*Original Research*

# Wistar-Kyoto and spontaneously hypertensive rat blood pressure after embryo transfer into different wombs and cross-suckling

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## Abstract

**Blood pressure (BP) varies based on genetic and environmental factors. To test genetic and environmental influences on body weight (BW) and BP, one-cell homozygous embryos were transferred into spontaneously hypertensive (SHR, pup:shr) or (Wistar-Kyoto normotensive [WKY], pup:wky) normotensive rats' oviducts (embryos: s,w; oviduct-uterine: S,W), cross-suckled at birth (nurses S,W) and weaned to normal diets at day-21. BP at day-120 was measured by radiotelemetry and analyzed by methods of linear least square rhythmometry and analysis of variance. Genetics dominantly affected shr BP, causing it to be significantly higher at birth ($24.6 \pm 1.8$ in sS versus $21.8 \pm 1.7$ mmHg in wW, $P < 0.005$), and at day-120 ($198 \pm 0.5$ in sSS versus $127 \pm 0.2$ mmHg in wWW, $P < 0.001$), with lower BW than those of wky ($5.3 \pm 0.2$ versus $5.7 \pm 0.2$ g at birth, $332 \pm 5$ versus $404 \pm 6$ g at day-120, both $P < 0.001$). Surprisingly, uterine-suckling milieus lowered shr BP significantly at day-120 ($198 \pm 0.5$ in sSS versus $178 \pm 0.5$, $147 \pm 0.6$, $179 \pm 0.5$ mmHg in sSW, sWS, sWW, respectively, all $P < 0.01$). BP was slightly elevated when wky-genetics were implanted into the S-uterine by 4 mmHg (wSW, $P < 0.05$), whereas implanting shr embryos into the W-uterine environment (sWS) lowered BP by 51 mmHg ($P < 0.001$). In summary, the hypertensive shr-strain showed significantly lower BP when provided with an WKY-uterine environment and/or by WKY-nursing mothers, indicating that environment can modify genetic influences; yet the shr MESORs (rhythm-adjusted 24-h mean: midline estimating statistic of rhythm) lowered by WKY environments remained above MESORs encountered in wky-donors.**

**Keywords:** embryo transfer, cross-suckling, spontaneously hypertensive and normotensive rat, ambulatory blood pressure

*Experimental Biology and Medicine* 2010; **235**: 1375–1384. DOI: 10.1258/ebm.2010.010081

## Introduction

Blood pressure (BP) levels vary widely due to predictable biological rhythms and unpredictable environmental factors.[1–7] Since variables are highly dependent upon accurate methods of measurement under stable conditions, the present study was carried out by a radiotelemetric monitoring method to obtain reliable circadian data in the cardiovascular parameters.[8] To test factors that influence body weight (BW) and BP, genetic and environmental factors were manipulated using genetic strains of spontaneously hypertensive (SHR)[9] and Wistar-Kyoto normotensive (WKY) rats in controlled oviductal-uterine and nursing milieus as environmental variables. The environmental variables interact with the embryos. Thus, genetics (embryo strains) and environmental factors (oviductal-utero-nursing strains) were manipulated in the current study using a randomized block design (Table 1a).

Considerable evidence suggests that maternal environmental factors, especially dietary factors acting either *in utero*, or through milk, may be important contributing factors to the development of hypertension in the SHR.[10,11] Different maternal nutritional environments may play a role in determining growth rate, BP and subsequent pathophysiology.[12,13] Maternal nursing behavior and nutritional delivery can be factors in SHR rats.[14] Embryo–maternal communications may influence the preimplanted embryo.[15] Some alterations of maternal milieu components, especially in the oviductal fluid composition may occur in hypertension. However, earlier embryo transfer studies using blastocyte stage embryos of 4.5 days' postcoitum, which were exposed for 1.5 days in the uterus, failed to show the expression of maternal environmental influences on BP.[13,16] They also used tail-cuff BP measurements. Embryos at a two-cell stage lack an active Na/H transporter and undergo important transformations in the membrane transporter system during the oviductal journey at this critical stage.[17,18] Na+ entry into the blastocyst is carrier-mediated and may involve several routes, including the Na+ /H+ exchanger and possibly the Na+ channel.[19] Therefore, in our current study design, one-cell homozygous

shr and wky embryos were cross-transferred into SHR or WKY ampullae before the tubal journey started. To examine environmental influence on the development of offspring, nursing milieus were introduced at birth to donors that were assigned to either an S- or W-nurse. After birth, we assessed endpoints at day-0, day-7 and day-120 for arterial BW and BP variations.

## Materials and methods

### Animals

SHR and WKY female rats (purchased from Taconic Farms, Germantown, NY, USA) were housed at the University animal facilities, with a light-cycle from 04:00 to 18:00, and standard rat chow (Purina Rodent Chow, St Louis, MO, USA) with freely accessible drinking water. They were stratified according to BW ($\pm 10$ g) and BP ($\pm 10$ mmHg) and randomly assigned to ensure balanced environmental conditions for all study groups.

