Endothelial function is impaired in fit young adults of low birth weight

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Abstract

Objective: Non-insulin-dependent diabetes, hypertension and ischaemic heart disease, with insulin resistance, are associated with low birth weight (the 'Small Baby Syndrome'). Common to these adult clinical conditions is endothelial dysfunction. We tested the hypothesis that endothelial dysfunction could precede their development in those of low birth weight. Methods: Endothelial function was measured by ultrasonic 'wall-tracking' of flow-related brachial artery dilatation in fit 19–20 year old subjects randomly selected (blind to the investigators throughout the study) from low (<2.5 kg) and normal (3.0–3.8 kg) birth weight subjects in the 1975–7 cohort of the Cardiff Births Survey and with no known cause for endothelial dysfunction. Results: Flow-related dilatation was impaired in low birth weight relative to normal birth weight subjects (median 0.04 mm [1.5%] [n=22] cf. 0.11 mm [4.1%] [n=17], p<0.05; 0.04 mm [1.5%] [n=15] cf. 0.12 mm [4.4%] [n=12], p<0.05 after exclusion of inadvertently included ever-smokers). Conclusion: The findings suggest that endothelial dysfunction is a consequence of foetal malnutrition, consistent with contributing to the clinical features of the 'Small Baby Syndrome' in later adult life. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Low birth weight; Endothelial function; Hypertension; Diabetes; Atheroma; Coronary artery disease; Insulin resistance

1. Introduction

Low birth weight has been reported to be associated with a high prevalence in adult life of non-insulin-dependent diabetes (NIDDM), hypertension and ischaemic heart disease, together with insulin resistance, glucose intolerance and hypertriglyceridaemia – the ‘Small Baby Syndrome’ [1–3]. This same constellation of conditions has been recognised also in the population at large as Metabolic Syndrome X [4–6] raising the possibility that nutritional factors in early development contribute significantly to the overall prevalence of these adult conditions.

Insulin resistance has come to be recognised as seminal to Metabolic Syndrome X [5] and thus to atherosclerosis [7], and it has been reported as early as the age of 4 years in low birth weight subjects [8]. The increased prevalence of NIDDM in adults of low birth weight appears to be mediated primarily by insulin resistance rather than by hypo-secretion of insulin [9]. Insulin resistance has been attributed at least in part to circulatory dysfunction since insulin sensitivity was shown to be related to capillary density in skeletal muscle biopsies [10] and glucose uptake was shown to be dependent on an insulin-induced increase in blood flow that is impaired in NIDDM [11,12]. The insulin-stimulated increase in blood flow has been shown also to be inversely related to blood pressure [13]. Insulin-stimulated vasodilatation is nitric oxide (NO) dependent, due to insulin stimulation of endothelial NO production [12] as well as to flow-related enhancement of NO production.

Endothelial dysfunction is common to the adult conditions which comprise the Small Baby Syndrome, and indeed to risk factors for atheroma–hypertension (and normotensive offspring of hypertensive patients [14], diabetes, smoking, dyslipidaemia and homocysteinaemia, as well as increasing age, oestrogen deficiency and lack of...
exercise. These conditions are characterised also by insulin resistance (for reviews, see [15,16]) to the extent that they have been investigated. The evidence in relation to hypercholesterolaemia per se is conflicting, however, in that it appears to be present in some conditions [17] but specifically not with familial hypercholesterolaemia [18]. The association of endothelial dysfunction with insulin resistance thus appears to be strong if not universal, reflecting the probable contribution of other factors and that endothelial dysfunction is unlikely to be a single entity with respect to its mechanisms and manifestations. Both endothelial dysfunction and insulin resistance are also found in chronic heart failure (a condition of limited life expectancy relative to the gestation period of atheroma). Their association is further circumstantially strengthened by its recent demonstration in microvascular angina [19–22] (in the absence of other known cause of endothelial dysfunction [22]), the coincidentally named ‘Syndrome X’ of the cardiologists [23]. Both endothelial function and insulin sensitivity can moreover be similarly modulated, as for example by changes in the level of physical fitness [24,25] It may be relevant too that the relationship of low birth weight to insulin resistance appears to be stronger in men than women [26], since women are also benefited from oestrogens which upregulate eNOS [27], increase NO production [28] and are protective of endothelial function [29].

