ARTICLES

Increased Elastic Tissue Defect Formation in the Growth Restricted Brown Norway Rat: A Potential Link Between In Utero Condition and Cardiovascular Disease

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ABSTRACT: Low birth weight for gestational age has been epidemiologically linked to cardiovascular mortality and morbidity in adult life. This study aimed to determine whether *in utero* growth restriction influences an early feature of atherosclerotic pathology; disruption of the aortic internal elastic lamina (IEL) in the adult Brown Norway (BN) rat. *In utero* growth restriction was induced by bilateral uterine artery ligation on day 18 of gestation, thereby decreasing newborn BN pup weight by approximately 14%. Restriction surgery significantly increased aortic IEL defect number at 8 wk of age in both sexes compared with no surgery animals ($p < 0.002$). At 16 wk of age placental restriction surgery significantly increased the number of defects in males compared with both no surgery and sham surgery control groups ($p < 0.001$). The total number of IEL defects was significantly correlated with several postnatal growth rate parameters, including 72-h postpartum weight. Neither blood pressure was significantly different between treatment groups, nor was it correlated with body weight or IEL defect numbers. The findings of this study seem to support the fetal origins of adult disease hypothesis, by demonstrating that a moderate growth restricting insult dramatically increases aortic elastic tissue defect formation via an apparently blood pressure-independent mechanism. (*Pediatr Res* 64: 125–130, 2008)

Many epidemiologic studies have demonstrated an association between low birth weight and increased mortality and/or morbidity from chronic atherosclerotic diseases (1–4). Most studies investigating the mechanisms behind this relationship have focused on how a poor *in utero* environment may influence cardiovascular risk factors; particularly diabetes, hypertension, and dyslipidemia. Few studies have investigated how a poor *in utero* environment may directly affect vascular structure. The atherosclerotic process has been shown to begin before birth in humans (5,6); the earliest changes being elastic tissue fragmentation and vascular smooth muscle cell proliferation.

In the mid 1920s, Wolkoff and Bork established that the splitting of the internal elastic lamina (IEL) and an associated migration of medial vascular smooth muscle cells via these defects, were the first events in the shift from a normal to atherosclerotic artery (7). Several studies have since demonstrated the involvement of these IEL defects in the initiation and progression of atherosclerosis (5,8), including an apparent correlation between the extent of intimal thickening and the degree of IEL disruption (9).

Spontaneous IEL defects have previously been reported in the caudal, renal, and cerebral arteries of several rat strains (10). Defects first appear from 1 mo of age and form most rapidly up to approximately 4 mo of age (11). The Brown Norway (BN) strain appears to have an increased predilection, having a significantly greater incidence of IEL defects compared with other rat strains (10). Recently IEL defects have been implicated as an early feature of the atherosclerotic lesions, which develop in the apolipoprotein E deficient mouse (12). Both the rat and mouse IEL defects appear morphologically identical to IEL defects observed in the arteries of young humans (8).

The rat bilateral uterine artery ligation model significantly reduces the blood supply to the fetuses and mimics placental insufficiency (13) in the human. A similar model, in the guinea pig, has implicated prenatal environment as a factor that may influence cardiovascular structure in adult animals (14).

This present study therefore investigated the hypothesis that *in utero* growth restriction, induced by bilateral uterine artery ligation in late gestation, may influence IEL defect formation in adult BN rats. The effect of exposure to a late gestational surgical manipulation, *versus* placental restriction, was determined by the inclusion of both no surgery and sham surgery control groups.

METHODS

In utero growth restriction model. All animal studies were approved by the University of Otago Animal Ethics Committee. The University of Otago

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Abbreviations: BN, Brown Norway; BP, Blood pressure; GD, gestational day; IEL, internal elastic lamina; PN, postnatal

BN colony was at generation 35 of inbreeding when received from the University of Pennsylvania in 1986 and have been continuously inbred thereafter. Thirty-one BN dams were used in this study, which were divided into three groups; no surgery controls ($n = 11$), sham surgery ($n = 9$), and placental restriction surgery ($n = 11$). The first day of pregnancy was confirmed by vaginal smears. Pregnancy was confirmed on gestational day (GD) 14 by weight gain (~ 30 g) and dams were randomly assigned into groups. On GD 18, dams in the sham and placental restriction surgery groups were anesthetized (2.5% halothane) and the uterus exposed via a midline anterior laparotomy. Restriction surgery dams had both the uterine arteries ligated (leaving the veins intact) at the origin of each horn using 5-0 prolene suture. The vessels in the sham surgery dams were manipulated, but not ligated. The abdominal wall was then closed in layers.

