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Jennifer A. Thompson
Western University

Ousseynou Sarr
Western University

Karolina Piorkowska
Western University

Robert Gros
Western University

Timothy R.H. Regnault
Western University, tim.regnault@uwo.ca

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Low birth weight followed by postnatal over-nutrition in the guinea pig exposes a predominant player in the development of vascular dysfunction

Jennifer A. Thompson[1,2], Ousseynou Sarr[2,3,4], Karolina Piorkowska[1], Robert Gros[1,5,6] and Timothy R. H. Regnault[1,2,3,4]

[1] *Department of Physiology and Pharmacology, The University of Western London, Ontario, Canada*
[2] *Department of Obstetrics and Gynaecology, The University of Western London, Ontario, Canada*
[3] *Lawson Health Research Institute, The University of Western London, Ontario, Canada*
[4] *Children's Health Research Institute, The University of Western London, Ontario, Canada*
[5] *Robarts Research Institute, The University of Western London, Ontario, Canada*
[6] *Department of Medicine, The University of Western London, Ontario, Canada*

Key points

- Suboptimal intrauterine conditions and consequent intrauterine growth reduction (IUGR), resulting in low birth weight (LBW), increase the risk for hypertension and cardiovascular disease in adulthood.
- LBW offspring who experience an accelerated growth in childhood have a higher risk of cardiovascular disease than those who grow more slowly, suggesting that the postnatal environment interacts with programmed deficits in organ function to influence disease risk.
- We show here that arterial stiffening in LBW guinea pig offspring is exacerbated with postnatal feeding of a Western diet, suggesting that IUGR confers heightened vascular susceptibility to postnatal risk factors and thus may contribute to an individual's total risk score.
- Our results also demonstrate that the independent effect of the intrauterine environment on vascular function in young adult guinea pigs is greater than the effect of a postnatal Western diet, thus highlighting the importance of prenatal factors on long-term vascular health.

Abstract The association between intrauterine growth restriction (IUGR) and hypertension is well established, yet the interaction between IUGR and other pathogenic contributors remains ill-defined. This study examined the independent and interactive effects of fetal growth reduction resulting in low birth weight (LBW), and postnatal Western diet (WD) on vascular function. Growth reduction was induced in pregnant guinea pigs by uterine artery ablation. LBW and normal birth weight (NBW) offspring were randomly assigned to a control diet (CD) or a WD. In young adulthood, length–tension curves were generated in aortic rings and responses to methacholine (MCh) were evaluated in the carotid and aorta using wire myography. Relative to NBW/CD, aortae of NBW/WD offspring were stiffer, as determined by a leftward shift in the length–tension curve, yet the shift in the LBW/CD curve was considerably greater. Aortic stiffening was most severe in LBW/WD (slope: NBW/CD, 1.97 ± 0.04; NBW/WD, 2.16 ± 0.04; LBW/CD, 2.28 ± 0.05; LBW/WD, 2.34 ± 0.07). Maximal responses (E_{max}) to MCh were significantly blunted in the aorta of LBW/CD *vs.* NBW/CD ($P < 0.05$) and in LBW/WD *vs.* NBW/WD offspring ($P < 0.05$); but WD alone had no influence on MCh responses. E_{max} values for carotid responses to MCh were reduced in LBW/CD *vs.* NBW/CD ($P < 0.05$). Thus, aortic stiffening was influenced more by LBW than by a postnatal WD and the most severe stiffening was observed in LBW/WD offspring. In contrast, blunted endothelial responses in LBW/CD offspring were not exacerbated by WD. IUGR may have a greater independent impact on vascular function than a postnatal WD.

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Corresponding author Jennifer A. Thompson: Department of Physiology, Georgia Regents University, 1120 15th St., Augusta, GA 30912, USA. Email: jthompson2@gru.edu

Abbreviations ANP, atrial natriuretic peptide; CD, control diet; ECM, extracellular matrix; IUGR, intrauterine growth restriction; LBW, low birth weight; MCh, methacholine; MCP-1, monocyte chemoattractant protein-1; αMHC, myosin heavy chain alpha; βMHC, myosin heavy chain beta; MMP-2, matrix metalloproteinase 2; MUFA, monounsaturated fatty acid; NBW, normal birth weight; PE, phenylephrine; PSS, physiological saline; PUFA, polyunsaturated fatty acid; RT-PCR, real-time PCR; SFA, saturated fatty acid; SNP, sodium nitropusside; SOD, superoxide dismutase; TGFβ_1, transforming growth factor beta 1; WD, Western diet .

Introduction

Advancement of vascular disease occurs over decades, the speed of which relates to the cumulative burden of risk factors. The initial exposure can be traced to as early as the intrauterine environment, as evidenced by the consistent observation that heart failure, hypertension and their subclinical markers occur more frequently in those born small (Barker *et al.* 1989, 1990; Curhan *et al.* 1996). Low birth weight (LBW) is a consequence of sub-optimal intrauterine conditions wherein the supply or delivery of maternally derived nutrients and oxygen is limited. In adaptation to chronic substrate deprivation, fetal strategy shifts to one of survival that involves departure from its growth trajectory and thus critical periods of development may fail to endow the organ systems with the structural integrity required for optimal efficiency. Shortly after birth, the organs no longer enjoy the plasticity that was granted *in utero* (Gluckman and Hanson, 2007) and thus any aberration in organ structure and function may be fixed going forward in postnatal life. This premature disadvantage may lead to progressive dysfunction that is accelerated with the burden of ageing and superimposition of postnatal risk factors.

Our group previously demonstrated aberrant extracellular matrix (ECM) remodelling in the aorta of hypoxic ovine fetuses (Thompson *et al.* 2011*a*) and aortic stiffening in LBW guinea pig offspring, the latter growth restricted by a reduction in maternal–placental blood flow (Thompson *et al.* 2011*b*). Given that postnatal ECM composition is largely a function of developmental remodeling (Mariencheck *et al.* 1995; Shapiro *et al.* 1991), these studies underline the intrauterine milieu as an important determinant of the buffering capacity of the proximal vasculature. Current thought holds that the influence of proximal stiffening on the risk for cardiovascular disease is primarily exerted at a late stage in life (O'Rourke, 2007; Kaess *et al.* 2012). Yet pre-existing structural abnormalities established *in utero* may lead to a premature cycle of wall stiffening and associated endothelial dysfunction.

Placental insufficiency and consequent chronic fetal hypoxaemia are the most common features of intrauterine growth restriction (IUGR) in developed countries (Ghidini, 1996). In such societies, the newborn which has endured deprived conditions in the womb is often met with an incongruent environment of nutrient overabundance in postnatal life. High intake of saturated fats and simple sugars has deleterious effects on the vasculature, which are largely mediated through oxidative stress and inflammation (Li *et al.* 2013; Renna *et al.* 2013). Redox and inflammatory signals serve as hypertrophic stimuli and thus may amplify pre-existing structural abnormalities in LBW offspring, thereby accelerating central stiffening and the progression of vascular dysfunction. In addition to *in utero* programmed structural abnormalities, deficiencies in antioxidant defences and unabated stimulation of inflammatory pathways in the vasculature of IUGR offspring may confer enhanced sensitivity to a postnatal Western diet. Indeed, a persistence of oxidant–antioxidant imbalances initiated *in utero*, in part due to programmed deficiencies in antioxidant capacity, have been demonstrated in LBW offspring (Cambonie *et al.* 2007; Mohn *et al.* 2007; Giussani *et al.* 2012).

Programmed changes in the vasculature in response to suboptimal intrauterine conditions may increase the risk for cardiovascular disease, not only through independent effects on vascular function but also through sensitizing the vasculature to a secondary postnatal insult. The present study thus aimed to investigate the independent and interactive effects of a suboptimal intrauterine environment and postnatal Western diet on vascular function in male and female guinea pigs at young adulthood. We tested the hypothesis that LBW and postnatal feeding of a Western diet each independently lead to aortic stiffening and endothelial dysfunction in young adulthood, and that when superimposed have synergistic effects on these functional parameters. We show that aortic stiffening in young LBW offspring is exacerbated by a post-weaning Western diet and intriguingly, that the independent effect of the intrauterine environment on postnatal vascular function is greater than that of a poor postnatal diet.

Methods

Ethical approval

All surgical and experimental protocols were conducted in accordance with the Canadian Council on Animal Care Guidelines and approved by The University of Western Ontario Animal Use Subcommittee.

Animals

Time-mated pregnant Dunkin–Hartly guinea pigs (Charles River Laboratories, Wilmington, MA, USA) were housed with a 12/12 h light–dark cycle with constant access to normal guinea pig chow (LabDiet diet 5025: 27% protein, 13% fat, 60% carbohydrates).

