Evidence for Sympathetic Origins of Hypertension in Juvenile Offspring of Obese Rats

Anne-Maj Samuelsson, Abigail Morris, Natalia Igosheva, Shona L. Kirk, Joaquim M.C. Pombo, Clive W. Coen, Lucilla Poston, Paul D. Taylor

Abstract—Maternal obesity in rodents is associated with increased adiposity, impaired glucose tolerance, and hypertension in adult offspring. In this study we investigated the influence of maternal obesity in the rat on blood pressure and blood pressure regulatory pathways in juvenile and adult offspring. Obesity was induced before pregnancy in female Sprague-Dawley rats by feeding a highly palatable energy-dense diet. In juvenile animals (30 days of age), before the onset of obesity and hyperleptinemia, basal nighttime mean arterial pressure was significantly raised in the offspring of obese dams (OffOb) relative to offspring of controls (OffCon; mean arterial pressure, males: OffOb, 121.8±0.6 mm Hg versus OffCon, 115.0±0.5 mm Hg, n=6, P<0.01; females: OffOb, 125.4±0.4 mm Hg versus OffCon, 114.4±0.5 mm Hg, n=6, P<0.001), as was the mean arterial pressure response to restraint stress (P<0.01). The pressor response to a leptin challenge was enhanced in OffOb rats (Δmean arterial pressure: OffOb, 9.7±0.8 mm Hg versus OffCon, 5.3±1.3 mm Hg; n=8; P<0.05). Renal tissue norepinephrine content (P<0.001) and renin expression (P<0.05) were markedly raised. Analysis of heart rate variability revealed an increased low:high frequency ratio in OffOb versus OffCon rats (P<0.05). At 90 days, hypertension in OffOb rats persisted and was abolished by α1- and β-adrenergic blockade, and cardiovascular responses to phenylephrine or sodium nitroprusside indicated altered baroreceptor function. The exaggerated pressor response to leptin in OffOb rats was maintained. Hypertension in the offspring of obese rats may arise from persistent sympathoexcitatory hyperresponsiveness acquired in early stages of development. (Hypertension. 2010;55:00-00.)

Key Words: hypertension ■ sympathetic activity ■ leptin ■ developmental programming ■ kidney

The influences of the worldwide obesity epidemic on reproductive health in women, particularly in relation to an adverse pregnancy outcome, present a significant health burden and a substantive healthcare cost.1 We now appreciate that consequences for the child may extend well beyond the perinatal period. Evidence from observational cohort studies has suggested that maternal obesity is an independent determinant of childhood obesity and metabolic syndrome, arising from adverse influences of the maternal “hypernutritional” environment on the developing child; however, because of a multiplicity of confounding variables, causality cannot easily be proven.2–5 Studies on experimental animals have provided the strongest evidence for a causal relationship.6–8 We have developed a rodent model of maternal obesity in mice9 and rats5 and have shown that adult offspring display many features of the metabolic syndrome, including hypertension at 3 months of age.9 We reported recently that hyperphagia and increased adiposity in adult rat offspring are associated with resistance to the anorectic and weight-reducing actions of leptin, as well as impaired leptin signaling in the arcuate nucleus of the hypothalamus,6,8 but the mechanism(s) underlying the etiology of hypertension secondary to maternal obesity remains to be elucidated. Theoretically, hypertension in the adult animal could arise from a direct influence of maternal obesity in fetal or neonatal life, during “windows” of developmental plasticity in cardiovascular homeostatic pathways, or it may be secondary to the development of increased adiposity and associated hyperleptinemia in adulthood.6,10 We, therefore, investigated blood pressure and pathways of blood pressure regulation in offspring of obese Sprague-Dawley rats from weaning to adulthood, using radiotelemetric monitoring. We hypothesized that altered central leptin sensitivity, which we have demonstrated previously in the development of hyperphagia in the offspring of obese dams,8 may also contribute to evolution of the hypertension observed. Hence, we have investigated cardiovascular responses to endogenous administration of leptin. To evaluate pathways of autonomic control, we have investigated cardiovascular reactivity to restraint stress, the renin-angiotensin system, renal catecholamine content, effects of α- and β-adrenergic receptor blockade, heart rate (HR) variability (HRV), and the baroreceptor response.
Methods

