Impaired Microvascular Vasodilatory Function in 3-Month-Old Infants of Low Birth Weight

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OBJECTIVE — Low birth weight has been linked to an increased risk of type 2 diabetes and cardiovascular disease in adult life. The fetal insulin hypothesis proposed that a genetic predisposition to insulin resistance may also influence vascular development. Therefore, impaired vascular function may be an intrinsic abnormality in low–birth weight infants that antedates clinical features of the insulin resistance syndrome.

RESEARCH DESIGN AND METHODS — Two groups of 3-month-old term infants were included in the study: 17 infants of lowest quartile birth weight (LQBW) and 21 infants of highest quartile birth weight (HQBW). Three aspects of skin microvascular function were examined; response to local heating, response to acetylcholine iontophoresis, and capillary density.

RESULTS — Median (interquartile ranges) birth weights of the LQBW and HQBW infants were 3,140 g (2,738–3,254) and 3,920 g (3,750–4,020), respectively. Skin maximal hyperemic response to local heating was 2.14 V (1.68–2.30) in the LQBW group vs. 2.44 V (1.96–2.90) in the HQBW group ($P = 0.020$), and the endothelium-dependent vasodilatory response was 1.03 V (0.62–1.32) in the LQBW group vs. 0.78 V (0.45–1.32) in the HQBW group ($P = 0.297$). Capillary density in the LQBW and HQBW groups were 46.3 mm^{-2} (40.1–53.7) and 44.1 mm^{-2} (41.7–56.0), respectively ($P = 0.736$).

CONCLUSIONS — Skin maximal hyperemic response was lower in LQBW infants, although no reduction in capillary density or defect in endothelium-dependent vasodilatation was observed. Such a lower maximal hyperemic response in early life in LQBW subjects who are at risk for type 2 diabetes and cardiovascular disease supports the hypothesis that impaired microvascular function is an early antecedent to diabetes in later life.

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Retrospective studies in the U.K. have shown that low birth weight is associated with the development of type 2 diabetes, hypertension, and increased cardiovascular mortality in adult life, which are clinical features of insulin resistance syndrome (1). This association is independent of social class and adult BMI and has been confirmed in studies conducted in other populations (2) and countries (3,4). Barker (5) proposed the fetal programming hypothesis to explain the association, stipulating that undernutrition in utero leads to impaired fetal growth, which may permanently program the structure and function of the pancreas, thus predisposing later development of type 2 diabetes. An alternative or complementary explanation is the fetal insulin hypothesis, which states that the same polygenic factors that increase insulin resistance in utero and in adult life produce two phenotypic expressions: an infant of low birth weight and an adult with an increased risk for diabetes and hypertension (6).

In parallel with these observations has been the understanding that endothelial dysfunction is a crucial biological determinant of cardiovascular disease (7). Furthermore, impaired endothelium-dependent vasodilatation has been linked to key components of insulin resistance syndrome (8–11). This increases the possibility that endothelial dysfunction could be an intrinsic feature of the insulin-resistant state (12), which could explain the association between insulin resistance and increased cardiovascular risk. In support of this hypothesis, impaired endothelial function has been reported in children (13) and young adults with low birth weight (14).

The fetal insulin hypothesis suggests that a genetic predisposition to insulin resistance may also affect vascular development (6). In support of this suggestion are the findings that skin capillary density and microvascular vasodilatory function were found to correlate inversely with blood pressure and positively with insulin sensitivity in young healthy adults (15).

In the present study, we examined microvascular vasodilatory function and capillary density in 3-month-old infants with birth weight in the highest and lowest quartiles to test the hypothesis that impaired microvascular structure or function may be an intrinsic abnormality present in infants with low birth weight in early life.

RESEARCH DESIGN AND METHODS

— Two groups of infants were recruited through the Maternity Unit of the Royal Devon and Exeter Hospital: lowest quartile birth weight (LQBW), birth weight <25th centile, and highest quartile birth weight (HQBW), birth weight >75th centile on the Castlemead growth chart (16). Only singleton infants born at term (i.e., gestational age

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Abbreviations: HQBW, highest quartile birth weight; LDPI, laser Doppler perfusion imager; LQBW, lowest quartile birth weight.

