Maternal food restriction in the second half of pregnancy affects vascular function but not blood pressure of rat female offspring

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Food restriction during pregnancy in rats induces intrauterine growth retardation with consequences persisting into adulthood. In the present study we have investigated the hypothesis that malnutrition in pregnant rats may lead to altered cardiovascular function in adult female offspring. Perinatal growth retardation was induced by a 50 % reduction of normal dietary intake in rats during the second half of pregnancy. Systolic and diastolic blood pressure values and heart rate were recorded in conscious female offspring (100 d old) using a femoral artery probe. No significant differences in heart rate, or in systolic and diastolic blood pressures were recorded between control offspring and offspring of nutritionally deprived rats. In order to ascertain whether cardiovascular variables in the offspring were influenced by lactation, subgroups of offspring from food-restricted dams were fostered with lactating dams fed on a normal diet. Blood pressure and heart rate were also found to be normal in these offspring. The rise in blood pressure associated with NO inhibition was similar in all groups. Isolated resistance artery function was assessed *in vitro* in offspring (100–120 d old) of a second group of semi-starved dams. Small mesenteric arteries from these animals showed reduced endothelium-dependent relaxation (to acetylcholine and bradykinin), but enhanced sensitivity to exogenous NO (sodium nitroprusside). We conclude that food restriction during the second half of pregnancy and/or lactation does not induce hypertension in adult offspring, but may effect subtle changes in vascular function.

Food restriction: Intrauterine growth retardation: Vascular function

The concept that some adult diseases may result from *in utero* 'programming' of the fetus has been the subject of much recent investigation. Early studies from one of our laboratories reported that experimentally induced insulin-dependent diabetes in pregnant rats was associated with disturbed glucose handling and gestational diabetes in the offspring (Aerts & Van Assche, 1979; Holemans *et al.* 1991). Studies in human subjects have since documented that low birth weight, particularly thinness at birth resulting from impaired fetal growth in mid to late gestation, is associated with hypertension (Barker, 1995), coronary heart disease (Phillips *et al.* 1994) and non-insulin-dependent diabetes (Barker *et al.* 1993; Leon *et al.* 1996) in later life. Furthermore, blood pressure in 40-year-old offspring is inversely related with the mothers' intake of animal protein

and carbohydrate in late pregnancy (Campbell *et al.* 1996). Prospective nutritional studies are few and necessarily confined to investigations in animals, but the hypothesis has been strengthened by studies in which rats exposed to malnutrition *in utero*, either in the form of protein deprivation (Langley & Jackson, 1994; Langley-Evans *et al.* 1996*a*) or of severe food restriction (Woodall *et al.* 1996), demonstrated significantly raised systolic blood pressure in adulthood.

In the present study we have evaluated a number of cardiovascular variables in adult female rats previously growth retarded *in utero*. Growth retardation was induced by food restriction (50 % normal diet) during the second half of pregnancy (with or without food restriction during lactation). In the first series of experiments heart rate, and

systolic and diastolic blood pressures were evaluated in conscious 100-d-old offspring of food-restricted rats and in age-matched controls. In a second series of experiments, constrictor responses and endothelium-dependent dilator function were determined in small mesenteric arteries from the adult offspring (100–120 d old) of food-restricted maternal rats.

Materials and methods

The entire protocol was reviewed and approved by the local ethics committee for animal procedures (Katholieke Universiteit Leuven, Belgium).

Study 1: heart rate and blood pressure measurements

Animals. The animals used in this study were 100–120-d-old female offspring of control and food-restricted maternal rats (Leuven/pfd, KULeuven Breeding Centre, Leuven, Belgium). The control group consisted of nine female offspring of three pregnant control Wistar rats (three female offspring of each maternal rat). During pregnancy and lactation the rats had free access to a standard non-purified diet (Trouw, Ghent, Belgium) with an approximate composition of (g/kg): 210 protein, 40 fat, 510 carbohydrate, 70 ash, 50 cellulose and 120 water. The food-restricted group consisted of nine female offspring of three food-restricted maternal rats (three female offspring of each maternal rat). Food-restricted maternal rats received 11 g/d of a standard non-purified rat diet from day 11 of pregnancy until the day of delivery (day 23) and 20 g/d of the same diet during the entire lactation period. This food restriction scheme ensured that the rats received 50 % of the *ad libitum* food intake (Aerts *et al.* 1990). To determine whether *in utero* events or the lactation period had greater influence on the cardiovascular variables studied, pups from three litters were nursed by their mothers (food restriction during pregnancy and lactation; O-FR). Others (pups from three litters) were cross-fostered immediately after birth on three control dams with free access to a standard non-purified diet (food restriction during pregnancy with control lactation; O-FR:CL). In both subgroups three female offspring from each dam were studied (nine in total).