Animals
Female Sprague-Dawley rats (30 days of age; 205±5 g; Banting & Kingman) were maintained under controlled conditions (light from 7:00 am to 7:00 pm; 21±2°C; 40% to 50% humidity) and fed either a highly palatable obesogenic diet (20% fat and 10% simple sugar [wt/wt]; energy: 4.5 kcal/g) or a standard chow diet (3% fat and 7% simple sugar [wt/wt]; energy: 3.5 kcal/g; RM3, Special Dietary Services) 5 weeks before mating and throughout pregnancy and lactation (n=12 per group, as described previously). Litter size was standardized to 8 pups (4 male and 4 female) 48 hours after birth. All of the offspring were weaned at 21 days of age and subsequently fed a standard maintenance diet (RM1, Special Diets Services) ad libitum. One male and 1 female from each litter were euthanized for blood and tissue collection at 30 and 90 days of age; cardiovascular function was evaluated in the remaining littermates. Standard principles of laboratory animal care were in accordance with the Animals (Scientific Procedures) Act (1986).

Radiotelemetry
The methods for radiotelemetry have been described previously. Briefly, adult offspring of control (OffCon) and obese dams (OffOb) at 82 or 172 days of age) were implanted with a telemetry transmitter (DSI PhysioTel PA-C10, Data Sciences International,) inserted into the abdominal aorta. Young offspring (23 days) were implanted with small animal transmitters (DSI PhysioTel mouse PA-C10) using a modified carotid artery placement described previously for mice. Chronically indwelling venous catheters were inserted into the jugular vein for IV infusions, as described previously. For reasons of practicality and welfare, this method was not feasible in the weanling (30-day-old) offspring.

HRV was analyzed from the telemetry record. Cardiovascular response to a brief restraint stress was recorded for 20 minutes with 120 minutes of recovery (please see the online Data Supplement at http://hyper.ahajournals.org).

At 90 days, autonomic control of blood pressure was explored by IP administration of terazosin (10 mg/kg) and propranolol (10 mg/kg; Sigma-Aldrich Ltd) to achieve α- and β-adrenergic blockade. Baroreceptor sensitivity was assessed by measuring HR responses to IV infusions of phenylephrine (1, 2, and 4 µg/kg) or sodium nitroprusside (5, 10, and 20 µg/kg), as described previously.

Leptin Challenge
The pressor effects of a single bolus dose of IP leptin (10 mg/kg of body weight) or vehicle were assessed at 30 and 90 days of age in OffCon and OffOb rats for 3 days after 1 injection, using scheduled sampling (10 seconds every 15 minutes).

Renal Tissue Norepinephrine Content
In OffCon and OffOb rats (30 and 90 days of age), the left kidney was weighed and homogenized in 0.01 mol/L of HCl in the presence of EDTA (1 mmol/L) and sodium metabisulfite (4 mmol/L). After centrifugation (8000g; 30 minutes), the supernatant norepinephrine concentration was determined (ELISA; ALPCO Diagnostics; nr. 17-NORHU-E01-RES). Norepinephrine was expressed as nanograms per gram of renal tissue weight.

Renal Renin mRNA Expression
Real-time PCR in renal tissue for renin was performed using standard laboratory techniques in RNA extracted from the right kidney from 30- and 90-day-old animals (please see the online Data Supplement). Tissue renin mRNA was expressed as a copy number/ geometric mean of 3 housekeeping genes.

Analytic Methods
Serum angiotensin-converting enzyme concentration was assessed after cardiac puncture in OffCon and OffOb rats (30 and 90 days of age) using a colorimetric assay (KK-ACK, Alpha Laboratories Ltd).