Endothelial function could thus relate to both the metabolic and the vascular consequences of foetal malnutrition. To test the hypothesis that endothelial dysfunction could be an intrinsic feature of the Small Baby Syndrome, we measured flow-related dilatation of the brachial artery in fit young adults of low birth weight before the potential development of its adult clinical features.

2. Methods

2.1. Subjects

The Cardiff Births Survey comprises all births in Cardiff since 1965 [30]. From the cohort of those born from September 1975 through August 1977 (excluding complicated deliveries, multiple pregnancies and congenital abnormalities) 30 subjects of low and 30 of normal birth weight (<2.5 kg, or 3.0–3.8 kg, respectively, at ≥38 weeks gestation) known to be previously fit and non-smokers, were randomly selected, blind to the investigators throughout the study, and invited to participate if still non-smokers. Forty-three attended, of whom three were excluded because of technical difficulty in measuring brachial artery diameter. One further subject was later excluded because of uncertainty about his initial birth weight. The study, conducted in 1996, included 22 low and 17 normal birth weight subjects (see Table 1).

Serum cotinine levels became available after completion of the study and unexpectedly showed evidence of recent smoking in some subjects, despite their initial denial of smoking, in that non-smoking was a written condition of participation and that smoking was denied on personal questioning at first attendance. All subjects were therefore recalled for more rigorous, confidential questioning. This revealed that some had in fact smoked, albeit rarely. The data were accordingly analysed both for the whole group and after exclusion of all whose cotinine levels gave evidence of recent exposure and/or who admitted to any active or significant passive smoking.

2.2. Clinical assessment

History and physical examination confirmed that all were fit, on no drug therapy, and with no known cause of endothelial dysfunction (hypertension, diabetes, active or heavy passive smoking [but see above]).

2.3. Blood samples

Fasting venous blood samples were taken for measurement of plasma lipids, glucose, homocysteine [31] (a cause of endothelial dysfunction), von Willebrand factor [32] (a
marker of endothelial damage), and plasma cotinine levels [33] (to check against unadmitted recent smoking).

2.4. Protocol

The protocol was established following preliminary exploratory studies [34]. All studies were performed in the morning in a temperature-controlled room (21–23°C) on fasting subjects following ≥15 min supine rest, with the arm held outstretched on a pneumatic cushion. Venous blood was sampled before carrying out the brachial artery study. Caffeine-containing beverages were avoided for 12 h before the study.

Measurements of internal brachial artery end-diastolic diameter and blood flow, and of blood pressure were made at baseline; during increased brachial artery flow induced by hand hyperaemia (see below); after confirming return to baseline ≥15 min later; and 3 min after sublingual glyceryl trinitrate (GTN) spray (400 µg). Preliminary studies having shown that the increase in diameter is appropriately represented by measurement at this time. The two interventions of increased flow and GTN were always performed in that order to avoid pharmacological carry-over effects, preliminary experiments having shown that the dilator response to GTN lasts ≥40 mm.