Only offspring of rats with eight or more fetuses were included in the study. After weaning, only the female offspring of each litter were kept and weighed at 21, 28, 35, 49, 70 and 91 d of postnatal age. To prevent maternal rejection, pups were not weighed at birth. All rats had free access to tap water and a standard non-purified diet for the remainder of the study.

Measurement of blood pressure and heart rate. Rats were transported to the Janssen Research Foundation (Beerse, Belgium) when 91 d old. After 1 week of acclimatization measurements of blood pressure and heart rate were carried out as previously described (Xhonneux *et al.* 1990). Briefly, the animals (100 d old) were anaesthetized with diethyl ether and a femoral artery and vein dissected free of surrounding tissue and cannulated. The rats were restrained in Bollman cages and local anaesthesia was induced by administration of lidocaine (20 ml/l; Astra, Huizingen, Belgium) to the wound. Heart rate and blood pressure measurements were recorded from the output of a pressure transducer (Janssen Scientific Instruments Division, Beerse, Belgium) in a femoral artery catheter. When the animals were fully awake (approximately 60 min after diethyl ether withdrawal), systolic and diastolic arterial blood pressures and heart rate were recorded continuously for an equilibration period of 60 min (MacLab®, AD Instruments PTY Ltd, Castle Hill, New South Wales, Australia), during which systolic and diastolic blood pressures and heart rate reached a stable plateau. Recording was then carried out for a further 60 min experimental period over which the mean values were calculated for the three variables using a customized computer program (Janssen Scientific Instruments Division). To evaluate the relative contribution of NO to tonic lowering of blood pressure in each group, 4·63 mmol Nω-nitro-L-arginine methyl ester (L-NAME; Sigma, Bornem, Belgium)/kg was then injected into the venous catheter, and systolic and diastolic arterial blood pressures and heart rate recorded for a further 120 min. The mean systolic and diastolic blood pressures and heart rate were calculated over the 120 min period. Blood pressure and heart rate were measured on three consecutive days in nine animals simultaneously. The rats were used in a randomized manner.

Study 2: vascular function in isolated arteries

Animals. Female offspring (*n* 11) between 100 and 120 d of age of a second group (*n* 6) of food-restricted maternal rats (O-FR) were studied for assessment of vascular function. Comparison was made with arteries isolated from 100–120-d-old female offspring (*n* 11) of six control maternal rats (O-CR).

Assessment of vascular function. Small mesenteric arteries were mounted as previously described (Mulvany & Halpern, 1977) on a small vessel wire myograph. Arteries were bathed in physiological saline solution (mmol/l: NaCl 119, KCl 4·7, $CaCl_2$ 2·5, $MgSO_4$ 1·17, $NaHCO_3$ 25, KH_2PO_4 1·16, EDTA 0·026, glucose 6·0) at 37° and gassed with CO_2–O_2 (5 : 95, v/v). The passive tension-internal circumference characteristics of the arteries were determined by stretching to achieve an internal circumference equivalent to 90 % of that which would be attained when relaxed *in situ* under a transmural pressure of 100 mmHg. To confirm viability of the arteries five contractions (2 min duration) were performed to 5 μmol/l noradrenaline (NA; Winthrop, Guildford, Surrey, UK), a solution of 125 mmol/l KCl in physiological saline solution, or a combination of both. Arteries failing to produce active tension equivalent to 100 mmHg were rejected. A cumulative concentration response to NA (10^{-9}–10^{-5} mol/l) was constructed and arteries then preconstricted with NA at a concentration (3–5 μmol NA/l) required to give a sub-maximal constrictor response. Vasodilator responses to 10^{-9}–10^{-5} mol/l acetylcholine (Sigma, Poole, Dorset, UK) were determined with additions at 2 min intervals, and after further preconstrictions to NA, responses to 10^{-9}–10^{-5} mol/l bradykinin (Sigma) and 10^{-9}–10^{-5} mol/l sodium nitroprusside (Sigma) were evaluated. In most experiments, two arteries from each rat were investigated

simultaneously and the results expressed as the mean of the data pair.

