylcholine (25–200 nmol/min) and sodium nitroprusside (2.5–20 nmol/min) both resulted in similar dose-dependent increases in forearm blood flow in the infused arm and forearm blood flow ratio (infused arm:control arm). The

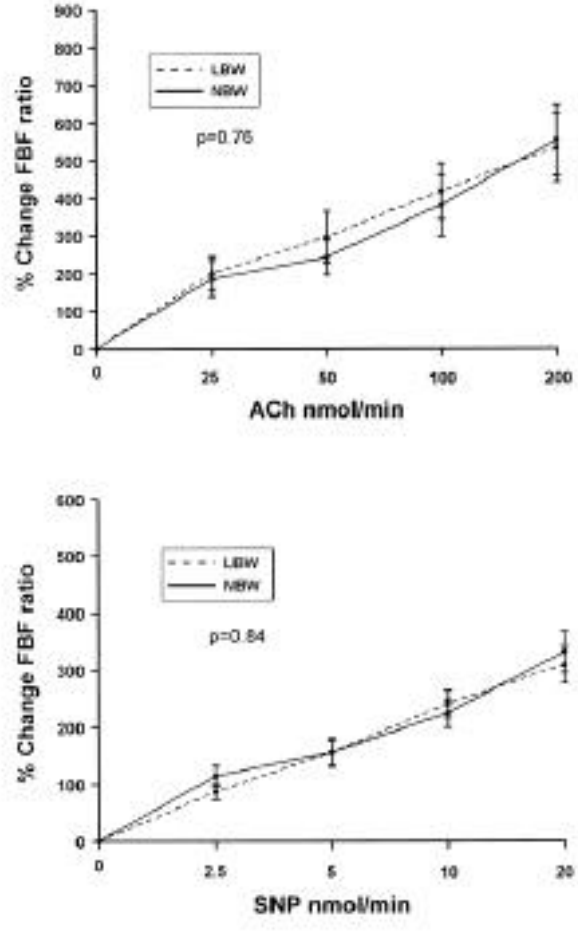

Figure 1—*Percentage changes in FBF ratio in response to incremental doses of acetylcholine (ACh) and sodium nitroprusside (SNP) in low birth weight (LBW) and normal birth weight (NBW) subjects. P value refers to comparison of area under dose-response curves.*

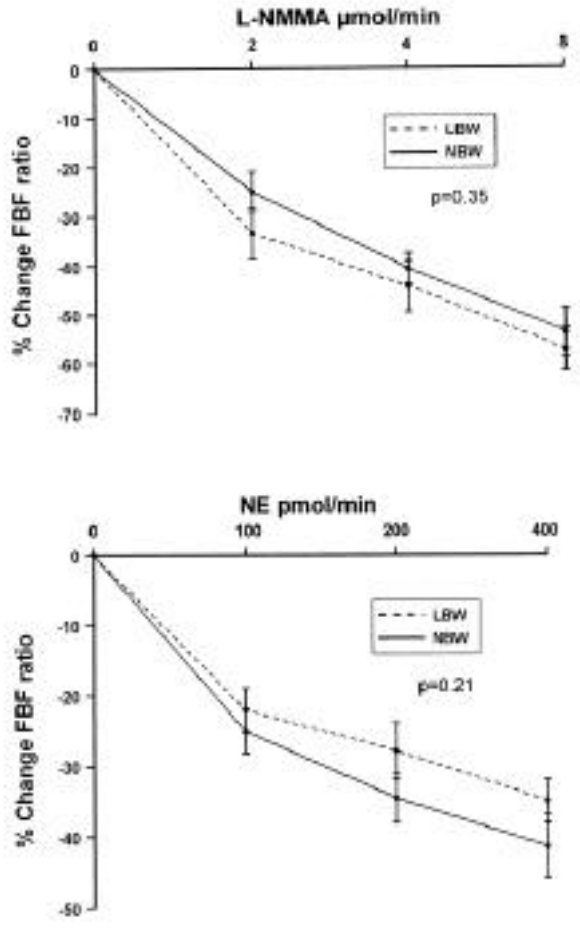

Figure 2—*Percentage changes in FBF ratio in response to incremental doses of* L*-NMMA and norepinephrine (NE) in low birth weight (LBW) and normal birth weight (NBW) subjects.* P *value refers to comparison of area under dose-response curves.*

responses to each agent, as summarized by the areas under the dose-response curves, were similar in each group (Fig. 1).

Effect on forearm blood flow of norepinephrine and L-NMMA

Norepinephrine (100–400 pmol/min) and L-NMMA (2–8 µmol/min) both consistently produced dose-dependent reductions in forearm blood flow ratio (infused arm:control arm) in the two groups of subjects. There were no differences in the percentage changes at any dose with either agent (Fig. 2).