Careful postoperative monitoring was conducted daily until the dam gave birth on GD23. At 24-h postpartum litter sizes were reduced to four (equal genders) to normalize postnatal (PN) milk access, pups were sexed and the feet tattooed for identification. Pups were weaned at 4 wk and the dams culled. Each litter was randomized to either 8- or 16-wk examination groups. Dam aorta were carefully removed and examined as described below.

Morphometry. Crown rump length (CRL) and body weight were recorded for each pup at 24, 72 h and at 1 wk of age. After this time CRL from all pups was recorded weekly until 4 wk of age and weight measured weekly for the remainder of the study.

Conscious blood pressure (BP) was measured in all animals weekly from 4 to 8 wk of age and again at 15 wk of age in the 16-wk group. Rats were acclimatized to the apparatus for 2 d before data collection. Peak systolic and diastolic measurements were made using a validated, noninvasive, tail-cuff BP system (model 29, IITC Life Sciences Inc.). Direct femoral intra-arterial mean BP was measured in all animals, under ether anesthetic, immediately before sacrifice, at 8 or 16 wk of age. In both BP methods five separate BP profiles were collected, the highest and lowest discarded and the remaining three averaged for analysis.

Anesthetized animals were euthanized and their aorta carefully removed following measurement of *in vivo* infrarenal abdominal aorta length. Kidney, liver, heart (including left ventricular mass), and brain weight was recorded. The aorta was pinned out to its *in vivo* length and opened longitudinally to allow *en face* examination of IEL defect number and severity as previously described (15). Aorta were then fixed in 10% formalin and embedded for histology.

Longitudinally orientated 4- μ m thick sections were cut and stained with Verhoeff's elastic stain. Using the aortic bifurcation as an anatomical reference point, a 10-mm region of the infrarenal aorta was identified and sites 11 mm (proximal), 6 mm (middle), and 1 mm (distal) from the bifurcation were identified. Images of these sites were digitally captured at 66 times magnification. Mean aortic wall thickness at each site was quantified using the ImageJ (v1.34, <http://rsb.info.nih.gov/ij/>) software package. The mean infrarenal aortic wall thickness was calculated as the average of the proximal, middle, and distal values. An infrarenal aortic wall taper ratio was calculated by dividing the mean distal wall thickness by its matching proximal wall thickness.

Statistical analysis. Comparisons of individual maternal weight, weight gain, pup weight, growth rate, organ weight, and BP measurements between each group were analyzed using ANOVA with Scheffe's post hoc test. Aortic IEL defect numbers were assessed using a negative binomial regression model, which is specifically used for count data, with the Bonferroni correction used to determine the level of significance for multiple comparisons. IEL defect severity comparisons were conducted using the Kruskal-Wallis independent samples test for multiple comparisons. Dunn's multiple comparisons post hoc test was used to determine significant differences between the groups. Differences between males and females within each group were assessed using the Mann-Whitney rank sum test. Relationships between IEL defect numbers and weight, growth, organ weight, and BP parameters were examined using the Spearman's rank correlation coefficient. Correlations between growth parameters, BP and organ weights were made using parametric correlation analyses. Multiple logistic regression was used to determine the independence of the aortic elastic defect/*in utero* treatment association from that other potential confounding variables. Values in the text and table are means and 1 standard deviation.

RESULTS

Placental restriction surgery outcomes. Maternal health parameters were assessed to ensure that these factors did not confound any observations that may occur in the offspring. The average age at which the dams became pregnant, average

prepregnancy weight and average weight gain did not significantly differ between the treatment groups (data not shown). Neither maternal IEL defect number was significantly different between treatment groups, nor was it a confounder of offspring IEL defect numbers.

Effect of placental restriction surgery on litter size, pup weight, and length. Live born litter size was significantly decreased in the dams exposed to placental restriction surgery compared with no surgery controls (Table 1). Placental restriction surgery offspring were significantly smaller than sham surgery controls at both 24 and 72 h postpartum (Table 1, Fig. 1). CRL was approximately 4% lower in restricted surgery offspring, compared with sham surgery, during the first week postpartum (Table 1); however, there was no significant difference between groups after 2–3 wk.