2.5. Brachial artery study

Endothelial function was assessed by measurement of flow-related brachial artery dilatation (FMD) [35] using ultrasonic ‘wall-tracking’ as previously described and validated [34,36]. The high resolution ‘wall-tracking’ system used in this study consists of a specially adapted duplex colour flow ultrasound machine (Diasonics Spectra™) with a 7.5 MHz linear phased-array transducer (giving high axial resolution ca. ±3 µm). The brachial artery was identified using the ultrasound transducer. A stand-off device containing ultrasound coupling gel was placed between the transducer to prevent compression of the anterior wall of the artery. The transducer was held in a stereotactic clamp and a two-dimensional B-mode image of the brachial artery obtained. The M-mode cursor was positioned perpendicular to the vessel and the horizontal distance between the cursor and the anatomical landmark recorded using the electronic calipers. The radio frequent (RF) signals from the M-mode were relayed to the wall tracking system (Vadirec™) and digitised. The sampling frequency of RF signals was 1 kHz, and each total recording time was 10 s. On completion of data acquisition the first RF-signal was displayed on the computer screen. From the sample volumes, the operator marked the positions of the anterior and posterior internal artery wall whose movements were tracked, using the stored RF signals, to give the distension waveform (intravascular diameter change as a function of time) beat-to-beat [37]. Changes in end-diastolic intravascular diameter were used as the measure of dilatation.

Blood flow was measured using an 8 MHz continuous wave Doppler probe mounted at an angle of 60° in a perspex block and positioned over the brachial artery distal to the 7.5 MHz probe. The Doppler signals were analysed by a spectrum analyser (SciMed Dopstation™) and stored on metal audio tape using a high performance recorder (Nakamichi B-100E™). Brachial artery blood flow was derived from the mean blood velocity (corrected for Doppler angle). Blood pressure was recorded by photoplethysmography (Finapres™) from a finger cuff on the middle finger of the ipsilateral arm.

Increased brachial artery flow was induced by hyperaemia of the hand. After taking baseline measurements, a paediatric sphygmomanometer cuff was inflated at the wrist to supra-systolic pressure for 5 min and then abruptly released. Blood flow and brachial artery flow were monitored continuously from 15 s before until 5 min after cuff release. Blood flow was recorded as the average of peak systolic flow velocity (corrected for calibre to give flow) over 15 s periods starting at cuff release (0 time) and at 1, 2, 3 and 5 min after cuff release. The increase in flow is expressed as a percentage of its preceding baseline level. Internal brachial artery diameter was measured for 10 s periods at (and spanning) 1, 2, 3 and 5 min after cuff release (figure). The increase in diameter is influenced by basal diameter [35,38] and is accordingly expressed in absolute terms. The time course of changes in flow and diameter during hand hyperaemia is shown in Fig. 1. The data presented in Table 2 and used for analysis record individual peak responses, i.e. during the first 15 s for flow, and at either 1 or 2 min after cuff release for diameter.

3. Statistics

Group data for patient characteristics are presented as mean±SD and compared using Student’s unpaired t-test (Table 1). Brachial artery flow and diameter data were shown to be not normally distributed and are accordingly presented as medians with inter quartile range and compared by Mann–Whitney test. p<0.05 is regarded as significant.

3.1. Ethical approval

The study was approved by the local research and ethics committee of Bro Taf Health Authority and all subjects gave informed consent. The investigation conformed with the principles outlined in the Declaration of Helsinki.
Table 2  
Brachial artery study

<table>
<thead>
<tr>
<th></th>
<th>Low birth weight</th>
<th>Normal birth weight</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Median</td>
<td>LQR</td>
</tr>
<tr>
<td><strong>All Subjects</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diameter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (mm)</td>
<td>2.61</td>
<td>2.35</td>
</tr>
<tr>
<td>FMD Δ(mm)</td>
<td>0.04*</td>
<td>-0.02</td>
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<tr>
<td>GTN Δ(mm)</td>
<td>0.55</td>
<td>0.41</td>
</tr>
<tr>
<td>Flow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (ml/min)</td>
<td>5</td>
<td>2.2</td>
</tr>
<tr>
<td>FMD Δ(ml/min)</td>
<td>27.6</td>
<td>15.28</td>
</tr>
<tr>
<td>GTN Δ(ml/min)</td>
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<td>263.5</td>
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<tr>
<td>GTN Δ (%)</td>
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<td>-3.15</td>
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<td></td>
<td>-12.29</td>
<td>-47.53</td>
</tr>
<tr>
<td><strong>Non-Smokers</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diameter</td>
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<td></td>
</tr>
<tr>
<td>Baseline (mm)</td>
<td>2.62</td>
<td>2.33</td>
</tr>
<tr>
<td>FMD Δ(mm)</td>
<td>0.04*</td>
<td>-0.02</td>
</tr>
<tr>
<td>GTN Δ(mm)</td>
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<td>0.41</td>
</tr>
<tr>
<td>Flow</td>
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<td></td>
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<tr>
<td>Baseline (ml/min)</td>
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<td>FMD Δ(ml/min)</td>
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<td>GTN Δ(ml/min)</td>
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</tr>
<tr>
<td>GTN Δ (%)</td>
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<tr>
<td></td>
<td>-13.51</td>
<td>-47.62</td>
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</tbody>
</table>