CONCLUSIONS — Although the evidence remains somewhat contradictory, in a number of populations, low birth weight has been linked to the various features of the insulin resistance syndrome—namely hypertension, type 2 diabetes, hyperlipidemia, and coronary artery disease (1–10). In addition, the presence of endothelial dysfunction in hypertension, diabetes, hypercholesterolemia, and coronary heart disease is now generally established (13–28), although again with some conflicting reports (20,21,41–43). In some cases, endothelial dysfunction has been demonstrated in apparently normal vessels, predating the development of atherosclerosis. While it may develop as a secondary feature of some conditions, in others, it seems to be a primary phenomenon (44).

The hypothesis that endothelial dysfunction may actually precede the features of the insulin resistance syndrome was also echoed in a recent editorial (45) published in conjunction with an article that reported impaired endothelium-dependent vasodilation in women with normal glucose tolerance but previous gestational diabetes (46). The advantage of studying a population of low birth weight individuals before the emergence of insulin-resistant features was specifically advocated (45). There has been one such study published, using a noninvasive ultrasound technique in 9- to 11-year-old children (47). Birth weight data were provided by parental recall and there was no endothelium-independent control incorporated. We chose rather to study young healthy adults of low and normal birth weight whose birth details could be accurately established from their mothers' obstetric records. Apart from birth weight and neonatal dimensions, the groups were well matched for clinical and maternal parameters (Table 2). In particular, there were no differences in maternal blood pressure, parity, or gestational age. Unfortunately, most of the obstetric charts had no record of smoking habits or nutrition during pregnancy. Endothelial function was examined using well-established methods.

We found that vWF was significantly elevated in low birth weight subjects (P = 0.016). The inverse correlation of vWF and birth weight persisted when all the subjects of both normal and low birth weight were grouped together ($r = -0.41$, $P = 0.04$). It has been claimed that vWF is currently the best available noninvasive marker for endothelial dysfunction (48). Elevated levels are reported in hypertension (49,50), diabetes (51,52), smoking (53,54), hypercholesterolemia (55,56), and ischemic heart disease (57), conditions in which abnormalities within the L-arginine–nitric oxide pathway have also been described. A recent prospective study of hemostatic factors in 14,477 patients from the Atherosclerosis Risk in Communities (ARIC) Study confirmed that elevated vWF levels were a risk factor for coronary heart disease (58).

Despite the increased vWF levels, we found no abnormality in either basal or stimulated NO production in subjects of low birth weight. The vascular responses to acetylcholine and sodium nitroprusside were virtually identical, with no trend towards a difference between the groups (P = 0.761 and P = 0.838, respectively). Similarly, there was no difference in basal NO production (P = 0.35).

Although a recent study of subjects presumably exposed to malnutrition in utero (during the Leningrad siege) demonstrated an elevation in vWF, reliable data on birth weight were not available (59). Despite the interest in vWF as a marker of endothelial dysfunction, there are no reports in the literature that have correlated it with NO production. Of course endothelium has many functions besides the production of NO, including the synthesis of other vasoactive

substances, such as prostacyclin and endothelin, and regulatory roles in hemostasis and cell growth. While it is possible that our assessment of the NO pathway simply failed to show an abnormality that was actually present, this seems unlikely because the findings were so similar in each group. Although the groups are not large, increasing the chances of a type II error, they are nonetheless similar in size to most of the invasive studies of endothelial function that have been published. While it is conceivable that abnormalities of this pathway might have been detected if subjects at older ages had been studied, it seems more likely that the elevation in vWF results from impairment of other aspects of endothelial function (perhaps involving the microcirculation) separate from the L-arginine–nitric oxide pathway.

There were no significant differences between the groups in fibrinogen ($P = 0.23$) or factor VII ($P = 0.84$), both recognized markers of increased vascular risk that have been studied in relation to weight at birth and at 1 year old (4), with variable findings. In addition, no significant differences were seen in lipid profiles, fasting glucose, HbA_{1c}, and fasting insulin (Table 1), differing from the findings of Hales et al. (3), although not all studies have reported differences in these variables. Blood pressure did not differ between the groups, despite the use of ambulatory monitoring. Serum cortisol was higher in the low birth weight group, but this was not statistically significant. However, interpretation of these various findings is limited by the comparatively small numbers. In addition, most of the previous reports of these associations have been in older age-groups.

In conclusion, we have shown a significant elevation in vWF in healthy young adult subjects who were of low birth weight, strongly supporting the presence of endothelial dysfunction in such individuals. This abnormality was in the absence of any of the conditions associated with increased vascular risk with which low birth weight has recently been linked. It would therefore appear that endothelial dysfunction precedes the development of these conditions, and it is thus conceivable that such dysfunction may explain association of these conditions with low birth weight. However, the endothelial abnormality, as assessed in the forearm vascular bed, does not appear to be related to the L-arginine–nitric oxide pathway.

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