Data and Statistical Analyses
Data are presented as mean±SEM. Cardiovascular analysis was performed by 2-way ANOVA for repeated measurements followed by the Tukey posthoc test. Baroreflex response was analyzed by a logistical sigmoid function (Graph Pad, Prism 4.02, GraphPad Software Inc) using the equation described previously. The magnitude of changes in HR evoked by changes in mean arterial pressure (MAP) was assessed by 2-way ANOVA followed by Student-Newman posthoc tests. Statistical significance was accepted at a level of P<0.05 (please see the online Data Supplement).

Results

Postweaning Body Weight and Fat Pad Mass
There was no significant difference between OffCon and OffOb rats in 30 day body weight or fat pad weight. At 90 days of age, OffOb rats were significantly heavier and had greater fat pad weights compared with OffCon rats (please see the online Data Supplement), and they had raised serum leptin levels, as reported previously.

Blood Pressure
At 30 days of age, nighttime (active phase) and daytime MAPs were higher in OffOb rats than in OffCon rats (Figure 1A).
Daytime (P<0.001) and nighttime (P<0.05) HRs were decreased in female OffOb rats only (please see the online Data Supplement).

At 90 days, OffOb offspring had increased nighttime and daytime MAPs than OffCon rats (Figure 1B). Daytime HR was reduced in male OffOb only (P<0.05; please see the online Data Supplement).

At 180 days, there were sex differences in blood pressure among the offspring. OffOb rats showed an increased nighttime MAP (MAP: OffCon males, 103.5±0.4 mm Hg versus OffOb males, 118.7±0.7 mm Hg, n=6, P<0.001; OffCon females, 113.9±0.3 mm Hg versus OffOb females, 121.2±0.5 mm Hg, n=6, P<0.01) and daytime MAP (MAP: OffCon males, 95.0±0.6 mm Hg versus OffOb males, 117.0±0.6 mm Hg, n=6, P<0.001; OffCon females, 109.0±0.3 versus OffOb females, 117.0±0.5 mm Hg, n=6, P<0.01). Thus, male OffOb rats showed a loss of diurnal blood pressure variation. There were no differences in HR at 180 days or locomotor activity between groups at any age (data not shown).

**Cardiovascular Reactivity to Stress and Renal Norepinephrine**

Responses to restraint stress at 30 and 90 days of age are shown in Figure 2A. ANOVA showed no sex differences in MAP; male and female data were, therefore, combined. At 30 and 90 days of age, restraint stress showed an increase in MAP (OffCon 20 min stress 120 min recovery **,** OffOb 20 min stress 120 min recovery **,** OffCon Male 100, Female 110, OffOb Male 110, Female 120, **,** P<0.01, **,** P<0.001, **,** repeated-measures ANOVA, n=6 to 8 per group. **,** P<0.001, **,** P<0.01, **,** P<0.05 vs control, ANOVA t test, n=6 to 8 per group.

Figure 2. A, MAP response to 20 minutes of restraint stress in offspring of control or obese dams and (B) renal tissue norepinephrine (NE) concentration in 30-day- and 90-day-old offspring of control dams (OffCon, open symbols) and obese dams (OffOb, closed symbols), **,** P<0.01, **,** P<0.05, repeated-measures ANOVA, n=6 to 8 per group, **,** P<0.001, **,** P<0.01, **,** P<0.05 vs control, ANOVA t test, n=6 to 8 per group.

At 180 days, OffOb offspring showed increased response to stress as measured by renal norepinephrine (NE) content (NE content: OffCon males, 300±50 ng/g kidney weight versus OffOb males, 400±60 ng/g kidney weight, n=6, P<0.01; OffCon females, 300±50 versus OffOb females, 400±60, n=6, P<0.01).

Figure 3. A, MAP response and (B) ΔMAP to α1-(terazosin) and β-(propanolol) blockade in 90-day-old offspring of control dams (OffCon, open symbols) and obese dams (OffOb, closed symbols), **,** P<0.01 vs control, ANOVA t test; **,** P<0.05 vs control (15 minutes, A; 60 minutes, B), repeated-measures ANOVA, n=8 per group.
age, OffOb rats showed a significantly higher MAP response to restraint (ΔMAP: 20.8 ± 0.9 and 17.7 ± 0.6 mm Hg, respectively), compared with OffCon (ΔMAP: 12.2 ± 0.9 and 8.2 ± 0.7 mm Hg, respectively). After 120 minutes, the MAP remained elevated in OffOb rats, whereas in OffCon rats MAP had returned to baseline.