Uterine artery ablation

Sixteen pregnant guinea pigs were studied; all underwent surgery as previously described. At mid-gestation (~32 days, term ~67 days) anaesthesia was induced using an anaesthetic chamber (4–5% isoflurane with 2 l min^{-1} O_2, followed by 2.5–3% isoflurane with 1 l min^{-1} O_2 for maintenance). Immediately after induction, a subcutaneous injection of Robinul (glycopyrrolate, 0.01 mg kg^{-1}; Sandoz Can Inc., Montreal, QC, Canada) was administered. A midline incision was made below the umbilicus to expose the bicornate uterus. At this time, sows underwent uterine artery ablation to induce placental insufficiency, as previously described (Turner and Trudinger, 2009). Arterial vessels feeding one horn of the uterus were identified and every second branch was cauterized using an Aaron 2250 electrosurgical generator (Bovie Medical, Clearwater, FL, USA). Immediately following surgery, a subcutaneous injection of Temgesic (buprenorphine, 0.025 mg kg^{-1}; Schering-Plough Co., Kenilworth, NJ, USA) was administered, and monitoring of sows continued after surgery. Sows delivered spontaneously, at which time pup weight, abdominal circumference, biparietal distance and body length were measured. After birth, pups were weighed daily until weaning, and thereafter body weights were measured biweekly. At the end of the pupping period, birth weight was collated and guinea pig pups were defined as normal birth weight (NBW) if their body weights were within the 25th and 75th percentile of all pups, and LBW if their body weights were below the 25th percentile. Based on this, NBW pup weights were greater than 90 g and LBW pups were below 85 g, in accordance with previously published data (Detmer and Carter, 1991; Kind *et al.* 1999).

Postnatal period

Five days prior to weaning at post-birth day 15, the postnatal diet was introduced to the pups through both the maternal feeding tray and the cage. At 15 days of age guinea pig offspring were separated by sex and housed in individual cages in a temperature- (19°C) and humidity- (30%) controlled environment, with a 12/12 h light–dark cycle. At this time guinea pig offspring were randomized to either a control diet (CD, TD: 110240; Harlan Laboratories, Madison, WI, USA) or a Western diet (WD, TD: 110239; Harlan Laboratories). The diets differed in kilocalorie density (3.4 *vs.* 4.2 kcal g^{-1}) but were matched for protein and macronutrients on the basis of kilocalorie density. Isolated soy protein served as the protein source. Carbohydrates comprised 60% of total kilocalories in the CD [distribution (% by weight): 10% sucrose, 40% corn starch] and 33% of total kilocalories in the WD [distribution (% by weight): 19% sucrose, 6.5% fructose, 9% corn starch]. Total fat constituted 18% of kilocalories in the CD [2.8% saturated fatty acids (SFAs), 4.4% monounsaturated fatty acids (MUFAs), 11.2% polyunsaturated fatty acids (PUFAs)] from soybean oil and 45% of kilocalories in the WD (31.7% SFAs, 11.8% MUFAs, 1.8% PUFAs) from a combination of coconut oil, lard and cocoa butter (9.5%, 5.5% and 5% by weight, respectively). Additionally, the WD contained 0.25% cholesterol. Each diet contained a stabilized form of vitamin C (0.61% ascorbyl-polyphosphate; Stay-C). To avoid litter effects, only one LBW/NBW animal per sex from a single litter was assigned to each diet. From the time of weaning, animal weights and food intake were monitored twice weekly until the animals were killed at ~150 days.

Tissue collection

At approximately postnatal day 150, which corresponds to young adulthood (Kind *et al.* 2003), offspring were killed by CO_2 inhalation following an overnight fast (Greulich *et al.* 2011). The thoracic cavity was opened, and the heart removed, stripped of fat and weighed. The right and left ventricles were dissected; the left ventricle was frozen for RNA extraction. A segment of the aorta from the aortic arch to the diaphragm and the carotid were carefully excised and immediately placed in 4°C physiological saline (PSS) containing: 118 mM NaCl, 25 mM $NaHCO_3$, 11.1 mM D-glucose, 4.71 mM KCl, 2.56 mM $CaCl_2$.$2H_2O$, 1.13 mM NaH_2PO_4.$2H_2O$, 12 mM $MgCl_2$.$6H_2O$; 0.114 ascorbic acid and 0.0297 mM disodium EDTA. The thoracic aortic segment was separated into proximal and distal sections and prepared for *ex vivo* measurements of length–tension and vascular reactivity, respectively. The carotid was used for vascular reactivity experiments.

Additional standard sections of the proximal aorta were placed in 4% paraformaldehyde for histological analyses and frozen in liquid nitrogen for later RNA extraction.

Length–tension curves

Three rings were cut from each proximal aortic section. Length–tension curves were generated as previously described (Thompson *et al.* 2011*a*b; Vafaie *et al.* 2014). Each ring was mounted isometrically onto two parallel stainless steel wires, one connected to a micrometer for fine distance adjustments and the other connected to a force transducer (FT03; Grass Instruments, Warwick, RI, USA) attached to a digital display (P11T; Grass Instruments). An initial pre-stretch of each vessel was performed by stretching the rings from the zero tension position to a maximal stretch of 3 mm in 500 μm increments, at 2 min intervals. Following the pre-conditioning, the aortic rings were allowed to equilibrate at zero tension for 15 min. A length–tension curve was then generated by increasing the distance by 500 μm at 2 min intervals from the zero tension position until no further response was observed.

Elastin content and structure of aorta

After deparaffinization in xylene, slides were rehydrated by passage through a decreasing ethanol series. Aortic cross-sections were stained for 30 min in 0.2% Orcein (Sigma-Aldrich Canada Ltd, Oakville, ON, Canada) for identification of elastic fibres. Stained cross-sections were captured on a microscope (Leica DM RB) at 10× magnification. Duplicates of three cross-sections per animal and 4–5 areas per cross-section were used for analyses. The area positive for protein was identified by colour thresholding using image analysis software (Image Pro 6.0; MediaCybernetics, Bethesda, MD, USA) and relative content within the arterial media was calculated as previously described (Thompson *et al.* 2011*a*). Wall thickness was measured as the distance between the internal and external elastic laminae. The perpendicular distance between the internal elastic lamina and the first elastic layer within the media was measured. Slides were blinded to the operator.

Vascular reactivity

Vascular reactivity studies were performed as previously described (Gros *et al.* 2000, 2007). Under a dissecting microscope, the distal aorta and carotid artery were cleared of perivascular fat and each cut into two 3 mm rings in a Petri dish filled with ice-cold PSS. Each ring was mounted in a myograph (DMT-USA, Ann Arbor, MI, USA) attached to two stainless steel wires, one attached to a force transducer and the other to a micrometer. Data were recorded by a PowerLab 4/SP data acquisition system (ADInstruments, Colorado Springs, CO, USA). The micrometer was adjusted to maintain a passive force of 1000 mg. Ring preparations were then equilibrated for 45 min in PSS at 37°C and continuously bubbled with 5% CO_2 and 95% O_2. Arterial viability was assessed first by stimulation with 120 mM KCl and vessels not reaching 300 mg of tension above baseline were excluded from analysis. All experiments were performed in intact vessels; preliminary studies determined that $>$ 50% relaxation in the guinea pig aorta and $>$ 90% relaxation of the guinea pig carotid occur in response to [1×10^{-3}] methacholine (MCh) when the endothelium is preserved. Following washing and stabilization to baseline, cumulative doses of phenylephrine (PE) (1×10^{-9} M to 1×10^{-3} M) were added to the bath. Subsequently, endothelium-dependent relaxation was assessed in PE (1×10^{-4} M) pre-contracted vessels by measuring the dilatory response to increasing doses of MCh (1×10^{-11} to 1×10^{-4} M). Endothelium-independent relaxation was then evaluated by pre-contraction with PE followed by addition of sodium nitropusside (SNP) (1×10^{-11} to 1×10^{-4} M). At least 10 min of washing and stabilization to baseline was allowed between each experiment. There were no differences between groups in the dried weight of the arterial rings. All chemicals used for vessel reactivity studies were purchased from Sigma-Aldrich.

RNA extraction and real-time PCR

Total RNA was extracted from frozen aortae and left ventricular samples with Trizol (Invitrogen Life Technologies, Burlington, ON, Canada). Following extraction each sample was assessed for RNA integrity using 1.2% agarose electrophoresis with ethidium bromide staining. Complementary DNA was synthesized from 1 μg of purified RNA from the aorta and 2 μg of purified RNA from the left ventricle, using oligo(dT) primers and the SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen Life Technologies). Three primer pairs were designed for each gene using BLAST. For myosin heavy chain alpha (αMHC) and myosin heavy chain beta (βMHC) primer design, sequences were identified using the UCSC genome browser and homology analysis was performed (rat *vs.* mouse *vs.* human *vs.* cow). Standard curves for each primer pair were generated from serial dilutions of cDNA for setting of optimal concentrations of input cDNA and determination of primer efficiencies. PCR efficiencies for each primer set were 90–100%. Melting curve analyses and the presence of a single amplicon at the expected size in 1.8% agarose gel confirmed amplification of a single product. cDNA products were used as templates for quantitative real-time PCR (qRT-PCR) for measurement of gene expression

levels using the SYBR green system (Bio-Rad Laboratories, Mississauga, ON, Canada) on a Bio-Rad CFX384 real time PCR detector. Ribosomal protein 15S was used for normalization; there were no differences in the expression of 15S between groups. Amplification was performed in triplicate at 95°C for 3 min, followed by 39 cycles at 95°C for 15 s, 59°C for 15 s and 72°C for 15 s. Expression of individual target genes is reported as fold changes relative to the control group using the $\Delta\Delta C_t$ method.