Biochemical analyses. Tail blood samples for measurement of plasma glucose and insulin concentrations were obtained from the dams at 20 d gestation. Tail blood samples were also taken from the offspring at 80 d of age after an overnight fast for determination of glucose, insulin, triacylglycerols and cholesterol. Plasma glucose was determined with a glucose analyser 2300STAT (Yellow Springs Instruments, Yellow Springs, OH, USA), plasma insulin by radioimmunoassay using rat insulin as a standard, plasma triacylglycerols (Triglycerides GPO-PAP) and cholesterol (Cholesterol CHOL-PAP) with kits from Boehringer (Mannheim, Germany).

Statistical analysis

Heart rate and blood pressure measurement. Data for blood pressure, heart rate and body weight are given as means with their standard errors (SEM). Statistical analyses for heart rate and blood pressure were carried out using a paired *t* test for sequential comparisons in the same animals or ANOVA for multiple comparisons followed by an unpaired *t* test, as appropriate, to assess differences within or between the groups. Two-tailed probabilities $P < 0.05$ were considered significant. Growth curves were analysed by ANOVA for multiple comparison followed by a *t* test ('Statistica'; Statsoft, Tulsa, OK, USA).

Vascular function in isolated arteries. All values are given as means with their standard errors. Tension was calculated as milliNewtons (mN)/mm artery length. To account for variation in artery diameter, concentration responses to NA were expressed as a percentage of the contractile response to a depolarizing K^+ buffer (124 mmol/l K^+-substituted physiological saline solution). Relaxation to acetylcholine, bradykinin and sodium nitroprusside was expressed as a percentage of the initial precontraction to NA. The concentration (mol/l) which elicited half the maximal response (EC_{50}), was calculated for each concentration response curve. Values are given as the pEC_{50}, the negative log of EC_{50}. When calculation of the pEC_{50} was not appropriate, responses to vasodilators were compared by the maximum responses to the agonist and curves were compared by summary score (Matthews *et al.* 1990). Summary values are calculated from individual data points as the sum of responses in each curve divided by the number of concentrations tested. Unpaired Student's two-tailed *t* test or the Mann–Whitney *U* test was used for comparison of parametric and non-parametric data respectively (both by 'Instat'; GraphPad Software Inc., San Diego, CA, USA). Significance was assumed if $P < 0.05$.

Results

Study 1: heart rate and blood pressure measurement

Litter size and mortality. Litter sizes were not significantly altered by food restriction of the maternal rat (pups per litter: controls 10·14 (SE 0·14); food restricted, 10·0 (SE 0·62)). Pup mortality rates per litter in O-FR and O-CR groups were not significantly different (4·6 (SE 4·6) *v.* 1·4 (SE 1·4) %; $P = 0.465$).

Growth rates in offspring of food-restricted rats (Fig. 1). Body weight was significantly reduced in weaned (21-d-old) female O-FR compared with O-CR ($P < 0.0001$). The growth rate subsequently paralleled that of O-CR, but body weight at each point of measurement remained significantly lower ($P < 0.0001$). Newborns of food-restricted rats, fostered by a mother on normal dietary intake (O-FR:CL) weighed more at 21 d than O-FR ($P < 0.0001$) but weights were lower than those of O-CR ($P < 0.01$). Although early growth rates paralleled those of controls, weights remained significantly lower in O-FR:CL at 91 d ($P < 0.001$).