Diameter/flow=brachial artery internal end-diastolic diameter/flow, FMD/GTN Δ=flow mediated/GTN induced increase in diameter.
* p<0.05 cf. normal birth weight.
LQR/UQR=lower/upper quartile.

4. Results

The low and normal birth weight groups appear well matched except in relation to birth weight and gender (Table 1). There was no evidence of dyslipidaemia, and serum von Willebrand factor (VWF) levels (a marker of endothelial damage) were normal, with no significant differences between the two groups. No subject had hyperhomocysteinaemia [normal<14 μmol/l] (a further cause of endothelial dysfunction) and although mean homocysteine levels differed slightly between low and normal birth weight subjects, it was the normal birth weight subjects who had the higher levels. At the time of these subjects’ birth, maternal age (recorded in 2 or 4 year bands) and the number of previous pregnancies showed no difference between low and normal birth weight subjects (Mann–Whitney test, p>0.05). Body length at birth was not recorded.

Data are given both for the whole group (of supposed non-smokers) and after exclusion of those subjects who were inadvertently included on the basis of denial of smoking but subsequently admitted to ever-smoking (see Section 2).

Flow-related dilatation was significantly impaired in low birth weight compared with normal birth weight subjects, both with and without exclusion of ever-smokers (Table 2, Fig. 1). GTN-induced dilatation was similar in low and normal birth weight subjects. Brachial artery blood flow increased 5 to 6-fold during hand hyperaemia in all subgroups, with no difference between low and normal birth weight groups. Basal brachial artery diameter was slightly (3–4%) but not significantly smaller in small birth weight subjects.

![Fig. 1. Time course of change in brachial artery internal diameter (mm) following release of wrist cuff (time 0–5 min), i.e. during hand hyperaemia. Data shown as median±interquartile range in the ‘never-smokers’ groups. Normal birth weight subjects (solid symbols, n=12); low birth weight subjects (open symbols, n=15).](https://academic.oup.com/cardiovascres/article-abstract/40/3/600/275863)
5. Discussion

The records of the Cardiff Births Survey [30] provided the opportunity prospectively to test the hypothesis that low birth weight might be associated with endothelial dysfunction not secondary to any other known cause. Endothelial function was assessed by measuring flow-related dilatation in fit young adults, aged 19–21 years, randomly selected as having been of either low (<2.5 kg) or normal (3.0–3.8 kg) birth weight. They were thus studied before the development of any of the conditions which in later adult life can characterise the ‘Small Baby Syndrome’ [1–3] The two groups were otherwise acceptably matched (except in gender) and it was confirmed that no subject had any condition known to be associated with endothelial dysfunction (except for the inadvertent inclusion of some occasional ever-smokers despite initial denial, illustrating the unreliability of smoking history in this age group).

The study shows that flow-related but not GTN-induced dilatation was impaired in fit subjects of low birth weight compared with those of normal birth weight, despite the absence of any identified cause of endothelial dysfunction. The difference was greater and more significant after exclusion of any subjects exposed to active or passive smoking which might have accounted for impairment of endothelial function.

The loss of flow-related dilatation cannot be attributed to the preponderance of females in the low birth weight group because flow-mediated dilatation is no less in premenopausal women than in men [39]. Nor can it be attributed to the slight difference in brachial artery basal diameter because this would have accounted potentially for a slight increase, rather than a decrease in flow-mediated dilatation [35,38].