Nitrotyrosine staining

Nitrotyrosine immunoreactivity was assessed using immunofluorescence. Cross-sections of the aorta were deparaffinized and incubated for 8 min in Background SNIPER (Biocare Medical, Concord, CA, USA) to reduce non-specific background staining. Sections were then incubated in rabbit anti-nitrotyrosine antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at a dilution of 1:1000 overnight at 4°C. After washing in PBS, slides were incubated with secondary antibody, goat anti-mouse Alexafluor 568 (1:200; Life Technologies) at room temperature for 30 min in a black covered humidity chamber. Replacement of the primary antibody with PBS or IgG was used as negative control. Fluorescence VectaShield mounting medium (Vector Laboratories, Burlington, ON, Canada) was used for mounting. Slides were stained in duplicate and simultaneously to minimize variation in staining intensity. The nitrotyrosine antibody was tested for specificity by Western blot. Sections were imaged on a Zeiss Z1microscope and captured at 20× magnification using a camera and software for image capture and analysis (Axiovision 4.0, Carl Zeiss Micro-imaging LLC, Thornwood, NY, USA). The intensity of staining was measured using image analysis software (Image Pro 6.0). Slides were blinded to the operator.

Statistical analyses

Statistical analyses were performed using one-way ANOVA for comparison between the four groups with respect to growth parameters before weaning and for EC_{50} determination. A three-way ANOVA (SPSS version 22) determined that sex was not a significant source of variation and therefore males and females were grouped together and differences between the groups were determined using a two-way ANOVA and a Bonferroni *post hoc* test with birth weight and diet as sources of variation. The length–tension relationship was fitted by a linear equation, the slope of which relates directly to compliance. For calculation of the half maximal effective concentration (log EC_{50}), concentrations were log-transformed, normalized to percentage maximal response and fitted to a four-parameter logistic curve using a non-linear interactive fitting program (Graph Pad prism 4.0; GraphPad Software Inc., La Jolla, CA, USA). Values of $P < 0.05$ were considered statistically significant. Data are expressed as mean ± SEM; *n* values represent the number of animals.

Results

LBW guinea pigs display signs of growth restriction

In the clinical setting, LBW is considered a proxy measurement of suboptimal intrauterine growth and changes in relative body dimensions are used as indicators of growth restriction (William, 2006). The NBW group comprised 33 guinea pig pups (female/male = 20:13) and 24 guinea pig pups were defined as LBW ($n = 24$; female/male = 11:13). The LBW group had significantly lower birth weights than NBW animals (NBW: 106.5 ± 1.1 g; LBW: 76.6 ± 1.0 g, $P < 0.0001$); this growth restriction was similar in male *vs.* female pups. Abdominal circumference was significantly reduced in the LBW relative to the NBW group (NBW: 11.56 ± 0.22 cm; LBW: 10.23 ± 0.26 cm, $P < 0.0001$). Brain growth as reflected by biparietal distance was significantly decreased in the LBW group compared to NBW pups (NBW: 2.23 ± 0.08 cm; LBW: 1.87 ± 0.09 cm, $P < 0.0001$). Body length, a measure of skeletal growth, tended to be decreased in the LBW relative to the NBW group (NBW: 14.34 ± 0.27 cm; LBW: 13.28 ± 0.53 cm), but this was not statistically significant ($P = 0.059$). Body weights at put-down were NBW/CD: 715.3 ± 32.3 g; NBW/WD: 640.9 ± 22.3 g; LBW/CD: 695.2 ± 37.2 g; LBW/WD: 582.2 ± 28.8 g. Despite catch-up growth in LBW animals in early postnatal life, LBW/WD animals had lower body weights at put-down compared to NBW/CD ($P < 0.05$).

The independent effect of the intrauterine environment on aortic compliance is greater than that of WD

Length–tension curves were generated in isolated aortic rings from guinea pig offspring and are considered a direct measurement of arterial wall compliance (Vafaie *et al.* 2014). As no sex differences were observed in the length–tension curves, male and female data were grouped together. The length–tension curve of each group and corresponding slope are shown in Fig. 1 With respect to slope of the length–tension curve, there was a significant effect for both birth weight and diet as sources of variation and the interaction was statistically significant ($P < 0.0001$). The length–tension curve of NBW offspring fed a WD was significantly shifted to the left relative to that of NBW/CD offspring (slope 1.97 ± 0.04 *vs.* 2.16 ± 0.04; length 1.5 mm, 2 mm, 2.5 mm, $P < 0.001$; length 3 mm,

$P < 0.05$), indicating a reduced compliance of these vessels. A significantly greater shift in the length–tension curve was observed in the LBW/CD animals (NBW/CD *vs.* LBW/CD: slope 1.97 ± 0.04 *vs.* 2.28 ± 0.05; length 1 mm, 1.5 mm, 2 mm, 2.5 mm, $P < 0.01$; length 3 mm, $P < 0.01$). The greatest shift in the length–tension curve was in the LBW animals fed a WD (NBW/CD *vs.* LBW/WD: slope 1.97 ± 0.04 *vs.* 2.34 ± 0.07; length 1 mm, 1.5 mm, 2 mm, $P < 0.001$; length 3 mm, $P < 0.01$).

Elastic fibre content is reduced in the aorta of LBW offspring fed a postnatal WD

Aortic compliance is a function of the passive properties of the arterial wall and thus reflects the relative content of the extracellular matrix fibres and media thickness (Cohn 2001). Relative elastic fibre content was 0.83 ± 0.18 AU in NBW/CD, 0.55 ± 0.03 AU in LBW/CD, 0.72 ± 0.18 AU in NBW/WD and 0.40 ± 0.07 AU in LBW/WD animals. Absolute wall thickness was 863.7 ± 79.9 μm in NBW/CD, 858.6 ± 44.3 μm in NBW/WD, 746.3 ± 70.4 μm in LBW/CD and 943.3 ± 107.2 μm in LBW/WD. Wall thickness adjusted for body weight was 1.29 ± 0.17 in NBW/CD, 1.40 ± 0.19 in NBW/WD, 1.08 ± 0.11 in LBW/CD and 1.54 ± 0.17 in LBW/WD. There were no differences in the ratio of the intima/media thickness (NBW/CD: 0.05 ± 0.01 μm; NBW/WD: 0.05 ± 0.01 μm; LBW/CD: 0.06 ± 0.01 μm; LBW/WD: 0.05 ± 0.00 μm) nor in the thickness of the internal elastic lamina (NBW/CD: 13.62 ± 0.56 μm; NBW/WD: 15.42 ± 1.17 μm; LBW/CD: 13.97 ± 1.17 μm; LBW/WD: 15.62 ± 2.19 μm).

Endothelium-dependent relaxation is blunted in LBW offspring, but not affected by WD alone

Endothelial dysfunction is associated with arterial stiffening and contributes to the vascular dysfunction that underlies cardiovascular disease (Cohn 2001). Contractile responses to PE in the aorta and carotid were similar between groups and between sexes (Fig. 2). Next we examined the relaxation responses to cumulative doses of MCh and SNP in isolated intact vessels as an indicator of endothelium-dependent and endothelium-independent relaxation, respectively. Relaxation responses to MCh in the aorta and carotid are shown in Fig. 3 and the EC_{50}

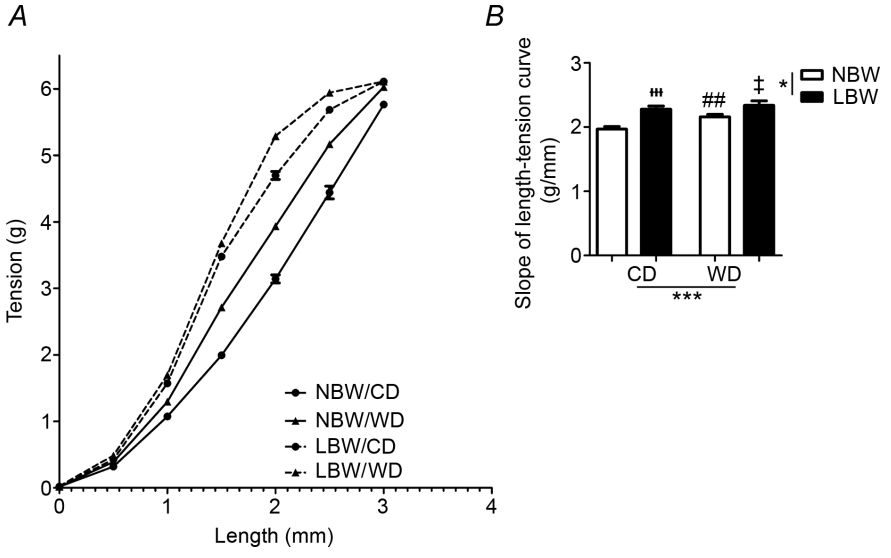

Figure 1. Three rings of the descending aorta excised from guinea pig offspring were used to generate length–tension curves, a direct measurement of arterial wall compliance
Average length–tension curves (*A*) and slope of the curves (*B*) are shown for each group ($n = 10$–17 per group). $^{\dagger}P < 0.05$ in Bonferroni *post hoc* test comparing CD, LBW *vs.* NBW; $^{\ddagger}P < 0.05$ in Bonferroni *post hoc* test comparing WD, LBW *vs.* NBW; $^{\#}P < 0.05$ in Bonferroni *post hoc* test comparing NBW, WD *vs.* CD; $^{*}P < 0.05$ for each source of variation (diet or birth weight).