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

≥37 weeks, confirmed by ultrasonography) were recruited. The exclusion criteria were maternal history of heart disease, hypertension, preeclampsia, diabetes, epilepsy, alcoholism, chronic drug treatment (e.g., steroids, anticonvulsants), any serious infections/sepsis during pregnancy, as well as any known genetic/chromosomal or metabolic disorders in the infants.

Mothers were approached by mail with a letter and a study information sheet during the first 4 weeks after the birth of the infants. Mothers who were interested in the study were requested to return a reply slip inviting further contact. Information about the mother's pregnancy and smoking habits, family history of diabetes, and the infant's anthropometric measures (birth weight, head circumference, and length) were obtained from the obstetric records. Written consent was obtained from the mothers. The study was approved by the Exeter Medical Research Ethics Committee.

The infants were studied after eating, while lying in a cot in a temperature-controlled environment (21.5–22.5°C). A thermocouple (Comark Electronic, Littlehampton, Sussex, U.K.) was used to record skin temperature on the abdomen and thigh. Heart rate and blood pressure were also recorded in subjects who tolerated the procedure using a semiautomatic blood pressure recorder (Dinamap; Critikon, Tampa, FL). An electrocardiograph (Sicard 440; Siemens) was used to assess heart rate in infants who did not tolerate blood pressure measurement. Skin vascular function tests were modified from protocols used in previous studies on adults.

Table 1—*Characteristics of the study subjects*

Parameter	LQBW	*n*	HQBW	*n*
Birth weight (g)	3,140 (2,738–3,254)	17	3,920 (3,750–4,020)	21
Sex (M/F)	7/10	17	12/9	21
Heart rate (min^{-1})	139 (129–160)	16	151 (142–157)	16
Systolic blood pressure (mmHg)	100 (94–109)	10	101 (90–112)	12
Diastolic blood pressure (mmHg)	63 (50–72)	10	67 (53–83)	12

Data are median (interquartile range) or *n*; *n* refers to the number of subjects from whom information was obtained.

Maximal hyperemic response

The infant's thigh was warmed gently using a hair dryer to a temperature of ~36°C. A small brass heater of 1 cm^2 in diameter was then applied to the thigh. The heating element was modified from a transcutaneous oxygen tension monitor used routinely in special-care infant units and has been described in detail elsewhere (17). The heater warmed the skin to between 42 and 44°C, at which temperature maximal vasodilatation is achieved (18). After a 20-min warming period, the heater was removed and the cutaneous hyperemic response was assessed by scanning the heated area of the thigh with a laser Doppler perfusion imager (LDPI) (Lisca Pim 1.0; Lisca Development AB, Linkoping, Sweden). The responses obtained by the LDPI were recorded on a computer as color-coded images of red-cell flux and subsequently analyzed using Pim 2.3 software.

Cutaneous microvascular endothelium-dependent vasodilatation

To examine endothelium-dependent vasodilatation, the response of the abdominal skin microcirculation to the iontophoresis of acetylcholine was determined. The abdominal skin was chosen to minimize movement artifact and to provide a relatively flat surface for the adherence of the iontophoresis chamber. This technique has been described in detail elsewhere (19). Briefly, a perspex chamber was placed on the abdominal skin and filled with the study solution. An indifferent electrode was placed on the left lower thigh. A small electrical charge was applied using a battery-powered iontophoresis controller (MIC 1; Moor Instruments, Axminster, Devon, U.K.) to transfer the study substance across the skin. Iontophoresis of 3% mannitol (acetylcholine carrier) (Royal Devon and Exeter Hospital Pharmacy, Exeter, Devon, U.K.) was performed on one skin site followed by 1% acetylcholine (Miochol, Bracknell, Berks, U.K.) on another abdominal skin site. The protocol involved the application of five 20-s pulses of 100-μA current with intervening periods of 60 s, with no current between each pulse. The skin microvascular response to the substances was quantified by using the LDPI at the end of the protocol. Skin red-cell flux to acetylcholine was expressed as the absolute response to acetylcholine minus the response to the mannitol in arbitrary units of volts (V) and analyzed in the same fashion using the Pim 2.3 software package.