The study was approved by the institutional animal care and use committee, and all of the study protocols followed NIH and the University of Minnesota Animal Use guidelines.

### Study design

To test genetic and environmental influences on BW and BP, the current study was designed for one-cell homozygous embryo transfer and cross-suckling (Table 1a). This was done to manipulate genetic (SHR/WKY strains; embryos: s,w; donors: shr/wky) and environmental factors (uterine–nursing: S,W), which were tested at three different developmental stages: at day-0 (birth) as uterine-parturition events; at day-7, cross-suckled at birth with diets exclusively through maternal milk; and at day-120, when donors were on regular diets for about 14 weeks. The first environmental manipulation occurred 12–14 h postcoitum at the one-cell stage embryos, which had not yet started the tubal journey. The one-cell embryos were implanted into SHR or WKY surrogate mothers' ampullae. The second environmental manipulation occurred at birth, when donors were assigned to either an S- or W-nurse.

### Procedures

#### *Female synchronization, embryonic transfer and offspring procedures*

Twelve- to 14-week-old SHR and WKY females were tested for behavioral estrous at 20:00 h with vasectomized sterile-proven males. Then, 'receptive' donors and recipients were mated overnight with the same strain of fertile or vasectomized males. At 12–14 h postcoitum, embryos were harvested from donors' oviducts in Whitten's media,[20] counted and assessed, and then 6–9 embryos were injected into each recipient's ampullae under Brevital anesthesia (methohexital, 50 mg/kg BW).[4,6,10,21–23]

Rats were caged one to three per box following transfer surgery and caged alone at 19 d post-transfer to prepare for the pups' arrival. Since rats give birth during the day, signs of parturition were monitored at light-on from day-20 and thereafter. Only male pups were included in this experiment to control sex variability. At birth, pups were randomly assigned 6–7 pups per nurse to each group according to similar birth times and BWs to ensure a group balance of at least five pups at the end of the suckling stage. Food was placed at a height accessible only to mothers to ensure that pups received only maternal milk.

#### *Electrolyte measurement procedure for the oviduct/bursha/uterus*

Using another set of rats, we assessed the maternal environmental pH changes in the initial stage of pregnancy. Rats were placed on a low degree heating pad; under Inactin anesthesia intraperitoneally, pH was measured in both sites of the oviduct, bursha and uterus by pH- and reference-electrodes and Electro-Chem Analyzer (Diamond General, Inc, Indianapolis, IN, USA). Electrode sensitivity was tested with pH 7.0 and 8.0 before each use. After the measurements, eggs were searched to confirm the pregnancy. For pH calculations from *mv* values obtained, $E1 = E0 + (2.3\ RT/nF)$log pH was used.

#### *Telemetry transmitter implants and measurements of BW and BP*

Since BP expression with age was the theme of our hypothesis, accurate BP measurements were of core importance in this study. Because the radiotelemetric methodology was limited to animals with a minimum BW of 140 g, BP was measured manually within 6–14 h after birth and at day-7, using techniques and procedures employed extensively in our laboratories.[6,10,21–23] For BP measurements at day-0, pups were covered with a cotton 'blanket,' fastened to an $8 \times 10$ cm glass plate and placed on a thermoregulated board. The femoral artery was exposed with a small needle probe under ether anesthesia. A glass micropipette tip thinned by a burner heat was used to access the artery internally by a micropuncture technique, and stable BPs were recorded by the Servo-null micropipette transducer system. The accuracy of the Servo-null micropipette transducer system was verified using a standard mercury sphygmomanometer, and stable intra-arterial BP recordings were observed and reproducible during the measurements. The pups' temperature was monitored in order to maintain it between 36 and 37°C using a rectal telethermister probe and a telethermometer (Yellow Spring Instruments, Yellow Spring, OH, USA). For day-7 BP measurements, carotid cannulation procedures were used under ether and 1% xylocaine local anesthesia for cautious nursing times of the seven-day-old pups' immature gastrointestinal tract.[10,24,25] To minimize the circadian variability of BW and BP, all measurements were carried out between 14:00 and 16:00 h for day-0 and day-7 data.

Radiotelemetry transmitter installation surgery was carried out at day-110 under methohexital anesthesia for the aortic catheter placement and affixing of transmitters to the abdominal wall.[8] Rats were returned to their home cage when they were fully awake and mobile with freely accessible food and acetaminophen in 5% glucose solution for the first 24 h postsurgery, followed by tap water. At day-120, data were collected continuously every four

minutes for five consecutive days to determine circadian BPs. By this time, all rats were gaining BW and BP was stabilized with the cardiovascular rhythm synchronization.