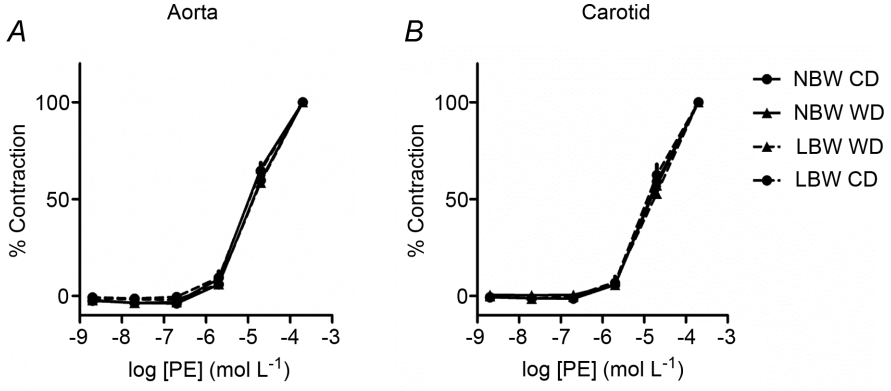

Figure 2. Cumulative concentration–response curves to phenylephrine (PE) generated in isolated vessels
Shown are responses to PE for the aorta (*A*) and carotid (*B*) of each group. ($n = 5$–10 per group).

values for responses to MCh calculated from transformed and normalized data are shown in Table 1. The E_{max} of aortic responses to MCh was significantly decreased in LBW/CD *vs.* NBW/CD (Fig. 3E), in LBW/WD *vs.* NBW/WD (Fig. 3G) and in LBW/CD *vs.* LBW/WD (Fig. 3I), but were not different in NBW/CD *vs.* NBW/WD (Fig. 3C). Birth weight, but not diet, was a significant source of variation for aortic responses to MCh ($P < 0.0001$). E_{max} values for carotid responses to MCh were significantly reduced in LBW/CD *vs.* NBW/CD offspring (Fig. 3F); and similar to the aorta, there were no differences between NBW/WD and NBW/CD animals

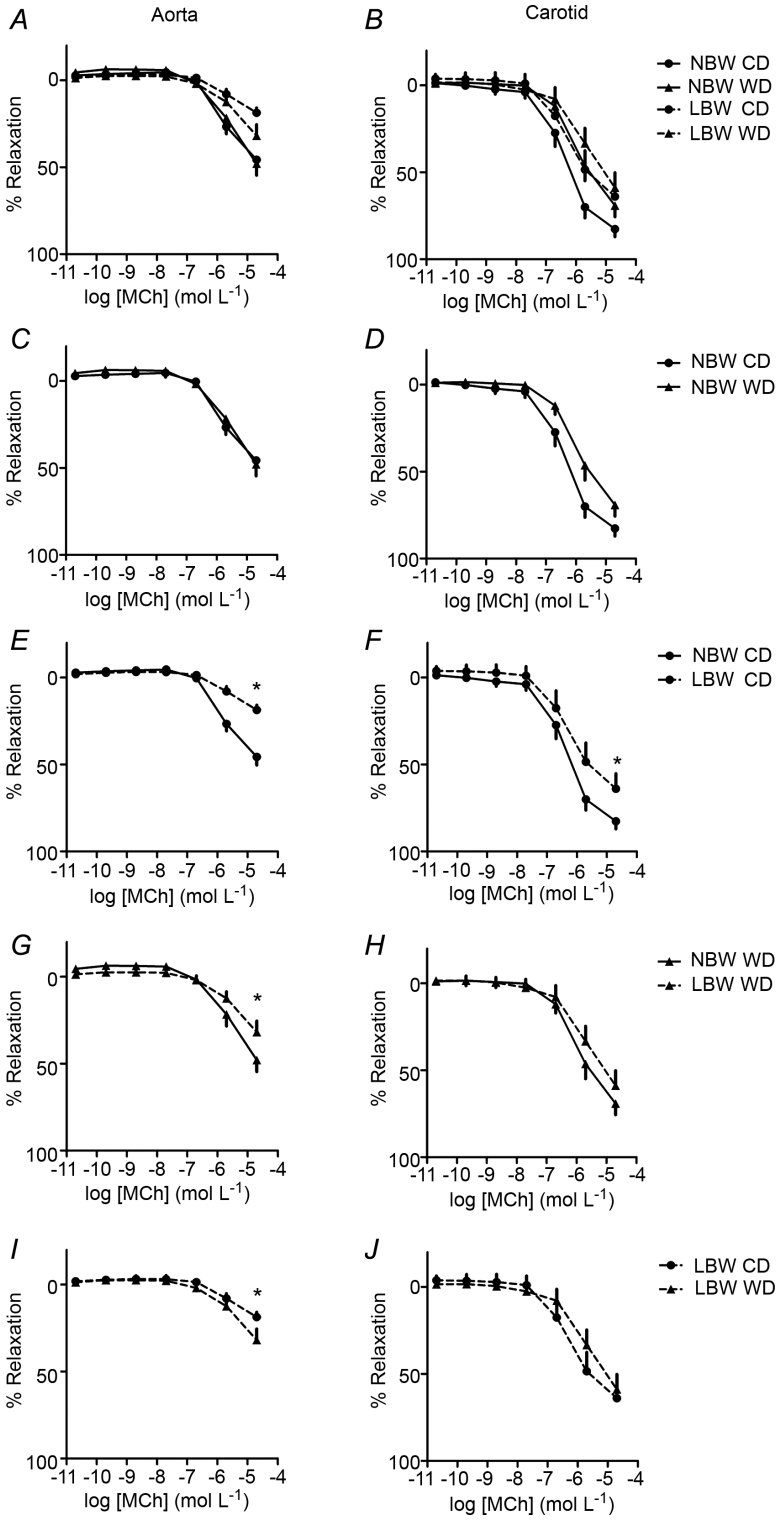

Figure 3. Relaxation responses to cumulative doses of methacholine
Relaxation responses to cumulative doses of methacholine (MCh) in the isolated aorta (*A*) and carotid artery (*B*). Responses to MCh in NBW/CD *vs*. NBW/WD in the aorta (*C*) and carotid (*D*); in NBW/CD *vs*. LBW/CD in the aorta (*E*) and carotid (*F*); in NBW/WD *vs*. LBW/WD in the aorta (*G*) and carotid (*H*); and in LBW/CD *vs*. LBW/WD in the aorta (*I*) and carotid (*J*) ($n = 10$–17 per group). $^*P < 0.05$.

Table 1. Sensitivity of endothelium-dependent and endothelium-independent relaxation responses of the aorta and carotid

		Aorta	Carotid
MCh	NBW CD	1.3×10^{-6} (8.9×10^{-7} to 1.9×10^{-6})	2.9×10^{-7} (1.4×10^{-7} to 6.1×10^{-7})
EC_{50} (mol L^{-1}, 95% CI)	NBW WD	1.6×10^{-5} (9.9×10^{-7} to 2.7×10^{-6})	4.2×10^{-7} (1.8×10^{-7} to 9.6×10^{-7})
	LBW CD	2.2×10^{6} (1.9×10^{-5} to 4.2×10^{-6})	5.8×10^{7} (1.7×10^{-7} to 2.0×10^{-6})
	LBW WD	1.9×10^{-6} (1.1×10^{-6} to 3.3×10^{-6})	2.2×10^{-6} (6.0×10^{-3} to 4.7×10^{-6})*
SNP	NBW CD	1.0×10^{-7} (5.1×10^{-3} to 2.0×10^{-7})	2.2×10^{-7} (1.1×10^{-7} to 4.5×10^{-7})
EC_{50} (mol L^{-1}, 95% CI)	NBW WD	1.2×10^{-7} (7.6×10^{-8} to 1.9×10^{-7})	1.1×10^{-7} (7.2×10^{-8} to 1.7×10^{-7})
	LBW CD	$4.9 \times 1o^{-8}$ (3.2×10^{-8} to 7.4×10^{-8})*,†	1.6×10^{-7} (9.1×10^{-8} to 3.0×10^{-7})
	LBW WD	2.3×10^{-8} (1.2×10^{-8} to 4.2×10^{-8})	2.9×10^{-7} (9.1×10^{-8} to 9.3×10^{-7})

LBW, low birth weight; NBW, normal birth weight; CD, control diet; WD, Western diet. **vs.* NBW CD. †*vs.* NBW WD.

nor between LBW/CD and LBW/WD offspring. Birth weight was also a significant source of variation for carotid MCh responses ($P < 0.05$). The EC_{50} for MCh responses in the carotid was increased in LBW/WD animals, compared to NBW/CD (Table 1). Relaxation responses to SNP in the aorta and carotid are shown in Fig. 4 E_{max} of aortic responses to SNP was significantly decreased in LBW/CD guinea pigs compared to both the NBW/WD ($P < 0.05$) and the LBW/WD groups (Fig. 4A, *I*). For aortic responses to SNP, diet, but not birth weight, was a significant source of variation ($P < 0.05$).

SOD2 mRNA levels are decreased in the aorta of LBW WD offspring

Given that the most marked functional abnormalities were observed in the vasculature of LBW offspring and that these abnormalities were exacerbated in LBW/WD animals, we expected that a programmed pro-inflammatory and pro-oxidant vascular phenotype underlies this susceptibility in LBW offspring. Therefore, we analysed the expression of genes associated with inflammation, arterial remodelling and oxidative stress in the thoracic aorta. Matrix metalloproteinase-2 (MMP-2) and transforming growth factor beta 1 (TGFβ_1), which are activated under conditions of vascular inflammation (Yasmin *et al.* 2005), regulate ECM remodelling and collagen deposition and thereby contribute to arterial wall stiffening. No changes in vascular mRNA levels of monocyte chemoattractant protein-1 (MCP-1), MMP-2, TGFβ_1, procollagen I and procollagen III were found (Table 2).