Capillary density

The infant's foot was visualized using a videomicroscopy system. The foot was illuminated under an objective lens (Leitz Wetzlar, U.K.; final magnification ×200) using a mercury vapor lamp and fiberoptic cable. The skin was coated with nail varnish to reduce light scattering. Images of the superficial dermal capillaries over a 1-cm^2 area were recorded on videotape via a video camera (Hitachi CCTV camera; Hitachi, Yokohama, Japan) and a videocassette recorder (Panasonic AG-6200; Panasonic, Osaka, Japan). The video images were examined by an independent blinded assessor to ensure that the qualities for the LQBW and HQBW groups were comparable. The video images were then analyzed by a single blinded investigator, who marked the presence of all capillary images within the defined area on an acetate sheet placed on the monitor screen. Capillary density for each subject was determined using the mean value of the capillary density in all of the images obtained for that subject.

Statistical analysis

Power calculations suggest that our sample size provided a 90% chance of detecting a 27% difference in maximal hyperemic response, a 43% difference in endothelium-dependent vasodilatation (acetylcholine), and a 14% difference in capillary numbers at 5% level of significance. A >50% reduction in maximal hyperemic response and a 15% reduction in capillary numbers were observed in a previous study of young relatives (age 23–33 years) of hypertensive patients who were at risk for developing hypertension in later life (20). A 63% difference in endothelium-dependent vasodilatation was previously reported between small-for-gestational-age and appropriate-weight-for-gestational-age infants (21). Comparisons of the microvascular func-

tion tests and other parameters between the low-birth-weight and high-birth-weight infants were made using the nonparametric Mann-Whitney *U* test. Spearman's rank-correlation coefficients (r_s) were calculated where appropriate. Data are presented as the median value with interquartile range in the text.

RESULTS — A total of 17 LQBW subjects (7 males and 10 females) and 21 HQBW subjects (12 males and 9 females) were included in the study. The median (interquartile range) birth weights, heart rates, and systolic and diastolic blood pressures of the LQBW and HQBW groups are listed in Table 1. No significant differences were shown in any of the parameters (other than birth weight) between the two groups. None of the infants had a parental history of diabetes. None of the mothers smoked during pregnancy, and only mothers of four infants (all in the LQBW group) were ex-smokers.

Basal skin temperature

There was no significant difference in the skin temperature on the abdomen or thigh between the two groups: LQBW abdomen 34.4°C (33.8–35.0), HQBW abdomen 34.1°C (32.9–34.7), $P = 0.719$; LQBW thigh 32.3°C (31.2–33.1), HQBW thigh 32.3°C (31.6–32.7), $P = 0.905$.

Maximum hyperemic response

The maximum hyperemic response was 2.14 V (1.68–2.30) in the LQBW group vs. 2.44 V (1.96–2.90) in the HQBW group ($P = 0.020$) (Fig. 1). The maximum hyperemic response was correlated with the birth length ($r_s = 0.437$, $P = 0.007$) (Fig. 2), head circumference ($r_s = 0.423$, $P = 0.009$) (Fig. 3), and placenta weight ($r_s = 0.417$, $P = 0.009$) (Fig. 4). The vasodilatory response was not correlated with the heart rate, systolic blood pressure, diastolic blood pressure, thigh skin temperature, or gestation period.

Response to iontophoresis of acetylcholine

The mean red-cell flux in response to iontophoresed acetylcholine was 1.03 V (0.62–1.32) in the LQBW infants vs. 0.78 V (0.45–1.32) in the HQBW infants ($P = 0.297$).

Capillary density

Due to the difficulty in keeping the subjects relatively immobile during video microscopy of the foot, good images of skin capillaries were recorded in only 16 LQBW and 17 HQBW infants. The mean capillary density for the LQBW and HQBW infants were 46.3 mm^{-2} (40.1–53.7) and 44.1 mm^{-2} (41.7–56.0), respectively ($P = 0.736$).