Systolic and diastolic blood pressures and heart rate (Fig. 2). During the first 60 min of recording after the equilibration period, heart rate and systolic and diastolic blood pressures were similar for the three groups studied. After administration of L-NAME, heart rate decreased significantly when compared with the value just before administration, and systolic and diastolic blood pressures increased significantly in all groups studied. There were no differences in the mean responses to L-NAME, nor in the maximal effect of L-NAME between groups.
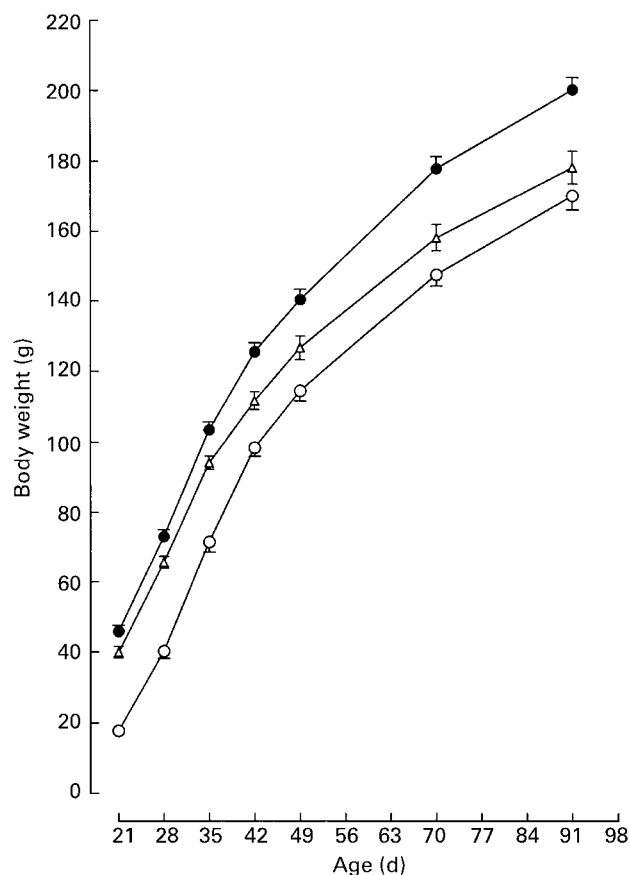

Fig. 1. Postnatal growth curves in female offspring of control rats (●), female offspring of rats food-restricted during pregnancy and lactation (○) and female offspring of rats food-restricted during pregnancy but not lactation (△). Values are means with their standard errors indicated by vertical bars for nine rats per group. For details of procedures, see p. 74.
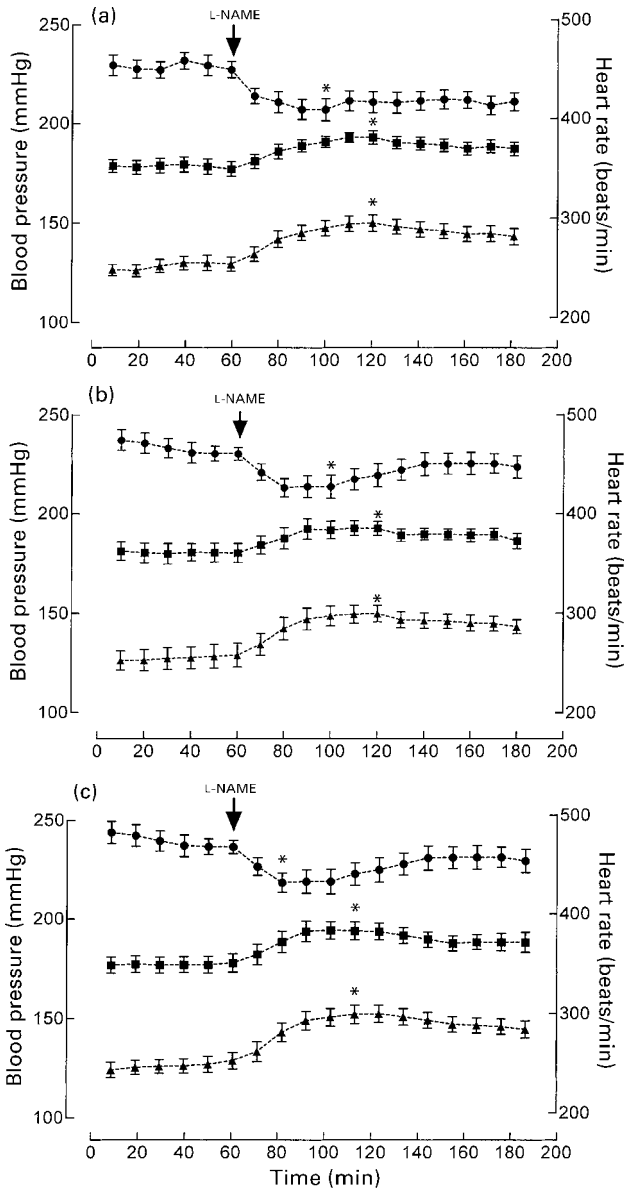
Fig. 2. Systolic (■) and diastolic (▲) blood pressures and heart rate (●) over a 180 min experimental period in (a) offspring of control rats, (b) offspring of food-restricted rats and (c) offspring of food-restricted rats suckled by control rats. For details of procedures, see p. 74. Values are means for nine rats, with their standard errors represented by vertical bars. (↓) Intravenous administration of Nω-nitro-L-arginine methyl ester (L-NAME). Mean values were significantly different from basal values, *$P < 0.05$.