There exists evidence to suggest that reduced antioxidant capacity and increased production of reactive oxygen species, particularly superoxide anion, underlie endothelial dysfunction in IUGR offspring (Yzydorczyk *et al.* 2006). Thus, we measured mRNA levels of the superoxide scavenging enzymes SOD1 and SOD2, expressed in the cytosol and mitochondria, respectively. Immunoreactivity of nitrotyrosine was used as an indicator of oxidative stress. Nitration of tyrosine residues is induced by the potent oxidant species peroxynitrite, which in turn is a product of the interaction between nitric oxide and superoxide (Pacher *et al.* 2007). There was a significant effect of birth weight on SOD2 mRNA levels in the aorta ($P < 0.05$) (Fig. 5C). Reduced mRNA expression of SOD2 was not accompanied by increased intensity of nitrotyrosine staining in aortic cross-sections (NBW/CD: 44.1 ± 1.1 AU; NBW/WD: 46.5 ± 0.8 AU; LBW/CD: 45.8 ± 1.1 AU; LBW/WD: 47.6 ± 2.1 AU).

Vascular dysfunction consequent to intrauterine growth impairment does not lead to compensatory left ventricular remodelling in young adulthood

Reduced compliance of the proximal arteries increases pulsatile load and thereby promotes left ventricular compensation (Cohn 2001). Therefore, to determine if the aortic stiffening observed in LBW offspring leads to left ventricular remodelling, we measured relative heart weight along with mRNA levels of cardiac myosin isoforms (αMHC and βMHC), which are regulated by haemodynamic load and altered in the hypertrophied heart (Xu *et al.* 2006). We also measured mRNA levels of atrial natriuretic peptide (ANP), a cardiac hormone that is up-regulated under conditions of compensatory remodelling. Additionally, we measured genes involved in the regulation of cardiac ECM remodelling (TGFβ_1, MMP-2, procollagen I, procollagen III). The ratio of heart weight-to-body weight was not different between groups (NBW/CD = 0.33 ± 0.01; NBW/WD = 0.33 ± 0.01; LBW/CD: 0.35 ± 0.02; LBW/WD: 0.34 ± 0.02). Also, there were no differences in mRNA levels of αMHC and βMHC, the ratio of αMHC/βMHC, ANP, MMP-2, TGF-β_1, procollagen I and procollagen III in the left ventricle (Table 2). Interestingly, in the left ventricle diet was a significant source of variation of SOD1 and LBW/WD animals had lower mRNA levels of SOD1 compared to LBW/CD animals (Fig. 5B).

Discussion

Adverse intrauterine events are now recognized as significant predictors of hypertension and heart disease, yet remain to be fully integrated into clinical risk profiling and prevention policy. An abnormal vascular phenotype appears to contribute to the progression of cardiovascular disease in growth-restricted offspring, as we along with other groups have previously reported vascular dysfunction in adult offspring that were born small (Cambonie *et al.* 2007; Morton *et al.* 2010; Thompson *et al.* 2011*a*b; Giussani *et al.* 2012). This programmed

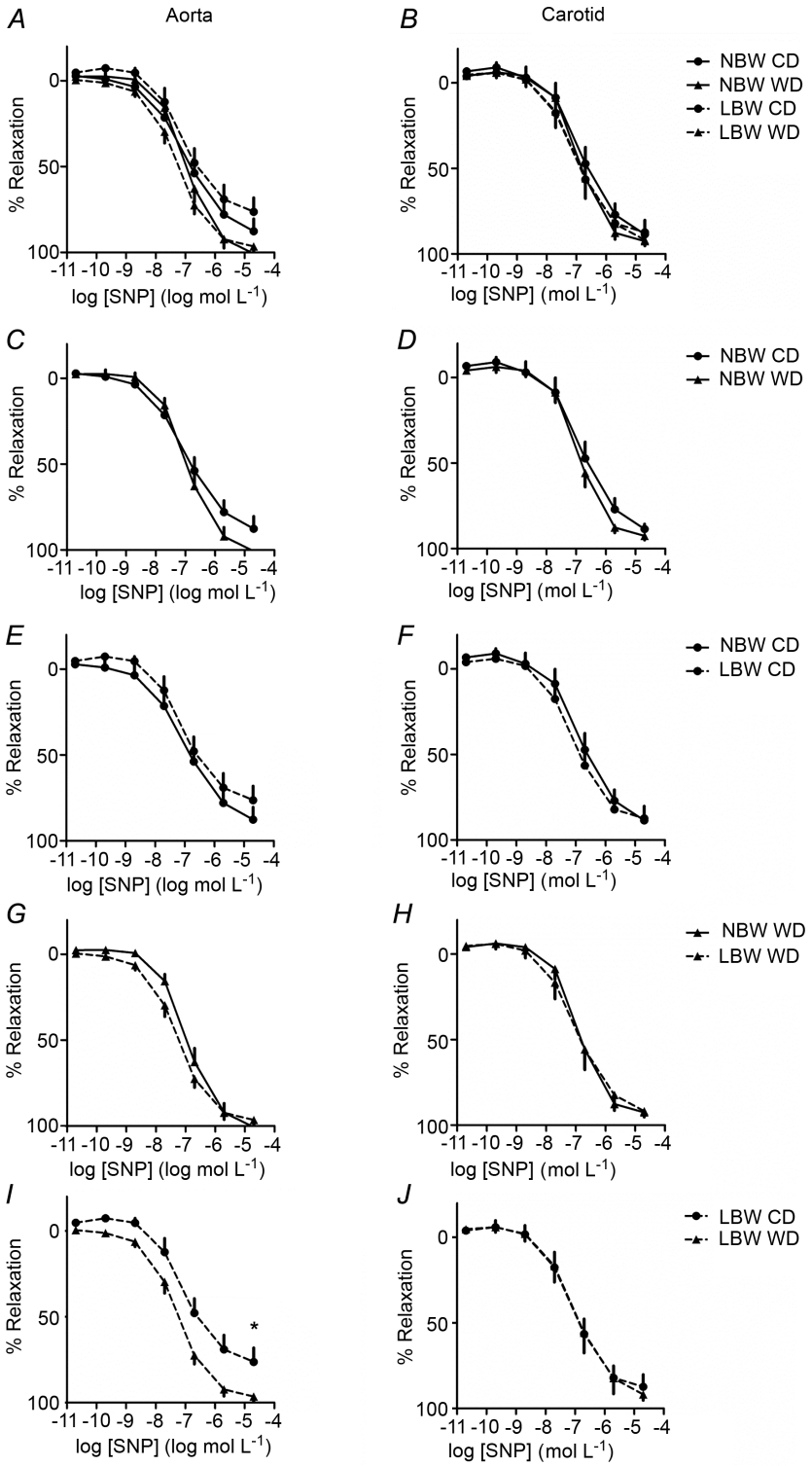

Figure 4. Relaxation responses to cumulative doses of sodium nitropusside
A and *B*, relaxation responses to cumulative doses of sodium nitropusside (SNP) in the isolated aorta (*A*) and carotid artery (*B*). *C–J*, responses to SNP in NBW/CD *vs*. NBW/WD in the aorta (*C*) and carotid (*D*); in NBW/CD *vs*. LBW/CD in the aorta (*E*) and carotid (*F*); in NBW/WD *vs*. LBW/WD in the aorta (*G*) and carotid (*H*); and in LBW/CD *vs*. LBW/WD in the aorta (*I*) and carotid (*J*) (n = 10–17 per group). *$P < 0.05$.

Table 2. Pro-fibrotic and inflammatory mediators in the left ventricle and aorta

	Left ventricle				Aorta			
	NBW/CD	NBW/WD	LBW/CD	LBW/WD	NBW/CD	NBW/WD	LBW/CD	LBW/WD
Procollagen I	1.00 ± 0.33	1.10 ± 0.37	0.86 ± 0.33	1.31 ± 0.68	1.00 ± 0.33	1.20 ± 0.34	1.62 ± 0.97	1.49 ± 0.65
Procollagen III	1.00 ± 0.04	1.06 ± 0.12	1.10 ± 0.10	1.20 ± 0.19	1.00 ± 0.10	0.79 ± 0.08	1.10 ± 0.07	0.92 ± 0.17
MMP-2	1.00 ± 0.19	0.84 ± 0.22	0.93 ± 0.19	0.60 ± 0.10	1.00 ± 0.18	0.94 ± 0.17	1.01 ± 0.24	1.06 ± 0.08
TGFβ_1	1.00 ± 0.11	1.64 ± 0.21	1.27 ± 0.36	1.33 ± 0.17	1.00 ± 0.15	1.02 ± 0.16	1.19 ± 0.34	1.19 ± 0.11
MCP-1	1.00 ± 0.14	0.54 ± 0.14	0.81 ± 0.17	0.47 ± 0.09	1.00 ± 0.26	0.56 ± 0.10	0.95 ± 0.35	1.44 ± 0.60
αMHC	1.00 ± 0.16	1.30 ± 0.12	0.71 ± 0.11	1.18 ± 0.21	—	—	—	—
βMHC	1.00 ± 0.09	0.93 ± 0.11	1.25 ± 0.25	0.98 ± 0.13	—	—	—	—
ANP	1.00 ± 0.33	1.10 ± 0.37	0.86 ± 0.33	1.31 ± 0.68	—	—	—	—

Data shown are fold change relative to the NBW/CD group, expressed as mean ± SEM.

predisposition to vascular dysfunction may confer susceptibility to a secondary insult during postnatal life and thus contribute to an individual's total risk status. The present study thus aimed to elucidate how superimposition of a postnatal WD, a ubiquitous risk factor in certain populations, impacts upon vascular function in young LBW offspring. We studied the independent and interactive effects of a suboptimal intrauterine conditions and postnatal WD on aortic stiffness and vascular reactivity in male and female guinea pigs.