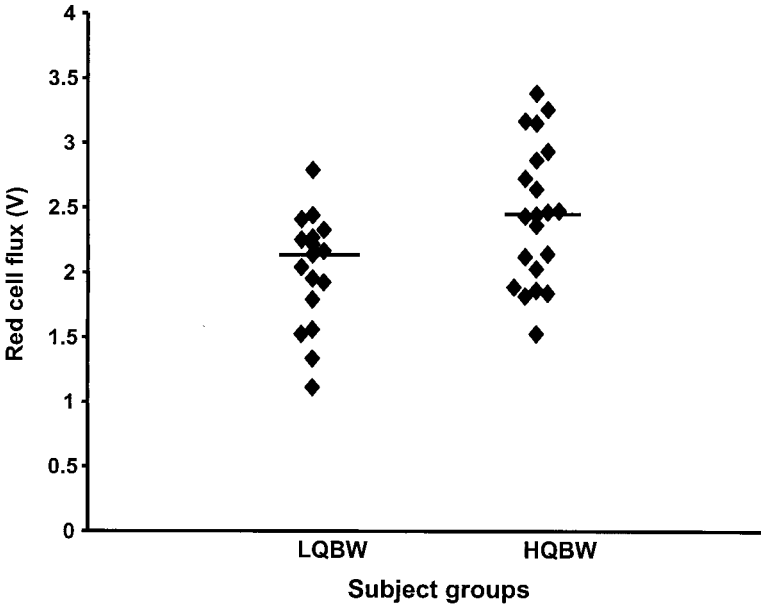

Figure 1—*Skin maximal hyperemic response to local heating in the LQBW and HQBW infants.*

Discussion

This study has demonstrated that cutaneous microvascular vasodilatory function (the maximum hyperemic response) is lower in LQBW infants compared with their HQBW counterparts at 3 months of age. In contrast, skin capillary density and the vasodilatory response to iontophoresed acetylcholine were similar in the two groups.

The small skin areas available and the desire to avoid close contact between the infants' eyes and the mercury vapor light source used in the estimation of capillary

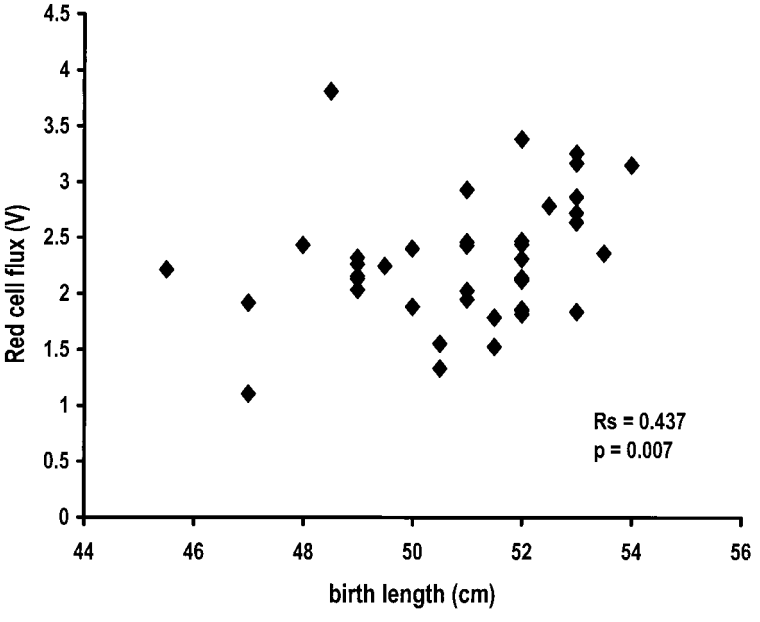

Figure 2—*Relationship between skin maximal hyperemic response to local heating and birth length.*