### *Statistics*

Telemetered BP data collected every four minutes using the Data Quest Program (Data Science International, St Paul, MN, USA) was converted into hourly averages and analyzed by the linear least square rhythmometry to obtain MESOR (rhythm-adjusted 24-h mean: midline estimating statistic of rhythm) and other rhythm characteristics.[26] For three-way-interaction comparisons, data were analyzed by the methods of the analysis of variance and the SPSS statistical package available at the University of Minnesota Computing Center. Data are expressed as mean ± SEM. A $P$ value of less than 0.05 was considered significant.

## Results

Embryo transfer study results are summarized in Table 1 and Figures 1–3. Genetic strains mostly dominated as the shr strain exhibited lower BWs and higher BPs. The results for BW and BP at three stages are summarized under subtitled categories for genetic and environmental effects in genetically 'susceptible' and 'non-susceptible' rat strains, although our overall results indicate that the effects of genetics and utero-nursing environmental factors are intertwined, contributing to the total BW and BP variations.

### Effects of genetics on BW and BP

At day-0, donor strains accounted for the BP variations ($F = 5.0$, $P = 0.036$). The shr genetic strain (sS) was a dominant factor, as the shr strain had significantly higher BPs ($P < 0.005$) and lower BWs ($P < 0.001$) than those of the wky donors (wW; Figure 1). At day-7, the donor strain again showed significantly affected BPs ($F = 6.84$, $P = 0.013$; Table 1a). The shr strain showed significantly higher BPs (sSS versus wWW, $P < 0.001$) and lower BWs than those of wky donors ($P < 0.005$; Figures 2a and b). All other group comparisons for BW and BP are presented in Figures 2a and b.

At day-120, the shr genetic was the single strongest factor in determining BP levels ($F = 108.45$, $P < 0.00001$; Table 1b). The shr donors (sSS) had significantly higher BPs as compared with wky donors (wWW): 198 ± 0.5 versus 127 ± 0.2, $P < 0.001$ (Figure 3b). The shr strain was a determining factor in lowering BW ($F = 91.09$, $P = 0.0001$), although BW did not show a consistent correlation with BP (Table 1b). All group comparisons on BW and BP are shown in Figures 3a and b.

### Effects of the uterine environment on BW and BP

Overall, the W-uterus lowered shr BPs at day-0. Nevertheless, the shr strain in the W-uterus (sW) had a similar BP level to that of wky donors in the S-uterus (wS), but with a larger SEM. BPs of wky donors in the S-uterus (wS) were significantly lower than those of shr donors in the S-uterus (sS) ($P < 0.05$; Figure 1). Neither the WKY nor SHR uterus affected donors' BW significantly at day-0.

At day-7, the S-uterus did not affect BW significantly in either shr or wky donors, while the uterine factor had a significant role on BP ($F = 11.36$, $P = 0.002$; Table 1a). Neither the W-uterus nor the S-uterus affected BW on their corresponding genetics (sSS versus sWS; wWW versus wSW;

**Table 1a** Offspring body weight and blood pressure after seven days' cross-suckling in genetic strains and uterine-nursing milieus in spontaneously hypertensive and normotensive rats

| Embryo strain | Uterus strain | Nurse strain | Donor type |
|---|---|---|---|
| shr | SHR | SHR | sSS |
| | | WKY | sSW |
| | WKY | SHR | sWS |
| | | WKY | sWW |
| wky | SHR | SHR | wSS |
| | | WKY | wSW |
| | WKY | SHR | wWS |
| | | WKY | wWW |

| | BW (Day-7) | | BP (Day-7) | |
|---|---|---|---|---|
| Analysis of variance | *F* | *P value* | *F* | *P value* |
| Embryo strain–A | 15.05 | 0.0003 | 6.84 | 0.013 |
| Uterus strain–B | 0.12 | 0.735 | 11.36 | 0.002 |
| Nurse strain–C | 60.69 | 0.0001 | 3.55 | 0.068 |
| A × B | 0.24 | 0.629 | 0.34 | 0.56 |
| A × C | 5.80 | 0.019 | 0.66 | 0.42 |
| B × C | 0.80 | 0.374 | 4.92 | 0.033 |
| A × B × C | 0.90 | 0.346 | 0.00 | 0.96 |
| BW | | | 2.07 | 0.16 |

Day-7, seventh day after born and cross-suckling; BP, blood pressure; BW, body weight; SHR, spontaneously hypertensive rat (donor: shr); WKY, Wistar-Kyoto normotensive rat (donor: wky); sSS, shr strain in S-uterine-nursing milieu; sSW, shr strain in S-uterine-W-nursing milieu; sWS, shr strain in W-uterine-S-nursing milieu; sWW, shr strain in W-uterine-nursing milieu; wWW, wky strain in W-uterine-nursing milieu; wWS, wky strain in W-uterine-S-nursing milieu; wSW, wky strain in S-uterine-W-nursing milieu; wSS, wky strain in S-uterine-nursing milieu
The wky and shr donors were assigned to S/W-uterine and/or S/W-nursing environment according to the design