The present study took advantage of the guinea pig for the study of developmental programming, as unlike other rodents but similar to the human and sheep, the guinea pig develops predominately in the prenatal period and is relatively mature at birth (Jones and Parer, 1983). Other analogies between the human and guinea pig lie in the structure and endocrine control of the placenta and thus in the vulnerability of the fetus to reductions in maternal blood flow (Mess, 2007). Uterine artery ablation leads to a decrease in maternal delivery of oxygen and nutrients and thus recapitulates the most common type of placental insufficiency in obstetrical patients characterized by abnormal Doppler waveforms in the uterine artery and placental hypoxia (Aviram, 2010). For the present study, a decrease in body weight and abdominal circumference without a significant change in body length in LBW guinea

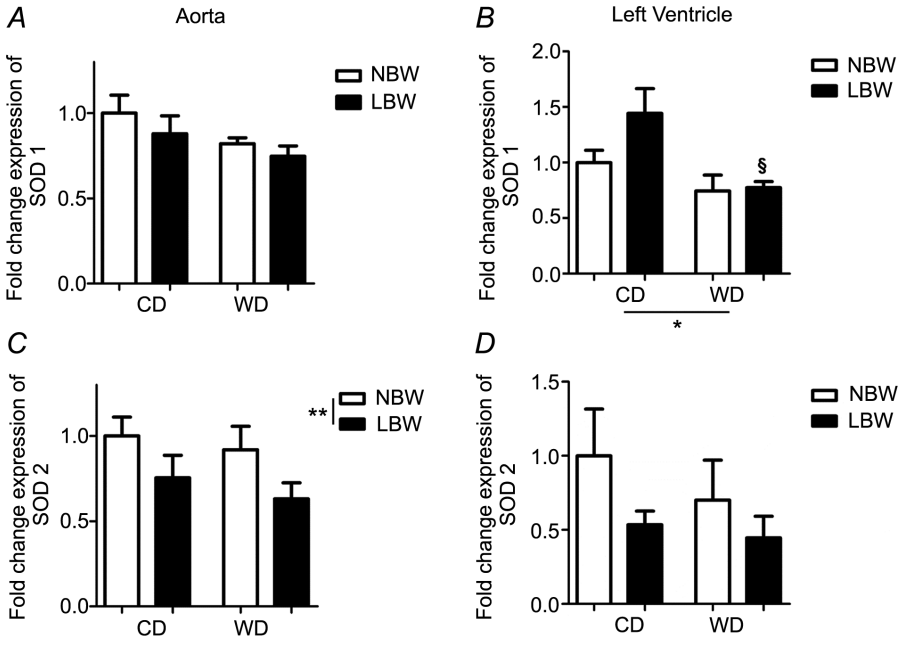

Figure 5. mRNA levels of SOD1 and SOD2 in the aorta and left ventricle
mRNA levels of SOD1 (*A*) and SOD2 (*C*) in the aorta and mRNA levels of SOD1 (*B*) and SOD2 (*D*) in the left ventricle, as measured by RT-PCR. There was a significant effect for diet ($P < 0.05$) and birth weight ($P < 0.05$) as sources of variation for SOD1 in the heart and SOD2 in the aorta, respectively, as calculated by two-way ANOVA ($n = 6$–9 per group). §$P < 0.05$ in Bonferroni *post hoc* test comparing LBW, WD *vs.* CD. * < 0.05 for each source of variation (diet or birth weight).

pig pups suggests that IUGR resulted from a reduction in placental blood flow.

Vascular function in isolated arterial segments was evaluated in terms of compliance and endothelial-dependent and -independent responses. Intriguingly, results of the current study suggest that a sub-optimal intrauterine environment has a greater impact on vascular function in young adulthood than chronic postnatal intake of a WD. Aortic compliance was reduced by the WD alone, but a markedly greater shift in the length–tension curve was observed in LBW animals fed a CD and the stiffest vessels were those of the LBW/WD animals. Stiffening of the proximal circulation, particularly the aorta, disturbs pulsatile haemodynamics and promotes ventricular hypertrophy and microvascular damage through arterial ventricular uncoupling (Abhayaratna *et al.* 2008). Proximal stiffening is an important antecedent to hypertension, as evidenced by several recent reports, including on a cohort of aged men and women from the Framingham Heart Study in which aortic stiffening preceded hypertension (Kaess *et al.* 2012). It is thought that fragmentation of elastic laminae due to repeated oscillatory stress underlies progressive central stiffening that in turn contributes to the age-related increase in risk for hypertension (Cohn, 2001; O'Rourke, 2007). Further evidence for this temporal relationship between central stiffening and hypertension was provided by another study which demonstrated that hypertension presents 5 months after the development of wall stiffening in diet-induced obesity (Weisbrod *et al.* 2013). Aortic stiffening in LBW offspring found in the present study corroborates our previous results, which showed aortic stiffening along with a reduction in elastic fibre content in aged (15 months) guinea pig offspring subjected to uterine artery ligation *in utero* (Thompson *et al.* 2011*b*). Importantly, however, the former highlights that aortic stiffening consequent to suboptimal intrauterine conditions manifests at an early age (5 months), thus providing further support to the notion that central stiffening is an important player in the propensity toward cardiovascular disorders including hypertension and atherosclerosis, which have been consistently observed in human adults born small. Although, stress–strain curves derived from a pressurized myograph system are a superior method for measuring arterial compliance compared to length–tension curves, we feel that the length–tension data presented in the current study still provide a measure of altered arterial stiffness. Although we did not measure blood pressure in the present study, other studies have demonstrated increased mean arterial blood pressure in adult rodents including guinea pigs that were growth restricted by experimentally induced reductions in uterine blood flow (Persson and Jansson, 1992; Battista *et al.* 2002; Alexander, 2003). It is possible that hypertension did not develop in guinea pig offspring used in the present study due to the young age at which they were studied. Nevertheless, our data suggest that a pre-hypertensive phenotype is present in LBW offspring.

Accelerated central stiffening may contribute to the increased risk for heart failure reported in LBW human adults (Barker *et al.* 1989, 1990). It has been previously demonstrated that fetal cardiomyocyte maturation is impaired under conditions of intrauterine hypoxia (Bubb *et al.* 2007; Louey *et al.* 2007; Morrison *et al.* 2007), and that in postnatal life IUGR offspring have a reduced capacity to recover from ischaemia reperfusion injury (Li *et al.* 2003). Therefore, limitations in the ability of the IUGR heart to compensate for an increase in pulsatile load consequent to central stiffening may lead to the development of heart failure and myocardial ischaemia. Compensatory left ventricular remodelling and hypertrophy were measured in the present study by markers including βMHC, αMHC and ANP (Xu *et al.* 2006). A change in the expression of these genes was not apparent in our young LBW offspring nor was a change in the relative heart weight. We attribute this apparent lack of left ventricular remodelling to the young age of the animals under study and would predict that compensatory cardiac remodelling would occur later in life.

It is known that arterial stiffening is accompanied by endothelial dysfunction and atherosclerosis and that inflammation and oxidative stress mechanistically link these vascular conditions (Campuzano *et al.* 2006; Anggrahini *et al.* 2009). In addition to aortic stiffening, endothelial dysfunction was present in LBW offspring fed a CD, as indicated by reduced E_{max} and increased EC_{50} of dose responses to MCh in the aorta and carotid artery. These findings are in agreement with other studies that have reported endothelial dysfunction in LBW offspring subjected to a range of prenatal insults (Mohn *et al.* 2007; Giussani *et al.* 2012). Investigation into programming of vascular dysfunction has primarily been conducted using rat models. Unlike the rat, but similar to the human, the guinea pig is a precocious developer and hence the present observation that endothelial dysfunction is present in the young adult LBW guinea pig offspring is significant. Note that studies examining the influence of intrauterine insults on postnatal vascular reactivity have not yielded consistent results, with variability between models, species and the arterial segment under study (Morton *et al.* 2010; Mazzuca *et al.* 2010). We assessed the function of the aorta and carotid artery given that these vessels are susceptible to atherosclerotic changes (Stary *et al.* 1992) and because we previously observed intima hyperplasia and altered ECM remodelling in the aorta but not in the atherosclerosis-resistant mesenteric artery of IUGR fetuses (Thompson *et al.* 2011*b*). Not only were abnormal endothelium-dependent responses apparent in each of these arteries, but also were apparent in both female and male offspring. Rat studies have overwhelmingly reported

a predominately male susceptibility to hypertension in IUGR offspring, yet evidence suggests that this sex-specific effect may be due to differential effects of prenatal nutritional deprivation on kidney development (Baserga *et al.* 2009; Ojeda *et al.* 2012). Our work implies that endothelial dysfunction in atherosclerotic-prone arteries is present at an early age in both female and male offspring growth restricted by placental insufficiency *in utero*, but not in normally grown offspring fed a WD from the time of weaning.