Study 2: vascular function in isolated arteries

General features. The serum glucose and insulin concentrations were lower in food-restricted maternal rats when compared with control pregnant rats (Table 1). The 80-d-old O-FR had lower body weights than O-CR, $P < 0.0001$ (Table 2). Glucose concentrations were higher in O-FR than in O-CR, whereas insulin concentrations were lower than in O-CR ($P < 0.05$). Fasting plasma triacylglycerol and cholesterol concentrations were normal in O-FR (Table 2).

Vascular function. Mean vessel diameters of the arteries from O-CR and O-FR were similar (303 (SE

Table 1. Study 2. Body weight and non-fasting plasma glucose and insulin concentrations in control and food-restricted maternal rats on day 20 of gestation

(Mean values with their standard errors for six rats per group)

	Control		Food-restricted	
	Mean	SEM	Mean	SEM
Body weight (g)	303	2	248***	3
Plasma glucose (mmol/l)	5·33	0·13	4·17**	0·11
Plasma insulin (nmol/l)	0·16	0·03	0·03**	0·01

Mean values were significantly different from control: **$P < 0.01$, ***$P < 0.0001$.

7) μm, *n* 22 and 276 (SE 11) μm, *n* 22 respectively). Maximum responses to 125 mmol/l K-buffer were significantly reduced in the O-FR compared with O-CR (4·01 (SE 0·20) *v.* 4·72 (SE 0·27) mN/mm; $P < 0.05$). There was no significant difference in the constrictor response to NA between O-CR and O-FR either in sensitivity (pEC$_{50}$: 5·63 (SE 0·06) in O-CR *v.* 5·72 (SE 0·05) in O-FR, $P = 0.293$) or in maximal response (97·85 (SE 1·90) % of maximum constriction to K$^+$ *v.* 102·53 (SE 2·80) %, $P = 0.170$). Maximal relaxation to the endothelium-dependent vasodilators, acetylcholine and bradykinin were significantly reduced in O-FR (acetylcholine 87·7 (SE 2·65) *v.* 95·51 (SE 1·36) % in O-CR, $P < 0.05$; bradykinin 29·93 (SE 3·15) *v.* 40·97 (SE 2·80) % in O-CR, $P < 0.05$) (Fig. 3(a and b)). In contrast, relaxation to the NO donor, sodium nitroprusside, was enhanced ($P < 0.01$, by summary score) in O-FR (Fig. 3(c)).