Effect of placental restriction surgery on growth rate parameters, organ weights and BP. The differences in weight gain between groups were reflected in relative weight gain (weekly weight gain, as a percentage of weight from the previous week). The 24 to 72 h weight gain in restriction surgery animals was significantly less than no surgery animals (25 versus 34%, $p < 0.05$). Sham animals increased by 28% during this period, which was not significantly different to the other groups. Once organ weights were adjusted for body weight, no significant differences were observed between groups, except for kidney weight in 16-wk male sham ($p < 0.03$) and restriction surgery ($p < 0.06$) compared with no surgery offspring. There was no significant difference in either conscious (tail-cuff) or unconscious (intra-arterial) BP measurements between groups (Table 1).

Effect of placental restriction surgery on abdominal aortic phenotype. The number of IEL defects observed in placental restriction surgery aorta at 8 wk of age was significantly increased compared with no surgery offspring (Fig. 2). Although 8-wk sham surgery females had fewer defects than placental restriction surgery females, this failed to reach statistical significance ($p = 0.0095$, with Bonferroni required $p < 0.00417$). At 16-wk placental restricted males had significantly increased defect numbers (Figs. 2 & 3) compared with both no surgery ($p = 0.0001$) and sham surgery ($p < 0.001$) controls. In contrast, defect numbers were not significantly different between placental restriction surgery females and either of the control groups at the 16-wk time point. Male 16-wk sham surgery offspring had a suggestive increase in defect numbers compared with no surgery controls ($p = 0.0185$, with Bonferroni required $p < 0.01667$).

Univariate logistic regression analysis indicated that placental restriction surgery was significantly associated with IEL defect number at both 8 and 16 wk. This remained independently associated following adjustment for gender, BP, and weight (birth and adult). Each additional defect above that present in the no surgery controls contributed an adjusted odds ratios of 1.18 (95% confidence interval 1.04–1.35, $p < 0.02$) and 1.11 (1.03–1.19, $p < 0.005$) at 8 and 16 wk, respectively. Interestingly a similar trend was observed for the 16-wk sham surgery group compared with no surgery controls with an adjusted odds ratio of 1.10 (1.03–1.17, $p < 0.08$).

Table 1. Comparative growth rate and blood pressure measurements

	No surgery (n = 11 litters)		Sham surgery (n = 9 litters)		Restriction surgery (n = 11 litters)	
Mean litter size (range)	6.5 (4–8)		5.0 (3–6)		4.3 (3–6)*	
	Females (n = 17)	Males (n = 28)	Females (n = 16)	Males (n = 25)	Females (n = 15)	Males (n = 17)
Body weight 24 h (g)	5.4 ± 0.4†	6.04 ± 0.49	6.02 ± 0.42	6.23 ± 0.73	5.20 ± 0.58†	5.51 ± 0.55‡
Body weight 72 h (g)	7.5 ± 1.0	8.21 ± 0.96	7.79 ± 0.90	8.06 ± 1.10	6.65 ± 0.90*§	6.90 ± 0.93‡
Body weight 1 wk (g)	12.7 ± 1.7	14.12 ± 1.71	13.61 ± 1.70	13.59 ± 1.67	11.82 ± 1.33†	12.44 ± 1.25‡
Body weight 4 wk (g)	52.8 ± 2.8	62.61 ± 4.38	54.64 ± 4.55	60.60 ± 5.02	50.63 ± 4.23§	57.75 ± 3.95*
Body weight 8 wk (g)	125.4 ± 4.5	189 ± 12.35	126.33 ± 10.87	183.03 ± 17.47	119.12 ± 10.13	176.28 ± 11.53‡
Crown rump length 72h (mm)	46.8 ± 2.0	49.1 ± 1.7	47.7 ± 2.2	48.1 ± 2.9	45.4 ± 1.7§	46.2 ± 1.7*§
Crown rump length 4 wk (mm)	98.6 ± 4.0	103.8 ± 4.0	99.2 ± 4.8	102.0 ± 4.5	97.1 ± 3.6	102.3 ± 3.7
Relative kidney weight (% of body weight)	0.89 ± 0.06	0.84 ± 0.08	0.93 ± 0.12	0.81 ± 0.07	0.88 ± 0.06	0.84 ± 0.07
Mean intra-arterial BP 8 wk (mm Hg)	70.9 ± 17.4	73.2 ± 18.1	76.0 ± 10.7	75.4 ± 18.7	77.0 ± 8.7	78.3 ± 10.9
Conscious peak systolic BP 8 wk (mm Hg)	122.3 ± 28.6	135.5 ± 27.0	130.0 ± 24.9	139.7 ± 34.5	131.5 ± 36.5	124.3 ± 29.9
16 wk data	Females (n = 7)	Males (n = 14)	Females (n = 8)	Males (n = 14)	Females (n = 7)	Males (n = 9)
Body weight 16 wk (g)	180.1 ± 10.2	295.3 ± 22.2	176.9 ± 12.6	295.6 ± 14.4	171.9 ± 11.0	275.0 ± 8.6*§
Relative kidney weight (% of body weight)	0.71 ± 0.03	0.67 ± 0.03	0.70 ± 0.03	0.64 ± 0.03*	0.67 ± 0.05	0.64 ± 0.03
Mean intra-arterial BP 16 wk (mm Hg)	89.0 ± 6.4	92.6 ± 8.3	87.3 ± 4.1	88.8 ± 5.6	87.9 ± 3.2	90.1 ± 2.2
Conscious peak systolic BP 16 wk (mm Hg)	131.8 ± 13.8	137.9 ± 20.7	136.9 ± 10.4	139.5 ± 9.1	131.7 ± 14.0	147.0 ± 21.2