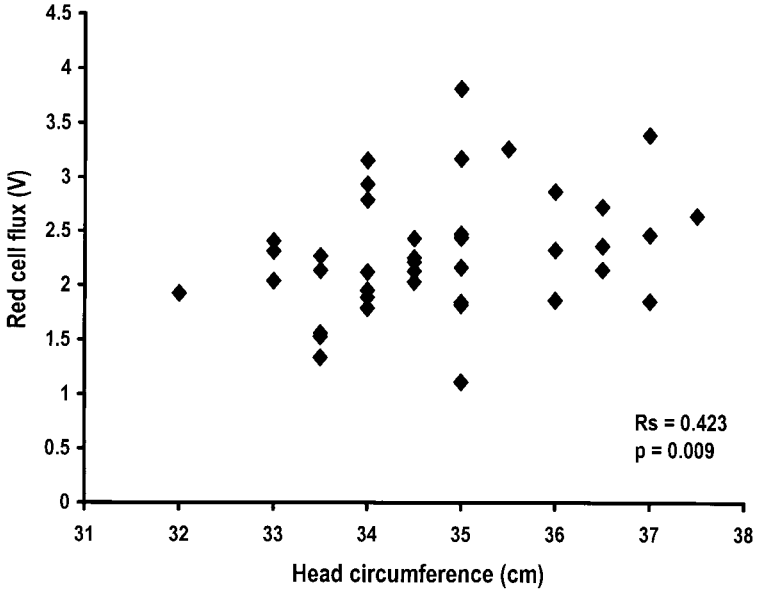

Figure 3—*Relationship between skin maximal hyperemic response to local heating and head circumference at birth.*

density made it impossible to perform all of the investigations of microvascular function on the same skin region. However, this is unlikely to alter the interpretation of the present data because previous studies have demonstrated impaired maximal hyperemic responses on the abdomen (17,22) as well as on the foot with a good correlation between the two measurements in type 1 diabetes (17) and impaired microvascular responses of both the foot and forearm of type 2 diabetic (19,23) and fasting hyperglycemic subjects (24,25).

A defect in cutaneous maximal hyperemia induced by local heating has been described in subjects with recently diagnosed type 2 diabetes (23) as well as in adults with fasting hyperglycemia (glucose concentration 5.5–7.8 mmol/l), who are at increased lifetime risk for developing type 2 diabetes (24). Another study revealed a correlation between maximal hyperemic response and calculated insulin sensitivity in subjects with fasting hyperglycemia (26), supporting the hypothesis that microvascular functional derangement may be linked with or a common antecedent of, insulin resistance syndrome. In normoglycemic adults, the maximal hyperemic response to local heating is inversely correlated with the level of plasminogen activator inhibitor 1 (27), which is a feature of insulin resistance syndrome (28).

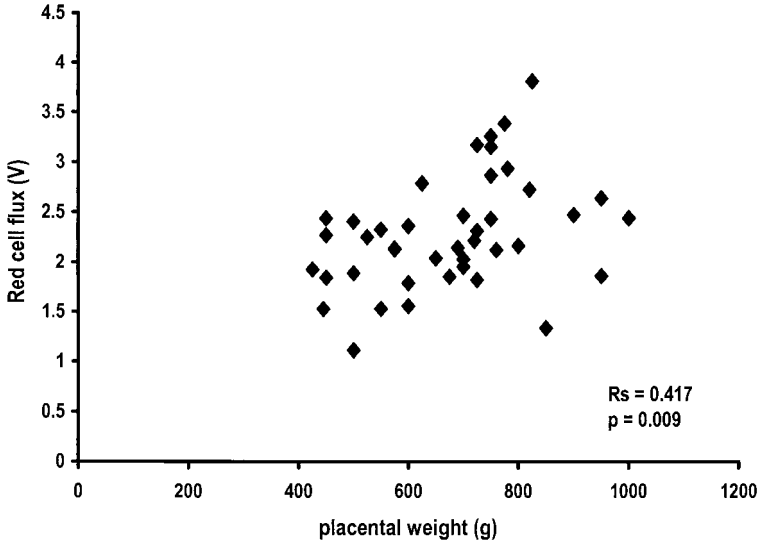

Figure 4—*Relationship between skin maximal hyperemic response to local heating and placental weight.*

The determinants of heat-induced vasodilatation remain to be elucidated but theoretically could involve structural factors (microvascular density and microvascular compliance) as well as neural, endothelial, and vascular smooth muscle function. Previous studies indicate that cutaneous capillary density is not reduced in normotensive type 2 diabetic patients (29), and in the present study, LQBW infants at risk for this condition had baseline capillary densities similar to those in the HQBW infants. It should be emphasized that the measurements of capillary density obtained in the present study were measures of the number of perfused capillaries at that time. It has been demonstrated previously that the application of a cuff to cause venous occlusion results in an increased number of visible capillaries, i.e., capillary recruitment occurs (30). In this study, inflation of a cuff around the infant's ankle to achieve capillary recruitment induced too much movement artifact. Therefore, it was not possible to confirm or refute the observations of Serne et al. (15), which suggested that skin capillary recruitment is correlated with insulin sensitivity and birth weight in young healthy adults.