Discussion

In this study we have shown that perinatal growth retardation in rats, induced by a 50 % restriction of food to the maternal rate during the second half of pregnancy, when fetal growth is maximal, is not associated with alterations in blood pressure or heart rate in adulthood (100 d). This would contrast, apparently, with a study by Woodall *et al.* (1996) which has shown a small but significant increase in systolic blood pressure (5–8 mmHg) in much older (30-week-old) offspring of severely dietary restricted rats (70 % restriction). The study differs from the present investigation as dietary restriction was imposed on the maternal rats from day 1 of pregnancy (Woodall *et al.* 1996), whereas food restriction was started on day 11 in the present study. The degree of food reduction or an age-related development of raised blood pressure could potentially explain the difference in results, and the findings might also suggest that dietary restriction in early pregnancy could be an important determinant of blood pressure in the offspring. The latter proposal is not supported by a study (Langley-Evans *et al.* 1996*c*) which observed no significant effect of maternal protein restriction in the first week of pregnancy on blood pressure of 7-week-old female offspring, and only a minor, but significant elevation in males. However, to our knowledge, no study has similarly evaluated the effect of reduction of all components of the diet in early pregnancy on the blood pressure of offspring of a similar age to those we have studied.

Table 2. Study 2. Body weight and fasting plasma glucose, insulin, triacylglycerol and cholesterol concentrations in 80-d-old female offspring of control maternal rats (O-CR) and of food-restricted maternal rats (O-FR)

(Mean values with their standard errors for eleven rats per group)

	O-CR		O-FR	
	Mean	SEM	Mean	SEM
Body weight (g)	179	4	134***	1
Plasma glucose (mmol/l)	6·41	0·18	6·92*	0·12
Plasma insulin (nmol/l)	0·10	0·01	0·04**	0·01
Plasma triacylglycerols (mmol/l)	0·61	0·04	0·72	0·04
Plasma cholesterol (mmol/l)	1·73	0·08	1·68	0·04

Mean values were significantly different from control: *$P < 0.05$, **$P < 0.01$, ***$P < 0.0001$.

In contrast to the small rise in blood pressure observed by Woodall *et al.* (1996) and the lack of rise in blood pressure in the offspring of dietary deprived rats in the present study, protein restriction (casein 60, 90 and 120 g/kg diet) throughout rat pregnancy results in a very significant increase in systolic blood pressure in 9–21-week-old offspring (Langley & Jackson, 1994). Our results may indicate, therefore, that balanced food restriction during pregnancy has a lesser effect on blood pressure in the offspring than the restriction of specific nutrients. Further studies of dietary deprivation of all constituents throughout pregnancy, and in early pregnancy alone, would determine the relative roles of protein and balanced dietary deprivation on offspring blood pressure.

Theoretically, the relatively small numbers of offspring investigated in the present study might prevent detection of small differences in blood pressure between the groups, but in contrast to the measurement of blood pressure with a tail cuff, as used in most similar studies, the variability in the measurement of the blood pressure using a femoral artery transducer was very small and renders this an unlikely explanation for the lack of difference observed.

The present study, in which blood pressure was evaluated by direct measurement from a femoral artery catheter is, to our knowledge, the first to report direct recording of arterial blood pressure from the offspring of nutritionally deprived rats. Whereas similar blood pressures in control rats have been reported previously (Janssen *et al.* 1989), the blood pressure was higher in our controls than that reported in some studies using the tail cuff method (Langley & Jackson, 1994; Langley-Evans *et al.* 1996a) and others with indwelling transducers (Schiffers *et al.* 1994). There is no obvious explanation for the high degree of variability between studies other than differences in the method of measurement and the use of different strains. The use of female rats alone, which were used in the present study in order to compare with earlier similar investigations from our laboratory, is not a likely factor since previous studies have suggested there is no sex difference (Langley-Evans *et al.* 1996b).