**Table 1b** Body weight and circadian blood pressure at day-120 in genetic strains and uterine-nursing milieus in offspring of spontaneously hypertensive and normotensive rats

| | BW (Day-120) | | BP (Day-120) | |
|---|---|---|---|---|
| Analysis of variance | *F* | *P value* | *F* | *P value* |
| Embryo strain–A | 91.09 | 0.0001 | 108.45 | 0.00001 |
| Uterus strain–B | 11.27 | 0.0013 | 24.13 | 0.0001 |
| Nurse strain–C | 0.52 | 0.47 | 6.81 | 0.013 |
| A × B | 0.13 | 0.86 | 8.0 | 0.005 |
| A × C | 0.00 | 0.97 | 0.3 | 0.352 |
| B × C | 0.15 | 0.70 | 18.7 | 0.0001 |
| A × B × C | 1.37 | 0.25 | 47.6 | 0.00001 |
| BW | | | 0.4 | 0.512 |

Day-120, 120th day after born
The wky and shr donors were assigned to S/W-uterine and/or S/W-nursing environment according to the design shown in Table 1a

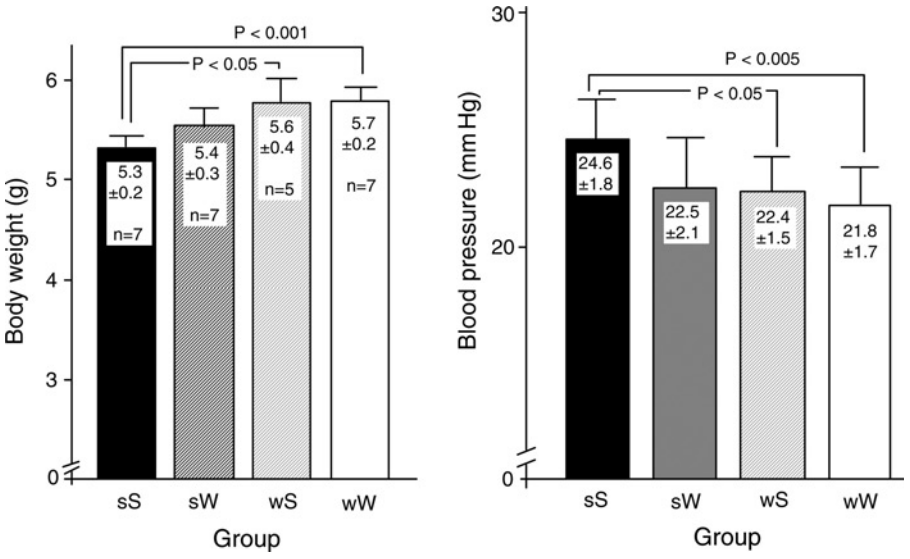



**Figure 1** Body weight and blood pressure comparison at birth (day-0) in spontaneously hypertensive and Wistar-Kyoto normotensive rats after embryo transfer into different wombs. The wky and shr donors were assigned to S- and/or W-uterine, and S- and/or W-nursing environments according to the design shown in Table 1a. Data are expressed as mean ± SEM. A *P* value of less than 0.05 was considered significant. *n*, rat number; day-0, within 14–16 h after donors born; SHR, spontaneously hypertensive rat (donor strain: shr); WKY, Wistar-Kyoto normotensive rat (donor strain: wky); sS, shr strain in S-uterine milieu; sW, shr strain in W-uterine milieu; wS, wky strain in S-uterine milieu; wW, wky strain in W-uterine milieu

Figure 2a). The W-uterus appeared to lower BP significantly in shr donors (sSS versus sWS, $P < 0.01$), while the S-uterus marginally increased wky donors' BP, but did not reach a significant level (wSW versus wWW; Figure 2b).

As shown in Table 1b, the uterine factor significantly influenced the overall BWs at day-120 ($F = 11.27$, $P = 0.0013$) and continued to play a significant role on BP ($F = 24.13$, $P = 0.0001$). Genetics and the uterine factor combined to be a strong factor on BP at this adult age ($F = 8.0$, $P < 0.005$). While the W-uterus increased the shr donor BW significantly, the S-uterus did not affect significantly on the wky donor BW (sSS versus sWS, $P < 0.05$; wWW versus wSW, $P =$ NS; Figure 3a). For BP comparisons, the altered uterine-milieus with the W-uterus significantly lowered circadian BPs in shr donors (sWS), as compared with the 'unaltered genetic-uterine milieu' (sSS) ($147 \pm 0.6$ versus $198 \pm 0.5$ mmHg, $P < 0.001$), while the S-uterus in wky donors (wSW) had a provocative effect on BP as compared with that of the W-uterus effect (wWW) ($131 \pm 0.3$ versus $127 \pm 0.2$ mmHg, $P < 0.05$; Figure 3b). BW did not show a consistent correlation with BP at all stages.