Renal tissue norepinephrine content was raised at 30- and 90-day–old OffOb rats versus OffCon rats (Figure 2B). An analysis of combined male and female responses showed an increased change in MAP after 1-(terazosin) and 1-(propanolol) blockade in OffOb versus OffCon rats (Figure 3A and 3B).

**Autonomic Function**

Spectral analysis of blood pressure tracings showed increased low-frequency oscillations in 30- and 90-day–old OffOb rats versus OffCon rats (Table). Low-frequency oscillations predominantly reflect enhanced sympathetic activity. The high low-frequency:high-frequency ratio observed is also indicative of a dominant increase in sympathetic activity. At 90 days, high-frequency oscillators were strongly attenuated in OffOb rats versus OffCon rats. High-frequency HR oscillations are generally mediated through parasympathetic pathways in rats. The SD (SD of normal to normal intervals) and root mean square of successive differences, time domain parameters describing HRV, were also reduced in OffOb rats (Table; please see the online Data Supplement).

**Baroreflex Sensitivity**

At 90 days, OffOb rats demonstrated an exaggerated fall in MAP in response to sodium nitroprusside and an enhanced pressor response to phenylephrine (Figure 4A). OffOb rats also had reduced baroreflex sensitivity, attenuating the tachycardia and bradycardia responses to sodium nitroprusside and phenylephrine analyzed by linear regression, resulting in a decreased slope of the curve of HR against MAP (OffCon rats, 2.41 ± 0.10 bpm/mm Hg versus OffOb rats, 1.22 ± 0.04 bpm/mm Hg; n=8; P<0.01; Figure 4B). This impairment in baroreflex sensitivity and the decrease in HR variability at 90 days of age were also observed at 180 days of age (data not shown).

**Blood Pressure Response to Leptin Challenge**

We have reported recently that serum leptin is not altered in 30-day–old OffOb rats compared with controls but is increased at 90 days of age. Administration of leptin IP increased MAP in both OffOb and OffCon rats (sex combined) within 2 hours of injection. MAP change from baseline (ΔMAP) was significantly higher over a period of 6 hours postinjection in OffOb rats at 30 days of age and 90 days of

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**Table. Spectral Analysis Data of HRV and Pulse Interval at 30 and 90 Days of Age**

<table>
<thead>
<tr>
<th>Frequency Spectrum</th>
<th>OffCon 30 d</th>
<th>OffOb 30 d</th>
<th>OffCon 90 d</th>
<th>OffOb 90 d</th>
</tr>
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<tbody>
<tr>
<td>LF, ms²</td>
<td>1.57±0.46</td>
<td>7.59±2.11*</td>
<td>4.07±1.75</td>
<td>8.45±2.17*</td>
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<tr>
<td>HF, ms²</td>
<td>12.60±7.84</td>
<td>14.53±6.84</td>
<td>20.00±6.79</td>
<td>5.21±1.06*</td>
</tr>
<tr>
<td>LF:HF</td>
<td>0.30±0.07</td>
<td>0.79±0.05*</td>
<td>0.30±0.19</td>
<td>1.58±0.07†</td>
</tr>
</tbody>
</table>

LF indicates low-frequency bands; HF, high-frequency bands.

*P<0.05, offspring of obese vs controls, ANOVA t test, n=4 to 6 per group.
†P<0.001, offspring of obese vs controls, ANOVA t test, n=4 to 6 per group.