To avoid maternal rejection, birth weights of the newborn pups from the three groups of offspring were not determined. However, we have previously shown body weight to be 20 % lower in 22-d-old fetuses of similarly food-restricted rats (Holemans *et al.* 1997b). Growth retardation was also clearly indicated by the postnatal growth patterns which showed that food restriction during pregnancy (with or without food restriction during lactation) had long-lasting effects on body weight of the adult offspring. It is unlikely that the reduced body weight in O-FR was the result of reduced food intake during growth as we have previously shown that their food intake (relative to body weight) is not different from that of controls (Holemans *et al.* 1997b). It is of interest that the growth curves for the offspring indicated that dietary deprivation during pregnancy alone was
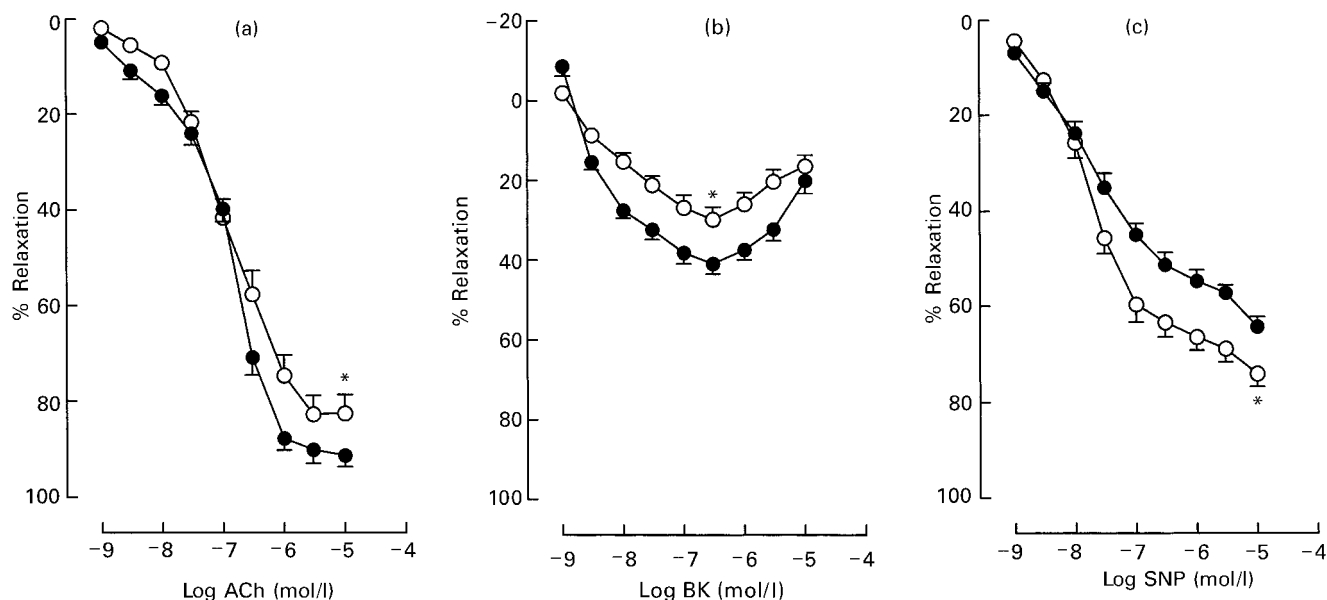

Fig. 3. Concentration-dependent relaxation to (a) acetylcholine (ACh), (b) bradykinin (BK) and (c) sodium nitroprusside (SNP) in mesenteric small arteries preconstricted with submaximal noradrenaline (3–5 μmol/l) from offspring of control rats (●) and offspring of food-restricted dams (○). Tension is given as percentage relaxation of the initial response to noradrenaline. Values are means for ten (control, SNP) or eleven rats, with their standard errors represented by vertical bars. *For ACh and BK, values for the two groups were significantly different ($P < 0.05$) by maximal relaxation and summary scores. For SNP, values for the two groups were significantly different ($P < 0.01$) by summary scores.

sufficient to induce a long-term effect on body weight. Indeed, in a recent study we have shown that the female offspring of rats nutritionally deprived (food restriction or low protein) during lactation alone demonstrated 'catch-up' of body weight (Holemans *et al.* 1997*a*).