Flow-related dilatation of the brachial artery has been shown to be mediated mainly by nitric oxide activity and to provide a measure of endothelial function [24,40]. The NO-dependent component was not measured in this non-invasive study but it is this which is lost in disease states where in recent studies it has been tested with intra-arterial L-NMMA [24]. Endothelial dysfunction thus measured appears to reflect a generalised deficiency of endothelial function in the cardiovascular system [16] and specifically of flow-mediated NO release, which has physiological relevance that differs from that of receptor-mediated stimulation of endothelial nitric oxide synthase (eNOS) and is mediated through different signalling pathways. The study thus supports the hypothesis that endothelial dysfunction could be an intrinsic feature of the Small Baby Syndrome, being found in the absence of known cause of endothelial dysfunction and preceding the potential development in later adult life of its clinical manifestations.

An association of endothelial dysfunction with small birth weight is supported also by a recently reported multicentre study of 333 children aged 9–11 years, in which similarly measured flow-mediated dilatation showed a significant, independent, albeit small correlation with birth weight [41].

The interest of the finding lies in the central role which endothelial dysfunction might potentially play in linking the features whose association characterises the syndrome associated with low birth weight. Endothelial dysfunction (i) predisposes to atherogenesis and thus to atheromatous coronary artery disease [15,16,42,43] and (ii) disturbs normal vascular control mechanisms, reducing distensibility of large arteries, increasing resistance of small arteries (thereby reducing vasodilator reserve) and leading to heterogeneity of microvascular tone and perfusion as demonstrated experimentally [44]. It can increase blood pressure [45]. It may prejudice the adequacy of perfusion by causing heterogeneity of tissue perfusion, even if total organ flow is maintained, as a result of patchy underperfusion and/or paradoxically increased total tissue flow [16,44]. This was shown in an experimental model of microembolisation to be associated with microvascular ‘steal’ attributable to increased local release of adenosine [46]. Endothelial dysfunction could therefore impair those functions which are flow-limited, limiting skeletal muscle glucose delivery and uptake, for example, and in this way contributing to insulin resistance [22]. Pancreatic β-cell dysfunction, as evidenced by high relative serum levels of proinsulin in patients with glucose intolerance, insulin resistance, NIDDM, hypertension and microvascular angina [22,47–49], may represent a further consequence of impaired microvascular perfusion, in this case, of the relatively poorly vascularised pancreatic β-cell clusters [50]. Functional disturbances in endothelial dysfunction do not exclude the chronic development of structural changes [51], whose presence could account for relative therapeutic irreversibility of consequences. Moreover, insulin is a foetal growth factor [52] and foetal insulin deficiency [53] could contribute to prejudicing endothelium-dependent angiogenesis and normal microvascular development.

Experimental evidence supports the concept of foetal programming. Prenatal exposure of rats to maternal dietary protein deprivation reduces neonatal β-islet cell mass and vascularisation [50], irreversibly alters the expression and activity of key insulin-sensitive hepatic enzymes [54,55], and leads in young adult offspring to insulin resistance [2,56,57] and hypertension [58]. The Thrifty Phenotype hypothesis is based on such irreversible developmental consequences of foetal diversion of scarce nutrients from visceral organs such as the pancreas and liver [2,59].

In conclusion, the present study suggests that endothelial dysfunction may be an early feature of the constellation of associated and inter-related consequences of foetal malnutrition, associated with insulin resistance (to which it may contribute) and preceding the development of hypertension, diabetes and ischaemic heart disease (to which it may contribute). An encouraging aspect of this hypothesis is the dynamic nature of endothelial function, suscep-
tible as it is to modulation. How foetal malnutrition might cause endothelial dysfunction remains unknown, as does the mechanism of the implied reduction in NO activity, whether this be from reduced production or increased inactivation by oxygen free radicals.

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