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![Figure 4. A, MAP response to sodium nitroprusside and phenylephrine (B) and baroreflex sensitivity in offspring of control dams (OffCon, open symbols; n=6) and offspring of obese dams (OffOb, closed symbols; n=6). Graph shows the regression analysis for the tachycardia and bradycardia responses in the offspring of control and obese dams. **P<0.001, *P<0.05 vs OffCon. **P<0.01 for slope vs OffCon using ANOVA t test. Data are presented as mean±SEM.](http://hyper.ahajournals.org/Downloaded from hyper.ahajournals.org)
Blood pressure returned to normal in all of the animals by 8 hours (Figure 5A).

**Renin mRNA Expression**
Renal renin mRNA expression showed a 3-fold increase at 30 days of age in male and female OffOb rats ($P<0.05$) and a 5-fold increase at 90 days of age in male OffOb ($P<0.01$) and female OffOb ($P<0.05$) rats compared with OffCon rats (please see the online Data Supplement).

**Angiotensinogen-Converting Enzyme Serum Analysis**
There was no significant difference in serum angiotensin-converting enzyme concentration at 30 or 90 days of age between the 2 groups (please see the online Data Supplement).

**Discussion**
This study reports several novel findings. First, we have extended our previous observations in mice and confirmed that maternal obesity induced by diet also results in persistent adult hypertension in the rat. Second, we have demonstrated that this increased MAP is already established in the juvenile offspring of obese dams at 30 days of age. This indicates that the hypertension arises as a direct consequence of in utero or postnatal exposure to maternal obesity and that it is not the consequence of increased adiposity or hyperleptinemia in the offspring, which becomes evident only in older animals. In addition, we show that the elevation of blood pressure in the young animals is accompanied by evidence for increased sympathoexcitatory activation, implicating abnormalities in autonomic control. The adult offspring similarly show evidence of sympathetic overactivation, which at this age may be influenced by the increased adiposity and hyperinsulinemia observed.

In human mother/child cohorts, 2 studies have shown a positive association between maternal body mass index in pregnancy and blood pressure of adolescent offspring; however, to our knowledge, none has explicitly compared offspring of clinically obese mothers with those of normal weight mothers. Offspring of diabetic mothers have been reported to have a higher blood pressure, but this relationship may be independent of maternal body mass index. Animal studies offer the opportunity to delineate cause-and-effect relationships. Many investigations have documented blood pressure elevation in adulthood induced by fetal nutrient restriction. Few have addressed the consequences of a maternal state of overnutrition; however, we have shown previously, using radiotelemetry, that adult offspring of nonobese dams fed a diet rich in animal fats are hypertensive, as are offspring of obese mice fed a highly palatable diet. Using the indirect tail-cuff method, Langley-Evans also reported elevated blood pressure in adult male rats exposed in utero to maternal high-saturated fat intake. No previous study has attempted measurement of blood pressure in the offspring before adulthood or before the development of other acquired abnormalities, including insulin resistance, increased fat mass, and hyperleptinemia. In this study, using a novel method of blood pressure measurement (ie, using mouse telemetry probes in juvenile rats), we were able to evaluate blood pressure in juvenile rats before the development of the adult phenotype.

To our knowledge, this is the first report to suggest that maternal diet-induced obesity in rats leads to hypertension of sympathetic origin. This was suggested by the enhanced cardiovascular stress responses to restraint, the hypersensitivity to the pressor effects of a leptin challenge, and the increased sympathetic component (high low-frequency:high-frequency ratio) of HRV in the juvenile animals. Altered sympathetic control has been implicated in several models of maternal undernutrition and, most recently, in a rat model of reduced placental blood flow, in which renal denervation abolished hypertension in growth-restricted off-
spring in adulthood. Importantly, there is some indication for parallel observations in humans, because a recent study has reported associations between fetal cardiac sympathovagal activation and maternal body mass index. Another study has reported similar abnormalities in baroreflex responses in obese children, but the extent to which this represents a primary "programming" event remains to be elucidated.