Despite normal blood pressures and normal heart rate, we chose to investigate vascular function in the offspring of food-restricted rats suckled by their own dams since previous studies from our laboratory have indicated abnormalities of glucose homeostasis in these animals (Holemans *et al.* 1996, 1997*b*), which could affect vascular function. Subtle changes were recorded in the small mesenteric arteries investigated. The difference in the maximal response to a depolarizing K^+ solution is likely to reflect smaller vascular smooth-muscle mass in growth-retarded offspring and the normal constriction to NA (once corrected for the differences in K contraction), did not suggest a disorder of constrictor function. Small, but significant, reductions were observed in the dilator responses to acetylcholine and bradykinin, indicative of a reduction in synthesis of the endothelium-dependent vasodilator, NO or a decrease in half-life as both agents evoke vasodilatation, at least in part, through NO release. It is relevant that previous studies in the same vascular bed of spontaneously hypertensive rats (Clozel *et al.* 1990; Luscher *et al.* 1992) have shown abnormalities of vascular endothelial dilator function that have been implicated in the evolution of raised blood pressure. In this study relaxation to sodium nitroprusside, which evaluates responsiveness of the vascular smooth muscle to exogenous NO, was enhanced. Theoretically, this could reflect a compensatory response to tonic NO depletion. We suggest that one abnormality offsets the other; the reduction in NO synthesis or availability being paralleled by increased sensitivity, so resulting in no net change of NO-induced vasodilatation, and thus no effect on blood pressure. These observations *in vitro* would accord with those observed after infusion of L-NAME *in vivo*, which showed a similar enhancement of blood pressure in all groups.

To our knowledge only one study has investigated the possibility that growth restriction in human pregnancy may be associated with vascular dysfunction and, in agreement with the present investigation, documented an apparent effect of growth restriction on endothelial function. Leeson *et al.* (1997) used a non-invasive method to evaluate endothelium-dependent dilatation in the brachial artery of children. The authors reported a significant, graded and positive association of birth weight with flow-mediated dilatation, whilst other childhood cardiovascular risk factors were unrelated to flow-induced response.

The mechanism underlying the reduced capacity to synthesize NO is nonetheless of interest as it suggests a defect of endothelial function. There is scant information regarding the effects of dietary deprivation on vascular function whereas dietary excess, in the form of a high lipid intake, has frequently been implicated in endothelial dysfunction. Possible candidates leading to endothelial malfunction in the offspring of the food-restricted animals include the mild degree of hyperglycaemia observed, as we and others (for reviews see Poston & Taylor, 1995; Tribe & Poston, 1996) have implicated hyperglycaemia in endothelial dysfunction associated with insulin-dependent and non-insulin-dependent diabetes mellitus. Hyperglycaemia, through a wide spectrum of biochemical pathways, may reduce endothelial synthesis of NO. The hypoinsulinaemia observed could also blunt NO synthesis as, in some vascular beds, insulin stimulates NO synthase (*EC* 1.14.13.39) (Chen & Messina, 1996). Recent studies in the offspring of food-restricted rats showed that the primary defect in glucose homeostasis appears to be at the endocrine pancreas (Garofano *et al.* 1997). Additionally, O-FR are also insulin resistant (Holemans *et al.* 1996). In contrast to the insulin-resistant patient, insulin resistance is attributable to decreased responsiveness of the liver to insulin, and normal peripheral glucose utilization (Holemans *et al.* 1996, 1997*b*). Thus, those earlier studies, together with the present investigation, lend some experimental support to retrospective epidemiological evidence proposing that low birth weight is associated with non-insulin-dependent diabetes in later life (Barker *et al.* 1993).

To our knowledge few studies have investigated the effect of dietary manipulation in pregnancy on vascular function in the offspring. It is of relevance that in two recent studies we have shown a very pronounced blunting of the response to acetylcholine in the neonatal vasculature from offspring of streptozotocin-diabetic rats on a high-fat diet (Koukkou *et al.* 1997) and in the adult offspring of streptozotocin-diabetic rats (Holemans *et al.* 1998). The abnormalities described in the present study and in our recent work suggest that *in utero* events may have lasting consequences in terms of *in vitro* vascular function. Whether these may contribute to overt adult disease remains to be determined. Further studies are indicated in which dietary restriction is imposed throughout pregnancy and at different stages in pregnancy, and in which the offspring are studied from birth to old age. More detailed evaluation of vascular function in a range of vascular beds would provide insight into the defects observed in this study and the likelihood of their contribution to overt vascular disease.

In conclusion, this study does not support the hypothesis that malnutrition of the fetus *in utero* may contribute to hypertension in adulthood. The modest disorders of vascular function described would tend to offset one another. Nonetheless, the demonstration that *in utero* events can alter vascular function opens an exciting avenue of research, not only in relation to undernutrition, but to a wide range of dietary extremes and pathological disorders of pregnancy that may affect the fetus.

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