High intake of SFAs and simple sugars and low intake of PUFAs are each independently associated with risk for cardiovascular disease and have deleterious effects on vascular function (Li *et al.* 2013; Vedtofte *et al.* 2012; Bray, 2013). A recent systematic review of 30 countries revealed that in the majority of those countries more than 50% of children and adolescents consumed over what is recommended by the World Health Organization for total fat and SFAs and also did not reach the recommended intake of PUFAs (Harika *et al.* 2011). Such changes in dietary habits have paralleled an alarming rise in the incidence of obesity and presence of cardiovascular risk factors in children (Corvalan *et al.* 2010). The WD used in our experiments was designed to replicate a classic WD as defined by a high relative contribution of saturated fats and simple sugars and was anticipated to induce adverse metabolic adaptations (Estranyi *et al.* 2012). The guinea pig is similar to the human not only in terms of developmental timing but also in many aspects of lipid metabolism and in metabolic and cardiovascular responses to a high-fat diet (Yang *et al.* 2011) and thus is an ideal model for studying the interaction between IUGR and postnatal high-fat diet. As expected, chronic intake of a WD exacerbated aortic stiffening in LBW offspring. These data provide insight into the epidemiological observation that fetal under-nutrition followed by postnatal over-nutrition or accelerated growth in childhood has a doubling effect on the later burden of cardiovascular disease (Eriksson *et al.* 1999). Interestingly, our data suggest that the intrauterine environment is the predominant player in this synergistic relationship between divergent prenatal and postnatal nutritional states.

The adverse effects of a WD on vascular function are largely mediated through inflammation and oxidative stress (Li *et al.* 2013; Renna *et al.* 2013). These pathways collaborate in the induction of genes and proteins that lead to modification in the responsiveness and mechanical behaviour of the vascular wall. Birth weight was a significant source of variation in aortic levels of SOD2, while diet was a significant source of variation in levels of SOD1 in the left ventricle. Thus, it appears that mitochondria SOD2 was influenced more by the intrauterine environment while cytosolic SOD1 was affected by the WD. A reduction in the antioxidant capacity of mitochdondria in LBW offspring may be due to abnormal *in utero* mitochondrial biogenesis in adaptation to insufficient oxygen and nutrient supplies. In support of this, Liu *et al.* (2012)) reported suppressed mRNA levels of genes involved in mitochondrial biogenesis and decreased SOD2 activity in the liver of IUGR piglets. Although we did not follow through with protein and activity assays, decreases in antioxidant enzyme activities have also been reported in human IUGR offspring (Mohn *et al.* 2007.) Contrary to studies in the rat (Cambonie *et al.* 2007; Giussani *et al.* 2012), we did not observe an increase in vascular oxidative stress as measured by nitrotyrosine staining in young LBW guinea pig offspring. Note that the major limitation of the current study in terms of identifying underlying mechanisms is that measurements of gene expression were made at a single time point after a chronic perturbation.

Conclusion

It is now recognized that hypertension and heart failure can be traced to a vulnerability established *in utero*, independent of the classical risk factors. Yet this pre-birth component of cardiovascular disease has yet to be fully integrated into clinical risk profiling and preventative policy. For instance, IUGR/LBW was not named a high-risk target in the American Heart Association's Impact Goals for 2020 (Lloyd-Jones *et al.* 2010). There remains skepticism regarding the contributing role of prenatal factors to cardiovascular health relative to prevailing postnatal risk factors such as diet and exercise (Gillman, 2002). Interestingly, the present study demonstrates that the independent effect of the intrauterine environment on vascular function is greater than that of a postnatal WD in young adult guinea pigs. Moreover, our data reveal that although vascular dysfunction does not arise through chronic intake of a WD alone, in utero insults and postnatal WD have synergistic effects on aortic stiffening. Thus, these results suggest that arterial wall compliance in postnatal life is largely a function of pre-birth factors and that a decline in vascular function follows exposure of a developmentally programmed pre-hypertensive phenotype to a secondary postnatal insult. Together, our data highlight the importance of the intrauterine environment in long-term vascular health and implore further investigation into its weight in total risk status and contribution to the world-wide epidemic of cardiovascular disease.

References

Abhayaratna WP, Srikusalanukul W & Budge MM (2008). Aortic stiffness for the detection of preclinical left ventricular diastolic dysfunction: pulse wave velocity versus pulse pressure. *J Hypertens* **26**, 758–764.

Alexander BT (2003). Placental insufficiency leads to development of hypertension in growth-restricted offspring. *Hypertension* **41**, 457–462.

Anggrahini DW, Emoto N, Nakayama K, Widyantoro B, Adiarto S, Iwasa N, Nonaka H & Rikitake Y (2009). Vascular endothelial cell-derived endothelin-1 mediates vascular inflammation and neointima formation following blood flow cessation. *Cardiovasc Res* **82**, 143–151.

Aviram R, Shental BT & Kidron D (2010). Placental aetiologies of foetal growth restriction: clinical and pathological differences. *Early Hum Dev* **86**, 59–63.

Barker DJP, Bull AR, Osmond C & Simmonds SJ (1990). Fetal and placental size and risk of hypertension in adult life. *BMJ* **301**, 259–262.

Barker DJP, Osmond C, Winter PD & Margetts B (1989). Weight in infancy and death from ischaemic heart disease. *Lancet* **2**, 577–580.

Baserga M, Bares AL, Hale MA, Callaway CW, McKnight RA, Lane PH & Lane RH (2009). Uteroplacental insufficiency affects kidney VEGF expression in a model of IUGR with compensatory glomerular hypertrophy and hypertension. *Early Hum Dev* **85**, 361–367.

Battista MC, Oligny LL, St.-Louis J & Brochu M (2002). Intrauterine growth restriction in rats is associated with hypertension and renal dysfunction in adulthood. *Am J Physiol Endocrinol Metab* **283**, E124–E131.

Bray GA (2013). Energy and fructose from beverages sweetened with sugar or high-fructose corn syrup pose a health risk for some people. *Adv Nutr* **4**, 220–225.

Bubb KJ, Cock ML, Black MJ, Dodic M, Boon WM, Parkington HC, Harding R & Tare M (2007). Intrauterine growth restriction delays cardiomyocyte maturation and alters coronary artery function in the fetal sheep. *J Physiol* **578**, 871–881.

Cambonie G, Comte B, Yzydorczyk C, Ntimbane T, Germain N, Oanh NL, Pladys P, Gauthier C, Lahaie I, Abran D, Lavoie JC & Nuyt AM (2007). Antenatal antioxidant prevents adult hypertension, vascular dysfunction and microvascular rarefaction associated with in utero exposure to a low-protein diet. *Am J Physiol Regul Integr Comp Physiol* **292**, R1236–R1245.

Campuzano R, Moya JL, Garcia-Lledo A, Tomas JP, Ruiz S, Megias A, Balaguer J & Asin E (2006). Endothelial dysfunction, intima-media thickness and coronary reserve in relation to risk factors and Framingham score in patients without clinical atherosclerosis. *J Hypertens* **24**,1581–1588.

Cohn JN (2001). Arterial compliance to stratify cardiovascular risk: more precision in therapeutic decision making. *Am J Hypertens* **14**, 258S–263S.

Corvalan C, Uauy R, Kain J & Martorell R (2010). Obesity indicators and cardiometabolic status in 4-yr-old children. *Am J Clin Nutri* **9**, 166–174.

Curhan GC, Willett WC, Rimm EB, Spiegelman D, Ascherio AL & Stampfer MJ (1996). Birth weight and adult hypertension, diabetes mellitus and obesity in US men. *Circulation* **94**, 3246–3250.

Detmer A, Gu W, Carter AM (1991). The blood supply to the heart and brain in the growth restricted guinea pig fetus. *J Dev Physiol* **15,** 153–156.

Eriksson JG, Forsen T, Tuomilehto J, Winter PD, Osmond C & Barker DJ (1999). Catch-up growth in childhood and death from coronary heart disease: longitudinal study. *BMJ* 318(7181), 427–431.

Estranyi ME, Proenza AM, Gianotti M & Llado I (2012). High-fat diet feeding induces sex-dependent changes in inflammatory and insulin sensitivity profiles of rat adipose tissue. *Cell Biochem Funct* **31**, 504–510.

Ghidini A (1996). Idiopathic fetal growth restriction: a pathophysiologic approach. *Obstet Gynecol Surv* **51**, 376–382.

Gillman MW (2002). Epidemiological challenges in studying the fetal origins of adult chronic disease. *Int J Epidemiol* **31**, 294–299.

Giussani DA, Camm EJ, Niu Y, Richter HG, Blanco CE, Gottschalk R, Blake EZ, Horder KA, Thakor AS, Hansell JA, Kane AD, Wooding FBP, Cross CM & Herrera EA (2012). Developmental programming of cardiovascular dysfunction by prenatal hypoxia and oxidative stress. *PloS One* **7**, e31017.

Gluckman PD & Hanson MA (2007). Developmental plasticity and human disease: research directions. *J Intern Med* **261**, 461–471.

Greulich S, Herzfeld de Wiza D, Preilowski S, Ding Z, Mueller H, Langin D, Jaquet K, Ouwens M, Eckel J (2011). Secretory products of guinea pig epicardial fat induce insulin resistance and impair primary adult rat cardiomyocyte function. *J Cell Mol Med* **15**, 2399–2410.

Gros R, Chorazyczewski J, Meek MD, Benovic JL, Ferguson SS & Feldman RD (2000). G-protein-coupled receptor kinase activity in hypertension: increased vascular and lymphocyte G-protein receptor kinase-2 protein expression. *Hypertension* **35**, 38–42.

Gros R, Ding Q, Armstrong S, O'Neil C, Pickering JG & Feldman RD (2007). Rapid effects of aldosterone on clonal human vascular smooth muscle cells. *Am J Physiol Cell Physiol* **292**, C788–C794.