Although growth restricted animals were significantly smaller than the control animals there was no apparent difference in BP parameters.

* $p < 0.05$ vs same sex no surgery controls.

† $p < 0.01$ vs same sex sham surgery controls.

‡ $p < 0.01$ vs same sex no surgery controls.

§ $p < 0.05$ vs same sex sham surgery controls.

Figure 1. The average body weight of *in utero* growth restriction surgery, sham surgery, and no surgery animals over the first PN week of life. Growth restriction surgery (□) significantly reduced early PN body weight compared with the no surgery (■) and sham surgery (■) groups (* $p < 0.01$ vs sham, † $p < 0.01$ vs no surgery, ‡ $p < 0.05$ vs no surgery). ANOVA, values are mean ± SEM.

Defect severity was not significantly different between treatment groups in either sex at either 8 or 16 wk, with the exception of 8-wk females where placental restriction surgery females had significantly more severe defects than sham surgery females ($p < 0.05$).

Variations in abdominal aortic wall thickness were assessed histologically. The distal wall thickness (as a proportion of proximal thickness) was significantly decreased in 16-wk placental restriction males compared with the no surgery control group (0.89 ± 0.14 versus 1.04 ± 0.14 , $p = 0.033$). There was no significant difference in 8-wk old animals.

Correlations between elastic tissue defects and growth parameters, organ weight and BP. Defect number was neg-

Figure 2. Total aortic elastic tissue defect numbers in treatment groups, divided by gender and age. *In utero* growth restriction (□) significantly increased elastic tissue defects numbers in males at both time points (* $p < 0.002$ vs no surgery (■), § $p < 0.01$ vs sham surgery (■), † $p < 0.001$ vs no surgery and sham surgery) and females at 8 wk of age (‡ $p < 0.001$ vs no surgery). Values are mean ± SEM.

atively correlated with weight and CRL parameters in all groups except 16-wk females. In both 8 and 16-wk males, weight at 72 h was most strongly (negatively) correlated with elastic tissue defect number (Fig. 4A & B). In 8-wk females, the percentage weight gain in the early PN period was negatively correlated with defect number (Fig. 4C).

Defect number (in any group) was not correlated with BP, using either conscious or unconscious methods, with the exception of 8-wk females where defect number was negatively correlated with diastolic pressure ($r = -0.48$, $p <$

Figure 3. Abdominal aortic elastic tissue defects in no surgery (A) and restriction surgery (B–D) 16-wk male BN rats. (A and B) Elastic tissue defects are visualized (*en face*) as dark transversely orientated discontinuities in the internal elastic lamina. Defects were significantly increased in 16-wk restriction males compared with their no surgery counterparts. (C) Distal and (D) proximal histology (matching regions indicated by double arrowheads). Mean wall thickness was 45.3 and 55.9 μm , respectively. Blood flow was right to left. Scale bars (A and B) 2 mm, (C and D) 200 μm .