In uterine-oviductal pH comparisons, the pH of both side oviducts of pregnant rats was significantly lower at around the implantation time at 3–4 d, as compared with that of non-pregnant controls ($7.51 \pm 0.08$ versus $7.59 \pm 0.09$ for left, $P < 0.025$; $7.46 \pm 0.08$ versus $7.54 \pm 0.08$ for right, $P < 0.05$).

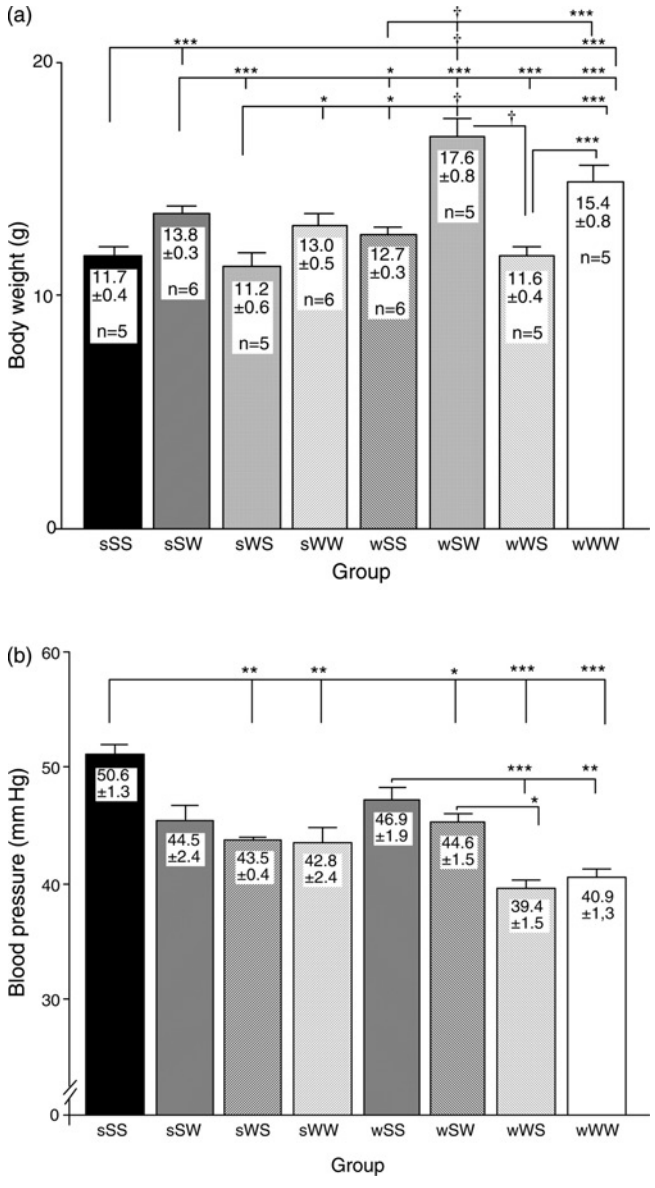
## Effects of the nursing environment on BW and BP

At day-7, nursing far outweighed other factors in the shr strain ($F = 60.69$, $P = 0.0001$; Table 1a). The W-nursing factor significantly increased shr donors' BW, while the S-nursing factor markedly reduced wky donors' BW (sSW versus sSS, wWS versus wWW, both $P < 0.001$; Figure 2a). The wky donors with the W-nursing milieu (wSW) had heavier BWs than those of wky donors with SHR nursing (wWS and wSS, both $P < 0.001$), and those of shr donors with SHR nursing milieus (wSW versus sSS and sWS, both $P < 0.001$; Figure 2a). Neither the S- or W-nursing factors on the same strain donor affected BP significantly (sSS versus sSW, wWS versus wWW, both $P =$ NS), while wky donors in W-nursing had much lower BPs as compared with those of shr donors in the S-nursing environment (sSS versus wWW, $P < 0.001$; Figure 2b).

At day-120, the nursing factor showed minor effects as compared with genetics, but the nursing factor still played a significant role on BP ($F = 6.81$, $P = 0.013$; Table 1b), while the donor strains determined almost all BW variability (Figure 2a). Altered nursing milieus with WKY significantly lowered circadian BP in shr donors (sSW), compared with those of 'unaltered genetic–environmental milieus' (sSS, $P < 0.01$), but did not reach the wky donor BP levels (sSS versus sSW, sWW, both $P < 0.01$; sSS versus wSW, wWW, both $P < 0.001$; sSW or sWW versus wSW and/or wWW, all $P < 0.001$; Figure 3b).