Additional evidence for a central role in the evolution of the raised blood pressure in the juvenile offspring of the obese dams is suggested by the raised renal norepinephrine concentration and renin expression, both of which are consistent with the enhanced central leptin sensitivity observed. Leptin increases blood pressure through an increase in hypothalamic and nucleus tractus solitarius efferent sympathetic tone via the renal nerve. Both systemic and central administrations of leptin increase renal nerve activity and MAP in the rat. Conversely, we have previously shown resistance to the appetite- and weight-reducing actions of exogenously administered leptin in juvenile OffOb rats. These contrasting effects of a leptin challenge on energy balance and pressor responses are indicative of selective leptin resistance, as described by others in overtly obese rodents. Because specific cardiovascular and appetite-regulatory actions of leptin can be attributed to different regions of the hypothalamus, regionally specific alteration in leptin sensitivity is considered to occur in different hypothalamic nuclei. We have shown, in relation to pathways involved in energy regulation, evidence for regional sensitivity in leptin signaling pathways in juvenile OffOb rats, with reduced phosphorylation of signal transducers and activators of transcription 3 in the arcuate nucleus but not in the ventromedial nucleus. Because the ventromedial nucleus is considered to play a focal role in the cardiovascular response to leptin, through sympathetic outflow to heart and kidney, this is consistent with the hypothesis of central selective leptin resistance in these animals.

The mechanisms that mediate renal sympathetic activation in response to leptin are still unclear. However, activation of the hypothalamic proopiomelanocortin system and its receptor types 3 and 4 (MC3/4R) appears to play an important role. Studies that have used pharmacological blockade of the MC3/4R, or those in transgenic mice which lack functional MC4R receptors, have shown that proopiomelanocortin-MC3/4R is required for leptin-induced sympathetic activation and to increase arterial pressure. Similar approaches in OffOb rats may also be informative of mechanisms leading to hypertension.

At 90 days of age, OffOb rats are hypertensive but also show a markedly increased fat pad mass and raised serum leptin, and the hypertension observed, at least in part, may be secondary to increased fat mass, recognized to enhance sympathetic efferent tone via hyperleptinemia. However, the blood pressure response to exogenous leptin was also enhanced, showing maintenance of the hypersensitivity observed in the young animals, despite the raised serum leptin concentration in adulthood. The additive effect of obesity on blood pressure could be delineated by pair feeding to control intake, because we have shown these animals to be hyperphagic. Administration of α- and β-adrenergic blockers immediately reduced MAP and negated the difference in blood pressure between obese and control animals, a finding consistent with increased sympathetic tone. Renal norepinephrine and renin expressions were also markedly increased, which would be also anticipated as a consequence of hyperleptinemia. The high-frequency domain of HRV, an index of vagal tone, was reduced in the 90-day-old OffOb rats, suggesting impairments of the parasympathetic control, which could contribute to a further increase in blood pressure. In the time-domain parasympathetic indexes, SD of normal to normal intervals and root mean square of successive differences were also reduced, which corroborates the parasympathetic dysfunction shown by spectral analysis. We also observed impaired baroreflex sensitivity in the adult hypertensive animals. The MAP-HR relationship was reset toward higher pressure (rightward shift). Resetting of the baroreflex has been reported in other models of developmental programming; prenatal exposure to dexamethasone in sheep or nutrition restriction in rat has been shown to lead to a blunted baroreflex control of HR in adulthood, and Wichi et al have demonstrated impaired baroreflex tachycardia elicited by decreasing MAP in offspring of streptozotocin-induced diabetic rats. However, the resetting of the baroreflex is commonly a consequence of hypertension but has also been implicated in the development of hypertension. The markedly enhanced pressor response in OffOb to phenylephrine may also contribute to the observed hypertension through enhanced sensitivity to endogenous α1-adrenoceptor agonists.

Sex-specific differences in a number of parameters studied were apparent, as shown in our previous studies in the offspring of obese rodents. Future studies are required in which attention is focused on the role of sexual maturity and circulating levels of sex steroids in offspring of obese rodents.