Harika RK, Cosgrove MC, Osendarp SJM, Verhoef P & Zock PL (2011). Fatty acid intakes of children and adolescents are not in line with the dietary intake recommendations for future cardiovascular health: a systematic review of dietary intake data from thirty countries. *Br J Nutri* **106**, 307–316.

Jones CT & Parer JT (1983).The effect of alteration in placental blood flow on the growth of and nutrient supply to the fetal guinea pig. *J Physiol* **343**, 525–537.

Kaess BM, Rong J, Larson MG, Hamburg NM, Vita JA, Levy D, Benjamin EJ, Vasan RS & Mitchell GF (2012). Aortic stiffness, blood pressure progression, and incident hypertension. *JAMA* **308**, 875–881.

Kind KL, Clifton PM, Katsman AI, Tsiounis M, Robinson JS, Owens JA (1999). Restricted fetal growth and the response to dietary cholesterol in the guinea pig. *Am J Physiol* **277**, R1675–R1682.

Kind KL, Clinfton PM, Grant PA, Owens PC, Sohlstrom A, Roberts CT, Robinbson JS, Owens JA (2003). Effect of maternal feed restriction during pregnancy on glucose tolerance in the adult guinea pig. *Am J Physiol Regul Integr Comp Physiol.* **284**: R140–R152.

Li G, Xiao Y, Estrella JL, Ducsay CA, Gilbert RD & Zhang L (2003). Effect of fetal hypoxia on heart susceptibility to ischemia and reperfusion injury in the adult rat. *J Soc Gynecol Investig* **10**, 265–274.

Li X, Gonzalez O, Shen X, Barnhart S, Kramer F, Kanter JE, Vivekanandan-Giri A, Tsuchiya K, Tsuchiya K, Handa P, Pennathur S, Kim F, Coleman RA, Schaffer JE & Bornfeldt KE (2013). Endothelial Acyl-CoA synthetase 1 is not required for inflammatory and apoptotic effects of a saturated fatty acid-rich environment. *Arterioscler Thromb Vasc Biol* **33**, 232–240.

Liu J, Yu B, Mao X, He J, Yu J, Zheng P, Huang Z & Chen D (2012). Effects of intrauterine growth retardation and maternal folic acid supplementation on hepatic mitochondrial function and gene expression in piglets. *Arch Anim Nutr* **66**, 357–371.

Lloyd-Jones DM, Hong Y, Labarthe D, Mozaffarian D, Appel LJ, Van Horn L, Greenlund K, Daniels S, Nichol G, Tomaselli GF, Arnett DK, Fonarow GC, Ho PM, Lauer MS, Masoudi FA, Robertson RM, Roger V, Schwamm LH, Sorlie P, Yancy CW & Rosamond WD, American Heart Association Strategic Planning Task Force and Statistics Committee (2010). Defining and setting national goals for cardiovascular health promotion and disease reduction: the American Heart Association's strategic impact goal through 2020 and beyond. *Circulation* **121**, 586–613.

Louey S, Jonker SS, Giraud GD & Thornburg KL (2007). Placental insufficiency decreases cell cycle activity and terminal maturation in fetal sheep cardiomyocytes. *J Physiol* **580**, 639–648.

Mariencheck MC, Davis EC, Zhang H, Ramirez F, Rosenbloom J, Gibson MA, Parks WC & Mecham RP (1995). Fibrillin-1 and fibrillin-2 show temporal and tissue-specific regulation of expression in developing elastic tissues. *Connect Tissue Res* **31**, 87–97.

Mazzuca MQ, Wlodek ME, Dragomir NM, Parkington HC, &Tare M (2010). Uteroplacental insufficiency programs regional vascular dysfunction and alters arterial stiffness in female offspring. *J Physiol* **588**, 1197–2010.

Mess A (2007). The guinea pig placenta: model of placental growth dynamics. *Placenta* **28**, 812–815.

Mohn A, Chiavaroli V, Cerruto M, Blasetti A, Giannini C, Bucciareli T & Chiarelli F (2007). Increased oxidative stress in prepubertal children born small for gestational age. *J Clin Endocrinol Metab* **92**, 1372–1378.

Morrison JL, Botting KJ, Dyer JL, Williams SJ, Thornburg KL & McMillen IC (2007). Restriction of placental function alters heart development in the sheep fetus. *Am J Physiol Regul Integr Comp Physiol* **293**, R306–R313.

Morton JS, Rueda-Clausen CF & Davidge ST (2010). Mechanisms of endothelium-dependent vasodilation in male and female, young and aged offspring born growth restricted. *Am J Physiol Regul Integr Comp Physiol* **298**, R930–R938.

Ojeda NB, Hennington BS, Williamson DT, Hill ML, Betson NE, Sartori-Valinotti JC, Reckelhoff JF, Royals TP & Alexander BT (2012). Oxidative stress contributes to sex differences in blood pressure in adult growth-restricted offspring. *Hypertension* **60**, 114–122.

O'Rourke MF (2007). Arterial aging: pathophysiological principles. *Vasc Med* **12**, 329–341.

Pacher P, Beckman JS & Liaudet L (2007). Nitric oxide and peroxynitrite in health and disease. *Physiol Rev* **87**, 315–424.

Persson E & Jansson T (1992). Low birth weight is associated with elevated adult blood pressure in the chronically catheterized guinea pig. *Acta Physiol Scand* **145**, 195–196.

Renna JF, Lembo C, Diez E & Miatello RM (2013). Role of renin-angiotensin system and oxidative stress on vascular inflammation in insulin resistance model. *Int J Hypertens* **2013**, 420979.

Shapiro SD, Endicott SK, Province MA, Pierce JA & Campbell EJ (1991). Marked longevity of human lung parenchimal elastic fibres deduced from prevalence of D-aspartate and nuclear-weapons-related radiocarbon. *J Clin Invest* **87**, 1828–1834.

Stary HC, Blankenhorn DH, Chandler AB, Glagov S, Insull W Jr & Richardson M (1992). A definition of the intima of human arteries and of its atherosclerosis-prone regions. A report from the Committee on Vascular Lesions of the Council on Atherosclerosis, American Heart Association. *Circulation* **85**, 391–403.

Thompson JA, Richardson BS, Gagnon R & Regnault TRH (2011*a*). Chronic intrauterine hypoxia interferes with aortic development in the late gestation ovine fetus. *J Physiol* **589**, 3319–3332.

Thompson JA, Gros R, Piorkowski K, Richardson BS & Regnault TRH (2011*b*). Central arterial stiffening in adulthood linked to aberrant aortic remodeling under suboptimal intrauterine conditions. *Am J Physiol Regul Integr Comp Physiol* **301**, R1731–R1737.

Turner AJ & Trudinger BJ (2009). A modification of the uterine artery restriction technique in the guinea pig fetus produces asymmetrical ultrasound growth. *Placenta.* **30**, 234–240.

Vafaie F, Yin H, O'Neil C, Nong Z, Watson A, Arpino JM, Chu MW, Wayne Holdsworth D, Gros R, Pickering JG (2014) Collagenase-resistant collagen promotes mouse aging and vascular cell senescence. *Ageing Cell* **13**, 121–130.

Vedtofte MS, Jakobsen MU, Lauritzen L & Heitmann BL (2012). The role of essential fatty acids in the control of coronary heart disease. *Curr Opin Clin Nutr Metab Care* **15**, 592–596.

Weisbrod RM, Shiang T, Al Sayah L, Fry JL, Bajpai S, Reinhart-king CA, Lob HE, Santhanam L, Mitchell G, Cohen RA & Seta F (2013). Arterial stiffening precedes systolic hypertension in diet-induced obesity. *Hypertension* **62**, 1105–1110.

William J (2006). Sonographic diagnosis of fetal growth restriction. *Clin Obstet Gynecol* **49**, 295–307.

Xu Y, Williams SJ, O'Brien D, Davidge SJ (2006). Hypoxia or nutrient restriction in pregnant rats leads to progressive cardiac remodeling and impairs post-ischemic recovery in adult male offspring. *FASEB J* **20**, 1251–1253.

Yang R, Guo P, Song Xin, Liu F & Gao N (2011). Hyperlipidemic guinea pig model: mechanisms of triglyceride metabolism disorder and comparison to rat. *Biol Pharm Bull* **34**, 1046–1051.

Yasmin, Wallace S, McEniery CM, Dakham Z, Pusalkar P, Maki-Petaja K, Ashby MJ, Cockcroft JR, Wilkinson IB (2005). Matrix metalloproteinase-9 (MMP-9), MMP-2 and serum elastase activity are associated with systolic hypertension and arterial stiffness. *Arterioscler Thromb Vasc Biol* **25**, 372.

Yzydorczyk C, Gobeil F, Cambonie G, Lahaie I, Le NL, Samarani S, Ahmad A, Lavoie JC, Oligny LL, Pladys P, Hardy P & Nuyt AM (2006). Exaggerated vasomotor response to ANG II in rats with fetal programming of hypertension associated with exposure to a low-protein diet during gestation. *Am J Physiol Regul Integr Comp Physiol* **291**, R1060–R1068.

Additional information

Competing interests

There are no conflicts of interest to report

Author contributions

The experimental model was conceived and designed by T.R. J.T., O.S. and T.R. carried out all tasks related to the experimental model. J.T. performed the functional and molecular experiments, with the exception of nitrotyrosine staining which was performed by K.P. Data analysis and interpretation was carried out by J.T. with input provided by T.R. and R.G. All authors participated in revision of the article. All authors qualify for authorship and have approved the final version of the paper.

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