## Effects of uterine–nursing combined factors on BW and BP

At day-7, utero-nursing factors together significantly affected BPs ($F = 4.92$, $P = 0.033$; Table 1a). In BW comparisons, the wky strain maintained significantly heavier BWs in the W-uterine–nursing environment than those of the S-uterine–nursing environment (wSS versus wWW, $P < 0.005$), while the W-uterine–nursing environments did not significantly affect shr donors' BW (sWW versus sSS, $P =$ NS; Figure 2a). Therefore, shr and wky donors displayed similarly low BWs in sSS, sWS, wSS and wWS groups. The shr donors had significantly lower BPs when




**Figures 2** Offspring body weight (a) and blood pressure (b). Comparisons in genetic strains and uterine–nursing milieus in spontaneously hypertensive and normotensive rats in the eight-study groups after seven-day cross-suckling. The wky and shr donors were assigned to S- and/or W-uterine and S- and/or W-nursing environments according to the study design shown in Table 1a. SHR, spontaneously hypertensive rat (donor strain: shr); WKY, Wistar-Kyoto normotensive rat (donor strain: wky); *n*, rat numbers; Day-7, seven days after donors born and cross-suckled; sSS, shr strain in S-uterine–nursing milieu; sSW, shr strain in S-uterine–W-nursing milieu; sWS, shr strain in W-uterine–S-nursing milieu; sWW, shr strain in W-uterine–nursing milieu; wSS, wky strain in S-uterine–nursing milieu; wSW, wky strain in S-uterine–W-nursing milieu; wWS, wky strain in W-uterine–S-nursing milieu; wWW, wky strain in W-uterine–nursing milieu. (a) $^{*}P < 0.05$; $^{**}$ $P < 0.01$; $^{***}P < 0.005$; $^{\dagger}P < 0.001$; $^{*}P < 0.05$; (b) $^{**}P < 0.01$; $^{***}P < 0.001$

W-uterine and W-nursing environments were provided (sSS versus sWW, $P < 0.01$), while wky donors in the S-utero–S-nursing environment had significantly increased BPs (wSS versus wWW, $P < 0.01$; Figure 2b). Both shr and wky donors' BPs were influenced by altered uterine and/or nursing environments as compared with those of shr donors in 'unaltered environments (sSS).' However, the W-uterus significantly lowered BPs in both shr and wky donors in either S- or W-nursing milieus (sSS versus sWS and sWW, both $P < 0.01$; sSS versus wWS and wWW, both $P < 0.001$). The SHR uterine and/or some combination of WKY/SHR nursing milieus did not significantly lower BP in some wky or shr donors (sSS versus sSW and wSS, $P =$ NS) but wky donors had increased BP when provided S uterine–S-nursing environments (wWW versus wSS, $P <$ 0.01; Figure 2b).

At day-120, the combined utero-nursing factors significantly affected BP ($F = 18.7$, $P < 0.0001$; Table 1b). Genetic strains were dominant factors, but the uterus was still a strong factor influencing both BW and BP, especially for shr donors in either W-uterine or W-uterine–nursing factors together (sSS versus sWW, $P < 0.05$ on BW in Figure 3a; sSS versus sWW, $P < 0.01$ on BP in Figure 3b). The factors expressed in a significant embryo–uterus–nursing three-way interaction indicated that all factors affected donors' BP ($F = 47.6$, $P \leq 0.00001$; Table 1b) in the partition components dominated by genetic strains, which accounted for over two-thirds of the total BP variability (78%), while the remaining part was due to uterine (15%) and nursing (7%) factors.

In BP fluctuations shown in Figure 3c (amplitude: a measure of one-half the extent of predictable change within a day, or half of the total predictable change in rhythm defined by rhythmic function fitted to data), shr groups showed markedly increased double amplitudes in the SHR uterine milieu ($7.5 \pm 0.6$; $8.3 \pm 0.7$ mmHg for sSS and sSW), while the same shr genetics in W-uterine or W-nursing-environments were significantly lower, compared with the unchanged environment ($7.5 \pm 0.6$: $3.18 \pm 0.9$ for sSS versus sWS, $P < 0.01$; $7.5 \pm 0.6$: $3.56 \pm 0.9$ for sSS versus sWW, $P < 0.05$). The shr donors with the altered environment(s) had somewhat lower amplitudes, compared with those of wky in W-uterine–nursing milieus, although they did not reach a significant level ($4.62 \pm 0.3$ in wWW versus $3.18 \pm 0.9$, $3.56 \pm 0.7$ mmHg for sWS and sWW, $P =$ NS). The S-uterine or S-uterine–nursing environment affected wky strain's amplitudes somewhat increased (wSW, wSS) compared with the unchanged environment (wWW), but did not reach a significant level.

All groups' peak times (acrophase: the timing of overall high values recurring each cycle in relation to a reference time) were similar during their active hours at night in this nocturnal animal (21:52 through 00:45; summary table in Figure 3b).