In conclusion, we have demonstrated for the first time that maternal obesity in the rat leads to hypertension in the offspring; we propose that alterations of sympathetic efferent pathways in early life play an important role. This study also adds to the increasing body of literature that suggests that leptin may be intricately involved in mechanisms of developmental programming of cardiovascular and metabolic dysfunction. We have reported previously on the presence of an amplified and prolonged neonatal leptin surge in this experimental model, which may permanently disrupt peptidergic systems and leptin signaling in the hypothalamus. We hypothesize that obesity in pregnancy and lactation may permanently affect the development of the fetal or neonatal brain leading to persistent alteration in homeostatic pathways involved in blood pressure control.

Perspectives
This study establishes a novel animal model of hypertension of developmental origin associated with abnormalities in autonomic regulation. Importantly, it suggests that maternal obesity may confer an increased risk of hypertension to the offspring. Relevance to the human condition cannot be immediately inferred, but the study presents a testable hypothesis for ongoing human birth cohorts, many of which are now focusing on the long-term consequences of maternal obesity.
Acknowledgments
We thank William Jefferson for assistance with serum analysis.

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Disclosures
None.

References
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EVIDENCE FOR SYMPATHETIC ORIGINS OF HYPERTENSION IN JUVENILE OFFSPRING OF OBESE RATS


Division of Reproduction and Endocrinology, King’s College London, London UK
Expanded materials and Methods

Telemetry and heart rate variability

Baseline mean arterial pressure (MAP), heart rate (HR) and physical activity were measured in OffCon and OffOb rats at 30, 90 and 180 days of age using radiotelemetry, as previously described. At 30 and 90 days of age heart rate variability (HRV) from BP signals (HR derived from pressure waves) was determined by spectral analysis. Briefly, analysis of time and frequency domains employing the HR variability Model for Chart Software (AD Instruments, Colorado Springs, CO) and power spectrum analysis identify differences in the high frequency (HF) domain (HF; 0.75–3.0 Hz), corresponding to parasympathetic activity, and in the low frequency domain (0.25–0.75 Hz) corresponding to preliminary sympathetic activity. Power (in ms²) was estimated as the area under the spectrum within these frequency ranges. Beat-by-beat time series of BP were generated, and series of pulse intervals (PI) were obtained by measuring the intervals between consecutive BP waves. In the time-domain, the following indices were obtained: R-R (mean RR interval), SDNN (standard deviation of normalized R-R intervals) and RMSSD (root mean square of successive differences of R-R intervals).

Restraint stress test

At 30 and 90 days of age, rats were subjected to brief restraint stress starting between 0900 and 1000 h. Restraint stress was performed by placing rats in a ‘humane rat restrainer’ comprising a clear Perspex cylinder, for 20 min; the length and diameter of the restrainer were selected on body size, with small-diameter restrainers being used in 30 days than 90 days. Mean arterial pressure was recorded for 10 min before and 20 min during stress and for 120 min during recovery in the home cage. Scheduled sampling was implemented for 10 seconds every 1 min (Dataquest LabPRO Acquisition System version 3.01, Data Sciences International, St Pauls, MN).

Real-time PCR

Total RNA was extracted from the right kidney of OffCon and OffOb rats (30 and 90 days of age) by standard TRizol (Sigma-Aldrich) method. RNA quantity and integrity were assessed by optical density (Nanodrop-1000 spectrophotometer; NanoDrop Products, Wilmington, USA). Reverse transcription was carried out using a QuantiTect Reverse Transcriptase Kit™ (cat. No. 205311; Qiagen®, Crawley, UK) according to manufacturers instructions; cDNA was stored at -80°C. Intron-spanning primers for renin for real-time PCR were designed using Universal Prolibrary® (Roche Diagnostics Ltd., Burgess Hill, UK) and Operon Biotechnologies GmbH. Amplification reactions were carried out (Thermal Cycler Corbett Rotorgene™ 6000 2-plex; Corbett research) and sample copy number determined with cDNA standard curves using rotorgene 6000 series software, and BestKeeper© used to validate stable housekeeping genes (β-actin, 18sRNA, RPL13A).
Data and statistical analysis