There is considerable evidence that impaired endothelium-dependent vasodilatation may precede the development of diabetes and may even contribute to the development of diabetes per se by interfering with insulin-induced muscle hyperemia in the postprandial state, thereby limiting glucose disposal (31). Impaired endothelium-dependent function, but not endothelium-independent function, has been demonstrated in adult subjects with impaired fasting glucose (glucose concentration 6.1–7.0 mmol/l) by measuring forearm blood-flow responses to intra-arterial infusion of vasoactive agents (32). These results contrast with findings in subjects with fully developed type 2 diabetes who showed defects in both endothelium-dependent and endothelium-

independent vasodilatation (19). Further evidence that endothelium-dependent vasodilatation might be impaired in subjects at risk for developing type 2 diabetes comes from studies of normoglycemic women with a history of gestational diabetes who were shown to have impaired flow-mediated dilatation (a measure of conduit artery endothelium-dependent vasodilation) (33) and cutaneous microvascular response to iontophoresis of acetylcholine (34). Of more relevance to the present study, Leeson et al. (13) revealed a correlation between flow-mediated dilatation and birth weight in healthy school children. Similarly, Goodfellow et al. (14) found an impairment in endothelium-dependent vasodilatation in the conduit arteries of young adults of low birth weight compared with normal-birth-weight counterparts. McAllister et al. (35) examined the forearm blood-flow response to intra-arterial infusion of acetylcholine and sodium nitroprusside in 12 young adults with low birth weight compared with control subjects of normal weight. In low-birth-weight subjects, the von Willebrand factor (regarded as a circulating marker of endothelial activation/damage) was elevated, but the blood flow responses to acetylcholine and nitroprusside were not impaired. Martin et al. (21) reported an impairment in endothelium-dependent vasodilatation in small-for-gestational-age infants compared with the appropriate-weight-for-gestational-age infants at 3 days of age. However, at this time, dramatic functional and structural changes are still occurring in the macrocirculation and microcirculation as adaptation to the extrauterine environment takes place (36). Indeed, previous studies using laser Doppler flowmetry have documented marked changes in skin blood flow during the first 5 days of life (37). At the age of 3 months, the transient changes in blood volume (38) and metabolism that follow parturition have largely stabilized, and the maturation of biological rhythms of the cardiovascular and thermoregulatory system would have occurred (39,40). Of particular relevance is the observation that hematocrit, a key determinant of blood viscosity and hence red-cell flux is reported to be high in low-birth-weight neonates during the first few days of life (41), which may have contributed to the profound reduction in skin blood flow in 3-day-old small-for-gestational-age infants observed by Martin et al. (21).

Although our study failed to demonstrate an impairment of endothelium-dependent vasodilatation in LQBW infants at 3 months of age, the results do not negate the possibility that low-birth-weight subjects could have an inherited propensity or been programmed to develop endothelial dysfunction. In addition to the production of vasoactive substances, the endothelium has several other functions, such as regulation of hemostasis and cell growth. Because we only examined the endothelium-dependent vasodilatory capacity in the skin microcirculation in our study, the possibility that other aspects of endothelial function could be impaired at the age of 3 months cannot be excluded. It is also plausible that endothelial dysfunction may only manifest in childhood or early adulthood in at-risk subjects as accompanying subtle metabolic changes emerge or perhaps through the accumulation of visceral fat, to which such individuals may be predisposed (42). Evidence of childhood metabolic changes comes from a study of 7-year-old children in Salisbury, which revealed higher plasma glucose levels in response to a glucose challenge in those who were thin at birth and greatest fasting and 30-min insulin responses in those who were heaviest at 7 years of age (43).

CONCLUSIONS — We have demonstrated for the first time that cutaneous maximal hyperemic response is lower in 3-month-old infants in a LQBW group who are at increased risk for developing type 2 diabetes and cardiovascular disease in adult life. This difference does not seem to be associated with reduced baseline number of perfused capillaries or endothelium-dependent vasodilatation in the cutaneous vascular bed at this stage in life. Nevertheless, the finding of a lower response in this LQBW group soon after birth is suggestive evidence that reduced microvascular vasodilatory function is an early antecedent to diabetes in later life.

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