## Discussion

Our experimental design established the two-stage maternal environmental changes induced by oviductal-uterine–nursing factors using pronormotensive/hypertensogenic strains. Our results show that both genetic strains and perinatal maternal environments are important determinants of BW and BP levels throughout postnatal developmental and adulthood stages in SHR and WKY rats. The results suggest that W-uterine–nursing factors are most likely pronormotensive, and the hypertensive expression requires embryo

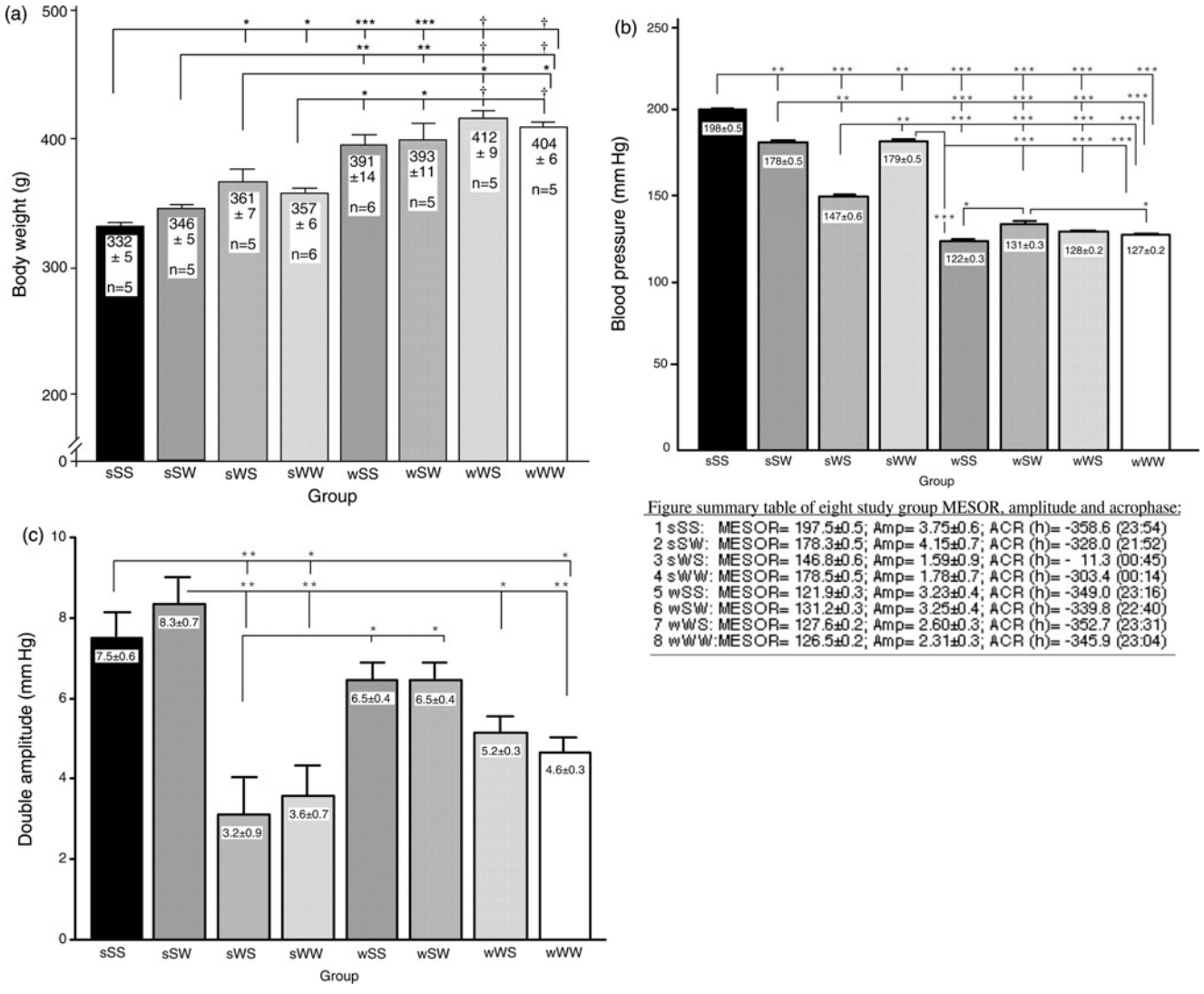



Figure summary table of eight study group MESOR, amplitude and acrophase:

1 sSS: MESOR= 197.5±0.5; Amp= 3.75±0.6; ACR (h)= -358.6 (23:54)
2 sSW: MESOR= 178.3±0.5; Amp= 4.15±0.7; ACR (h)= -328.0 (21:52)
3 sWS: MESOR= 146.8±0.6; Amp= 1.59±0.9; ACR (h)= - 11.3 (00:45)
4 sWW: MESOR= 178.5±0.5; Amp= 1.78±0.7; ACR (h)= -303.4 (00:14)
5 wSS: MESOR= 121.9±0.3; Amp= 3.23±0.4; ACR (h)= -349.0 (23:16)
6 wSW: MESOR= 131.2±0.3; Amp= 3.25±0.4; ACR (h)= -339.8 (22:40)
7 wWS: MESOR= 127.6±0.2; Amp= 2.60±0.3; ACR (h)= -352.7 (23:31)
8 wWW: MESOR= 126.5±0.2; Amp= 2.31±0.3; ACR (h)= -345.9 (23:04)