Data are presented as mean ±SEM. Baseline values of mean arterial pressure (MAP) and HR and cardiovascular response to stress or leptin were compared in OffCon and OffOb rats using one-way analysis of variance for repeated measurements followed by Tukey’s post hoc test. Variance in LF and HF and PI were analyzed using Model for Chart Software (AD Instruments, Colorado Springs, CO) and comparisons made using one-way analysis of variance. The relationship between MAP and HR was analyzed by a logistical sigmoid function (Graph Pad; Prism 4.02, GraphPad Software Inc, San Diego, CA) using the previously described equation. The sensitivity of the baroreflex was explored by plotting a sigmoidal curve of MAP response to PE and SNP. The magnitude of changes in HR evoked by changes in MAP was assessed by two-factor analysis of variance followed by Student-Newman- post hoc tests. The analyses were performed using Graph Pad; Prism 4.02 (GraphPad Software Inc, San Diego, CA). Where no significant differences between male and female data were observed, ANOVA collapsed to combine and increase the power. Statistical significance was accepted at a level of P<0.05.
References


Table S1. Body weight and Perirenal fat pad weight of 30 and 90 days old offspring.

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<td>female</td>
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<tr>
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<td>OffCon</td>
<td>OffOb</td>
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<td>Body weight</td>
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<td>Perirenal WAT weight</td>
<td>0.25±0.15</td>
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*P<0.05, †P<0.01, ‡P<0.001 ANOVA t-test, Values are means±SEM, n=8 per group. Abbreviation WAT-white adipose tissue
Table S2. Spectral analysis data of heart rate and pulse interval at 30 and 90 days of age.

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<th>Parameters</th>
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<td>OffOb</td>
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<td>Puls interval (mean ms²)</td>
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<td>SDNN (ms)</td>
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</tr>
<tr>
<td>LF (ms²)</td>
<td>1.57±0.46</td>
<td>7.59±2.11*</td>
</tr>
<tr>
<td>HF (ms²)</td>
<td>12.60±7.84</td>
<td>14.53±6.84</td>
</tr>
<tr>
<td>LF/HF</td>
<td>0.30±0.07</td>
<td>0.79±0.05*</td>
</tr>
</tbody>
</table>

NN interval, the mean of normalized R-R intervals, SDNN, The standard deviation of normalized R-R values, RMSDD The square root of the mean of the squared differences between adjacent NN-intervals, LF-low frequency bands HF, high frequency bands, *P<0.05, †P<0.001 offspring of obese versus controls, ANOVA t-test, n=4-6 per group.
Table S3. Serum ACE (IU/l) of 30 and 90 days old offspring.

<table>
<thead>
<tr>
<th>Serum</th>
<th>30 days</th>
<th>90 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>male</td>
<td>female</td>
</tr>
<tr>
<td></td>
<td>OffCon</td>
<td>OffOb</td>
</tr>
<tr>
<td>ACE</td>
<td>241.4±9.6</td>
<td>222.5±8.8</td>
</tr>
<tr>
<td>[IU/l]</td>
<td>187.5±10.2</td>
<td>188.1±12.9</td>
</tr>
</tbody>
</table>

Values are means±SEM, n=6 per group.
Figure S1

Figure S1. (A) Mean arterial pressure (MAP) at 30 day old and (B) 90 day old offspring of control or obese dams. Hourly averages measured over 24 hours (male, female), ***P<0.001, **P<0.01 offspring of obese dams (OffOb, closed symbols) versus offspring of control dams (OffCon, open symbols), RM ANOVA, n=6 per group, night (black bar).
Figure S2

Figure S2. (A) Heart rate at 30 day old and (B) 90 day old offspring of control or obese dams. Hourly averages measured over 24 hours (male, female) ***P<0.01, *P<0.05 Offspring of obese dams (OffOb, closed symbols) versus control dams (OffCon, open symbols), RM ANOVA, n=6 per group, night (black bar).
Figure S3. (A) Renal renin mRNA expression in 30 and (B) 90 day old offspring of control dams (OffCon, open symbols) and obese dams (OffOb, closed symbols). **P<0.01, *P<0.05 versus controls (ANOVA t-test). Data are presented as mean±SEM; n=6 per group.