Figure 4. Correlations between IEL defect number and growth rate parameters. No surgery ($\circ$), sham surgery ($\bullet$), and restriction surgery ($\square$) treatment groups. Abdominal aortic elastic defect number vs (A) weight at 72 h in 8-wk males ($r = -0.402$, $p = 0.019$), (B) weight at 72 h in 16-wk males ($r = -0.530$, $p = 0.002$), (C) percentage increase in weight over the first 2 wk of life in 8-wk females ($r = -0.386$, $p = 0.041$) and (D) total kidney weight in 16-wk males ($r = -0.601$, $p = 0.003$).

0.04). In 16-wk males, defect number was negatively correlated with kidney parameters (Fig. 4D), this effect being preserved following adjustment for body size.

DISCUSSION

The principal aim of the study was to determine whether altered *in utero* environment would influence the development of vascular connective tissue (IEL) defects in adult rats. Late gestational placental restriction surgery significantly reduced newborn pup weight (by 14% at 72 h) and was associated with increased IEL defects in adult animals. This outcome was

most pronounced in 8-wk old animals in which median defect numbers were increased by between 43% (males) and 88% (females) compared with no surgery control. This remained significant following adjustment for gender, 24 h postpartum weight, adult weight and BP. This seems consistent with the results from another rodent model of impaired *in utero* environment in which a maternal low protein diet was shown to be associated with decreased aortic wall thickness and loss of elastin protein in the adult offspring (16).

In this current study, 16-wk old sham surgery offspring also had an apparent increase in defect number compared with no surgery controls (Fig. 2). Although this just failed to reach statistical significance it should be noted that sham surgery has been shown to influence growth rate *in utero* (17) and in some studies is associated with a decrease in birth weight (18). Although in the present study, 24-h weight was not significantly reduced in the sham surgery group, a temporary drop in fetal weight cannot be completely excluded. Indeed the sham group seemed to have an intermediate phenotype in terms of body weight and early growth rates. It is therefore possible that this more subtle insult still influenced aortic defect formation later in life. It is possible that exposure to late gestation surgery may influence postoperative parameters such as dam food intake and lactation. Although litter sizes were normalized to minimize differences in offspring postpartum feeding this could not completely exclude this potential confounder. Future studies utilizing cross-fostering may help elucidate this effect. Nevertheless, such issues further highlight the need for both unmanipulated and sham surgery controls within the experimental design of this type of study.

Correlation analysis indicated that IEL defect numbers were associated with early growth parameters. For example, weight at 72 h was negatively correlated with the number of defects in males at both 8 and 16 wk of age. In 8-wk females, those that put on the least weight (relative to initial body weight) from 24 h to 2 wk of age developed the greatest number of defects in subsequent adult life. This may suggest that accelerated growth (over the first 2 wk of life) may be “protective” against defect formation. In contrast, increased growth later in life (over 10 wk of age) seemed to be associated with increased defect numbers in 16-wk animals. Such observations warrant further investigation to determine which specific PN time periods most significantly influence defect numbers. This differential association between PN growth profiles and IEL elastic tissue defect formation is pertinent given human epidemiologic studies reporting correlations between accelerated PN growth and cardiovascular disease or its associated risk factors such as BP (4,19,20).

In the present study, once organ weights were adjusted for body weight, differences between no surgery and placental restriction surgery groups were largely abolished, implying that organ weights later in life are equally proportioned relative to total body mass in this model. Because other uterine artery ligation studies have shown reduced organ weights at early time points (21–23), it is possible that BN rat organ weights are initially decreased in growth restricted pups, but by adulthood, had become similarly proportioned to body mass compared with that of the control groups. Of particular

interest was that 16-wk male kidney weight was negatively correlated with IEL defects. In addition, defect number remained negatively associated with kidney weight once adjusted for body weight. Because weight at 24 h was positively associated with adult kidney weight, it is speculated that reduced birth weight may be independently associated with both reduced kidney weight and increased aortic defect numbers. However, it is beyond the scope of this study to determine whether low birth weight *per se* causes the increased defect numbers or if unidentified *in utero* events are responsible.

In light of these findings, it was of interest that BP was not increased in placental restricted animals in comparison with controls. Nor was adult BP correlated with weight at either 24 or 72 h. In a unilateral uterine artery ligation model, others have found no association between birth weight and BP at 15 wk of age as measured by telemetry in rats (24). In contrast, the aortic clip model of growth restriction (where placental blood flow is reduced by 35–40%) has shown that mean arterial pressure is increased by 13–17% in growth-restricted animals (25). Although the degree of growth restriction initially appears similar between the aortic clip study (birth weight decreased by 12%) and the present study (72-h weight decreased by 14%), it appears that the aortic clip model is associated with a more severe impact on body weight later in life, which may in turn impact more severely on BP. The BP findings from the aortic clip model, support results from other more chronic models of growth-restriction in the rat, such as the low protein diet (26,27). Furthermore, dietary models of growth restriction have also been associated with significant increases in BP (28,29). It is possible that this study may have been underpowered to detect small increases in BP, because others have described a statistically significant but modest (6–7%) elevation in BP (using the tail-cuff method) with larger group sizes (29). Nevertheless, we also note that small changes in BP appear unlikely to cause the substantial alterations in elastic tissue defect numbers observed in the present study. For example, administration of various antihypertensive agents to BN rats, although lowering BP to a similar extent, differentially affect the number of aortic defects (30); the inference being that these drugs may exert their effects on vascular structure independently of BP. Previous work within our own group on F₂ hybrid BN rats, has shown that BP does not correlate with elastic tissue defect numbers (11). These studies suggest that BP *per se*, is not directly responsible for the number of IEL defects that develop in the aorta and support the hypothesis that poor *in utero* growth likely influences IEL defect formation via a BP-independent mechanism.

Taken together, these results indicate that a poor *in utero* environment may directly affect blood vessel structure, which could possibly lead to an increased risk of cardiovascular disease later in life. Although evidence directly supporting a link between *in utero* environment and atherosclerosis in humans is extremely limited it has been shown that intrauterine growth restricted newborns have a significantly increased aortic intimal-medial thickness (IMT) compared with those of normal birth weight (31). As aortic IMT essentially detects a change in the thickness of the tunica intima, it can be consid-

ered to represent a marker of preclinical atherosclerosis in children (32).

Growth rate seems to be intimately linked to elastic tissue defect formation in the BN rat, which implies that growth rate of the aorta may also be involved. Aorta growth plateaus in both male and female rats at 10 wk of age (33), lengthening by less than 10% from weeks 10 to 52. In comparison, body weight is still rapidly increasing at week 10. In the BN strain, females gained approximately 20% of their body weight from weeks 10 to 16 and males gained approximately 23% of their body weight during this period. Therefore, although the aorta has essentially finished lengthening by approximately 10 wk of age, it must still accommodate the increased blood flow associated with increasing body and organ mass. We would suggest that this places increased strain on the blood vessels; exacerbating tendencies for regions of structural weakness to fail over this period. The observation in this study of decreased distal infrarenal wall thickness (relative to proximal thickness) in 16-wk, but not 8-wk, placental restricted animals may therefore be consistent with the altered physiologic demands placed on this vessel between these two time points. The redistribution of blood flow, which occurs in growth-restricted fetuses, results in blood flow being preferentially maintained to the brain over other organs and tissues. Vessels that develop in these “reduced flow” conditions during gestation may therefore have a diminished capacity to accommodate for the changes in blood flow that occur during PN life when under-nutrition is no longer present. Indeed, the BN rat is known to have a deficit in flow mediated remodeling compared with other rat strains (34). It is interesting to note that flow mediated remodeling in the rabbit is also associated with the development of IEL defects, morphologically identical to those observed spontaneously in the rat. Rabbit defects form after an initial lag period, vary depending on the size of the fistula and are the foci of significant intimal alteration, including smooth muscle cell hyperplasia, monocyte/macrophage recruitment as well as foam cell and extracellular lipid accumulation (35).

This study implicates alterations to *in utero* and PN growth in the development of elastic tissue defect formation in the BN aorta. Foremost, this study demonstrated that aortic elastic tissue defect numbers are negatively associated with both early PN weight and weight gain early in life, an effect that seems to occur via a BP-independent mechanism. We hypothesize that blood vessels in these situations are less able to accommodate the altered hemodynamics associated with PN body growth, resulting in increased vascular fragility, manifesting in increased numbers of elastic tissue defects.

In conclusion, this model of *in utero* growth restriction provides further support for a possible relationship between a poor quality of the *in utero* environment and a subsequent deleterious effect on cardiovascular structure in adult life.

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