**Figure 3** Body weight (a), circadian blood pressure (b) and amplitude (c) comparisons at day-120 in genetic strains and uterine–nursing milieus in offspring of spontaneously hypertensive and normotensive rats. The wky and shr donors were assigned to S- and/or W-uterine, and S- and/or W-nursing environments according to the design shown in Table 1a. Circadian blood pressure was assessed in the eight-study groups in SHR and WKY rats by radiotelemetry at day-120.[8] Blood pressure was collected every four minutes using the Data Quest Program (Data Science International, St Paul, MN, USA) and converted into hourly averages. Data were analyzed by the Cosinor method and the linear least square rhythmometry to obtain circadian rhythm characteristics, allowing variation as a function of the data and expressed as MESOR.[26] Data are expressed as mean ± SEM. A $P$ value of less than 0.05 was considered significant. MESOR, rhythm-adjusted 24-h mean (midline estimating statistic of rhythm); amplitude (Amp), a measure of one-half the extent of predictable change within a day, or half of total predictable change in rhythm defined by rhythmic function fitted to data; acrophase (ACR), a measure of the timing of overall high values recurring each cycle in relation to a reference time. *n*, rat numbers; day-120, 120 days after donors born; sSS, shr strain in S-uterine–nursing milieu; sSW, shr strain in S-uterine–W-nursing milieu; sWS, shr strain in W-uterine–S-nursing milieu; sWW, shr strain in W-uterine–nursing milieu; wSS, wky strain in S-uterine–nursing milieu; wSW, wky strain in S-uterine–W-nursing milieu; wWS, wky strain in W-uterine–S-nursing milieu; wWW, wky strain in W-uterine–nursing milieu (a) $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.005$; $^{\dagger}P < 0.001$; (b,c) $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. Acrophase: not significant in all group comparisons

strains with a genetic propensity to hypertension. S-uterine factors are likely hypertensogenic, but their effects may require either age and/or other factors for hypertensive expressions as shown at birth, while the wky donors appear to be resistant to the hypertensogenic effects of the SHR uterus. At day-7, a cross-suckling maneuver on shr donors in homologous transfers (sSS versus sSW) reduced BP values by 12%. We expected that the day-0 vasodepressive properties of the WKY uterine milieu would be magnified at day-7 due to vasodepressive WKY milk effects.[10] However, the shr donors in the W-uterine–W-nursing environments did not lower BP significantly (sWW versus sSW). At day-120, the genetic composition of the embryos dominated the BP outcome, while the maternal environment still modulated BP variability. The wky strain with the S-uterus increased BP slightly but significantly (wSW versus wWW), while the uterine factor in the shr strain played a stronger role than the nursing factor in influencing BP levels (sWS versus sSW).

BP in the shr strain is lowered by the provision of a W-uterine environment and/or by W-nursing mothers, yet the lowered shr pressure MESORs influenced by the W-environments remained above the MESORs encountered in wky donors (Table 1b and Figure 3b). The approach of

extrachromosomally manipulating the shr genetics leads in the right direction, suggests two postconceptional extrachromosomal effects. The corresponding wky donors' BP relates perhaps to the uterine factor(s), which is relatively small. It should be emphasized that outcomes gauged by BPs monitored by telemetry every four minutes for five days provide a very solid result. It is remarkable that the telemetered BP of wky genetics by the SHR nursing showed no difference at 120 days of age. BP was only slightly elevated by no more than 4 mmHg (wSW) when wky genetics were implanted into the SHR uterus, whereas the opposite maneuver of implanting shr-embryos into the WKY uterus (sWS) lowered BP by 51 mmHg. Interestingly, W-uterine–W-nursing milieus in shr donors (sWW) showed fewer suppressed effects in lowering MESORs as the similar BP level seen in the W-nursing factor (sSW), which was much higher than that of the W-uterine–S-nursing milieu (sWS) ($P < 0.01$). The wky strain showed an opposite result in the supposedly provocative S-uterine–S-nursing environment (wSW versus wSS, $P < 0.05$). In these findings, unknown mechanisms in the W-uterine–W-nursing milieu acted as distractive or subtractive effects in shr donors' BPs, and the S-uterine–S-nursing environment acted as additive effects in lowering wky donors' BPs. The differences in MESORs were larger in shr donors in the W-uterine–S-nursing environment (sWW − sWS = 31 mmHg) than those of wky donors in the opposite combination (wSW − wSS = 9 mmHg). The perplexing, albeit consistent, result is that if both the uterine and nursing effects coexist, the result is not additive upon shr genetics, but rather reverses the beneficial effect (sWW) and the additive effect upon wky genetics (wSS). In BW comparisons, the nursing factor was most prominent during suckling, but became a non-factor by day-120, with almost all BW variability by donor strains.

Many studies have reported perinatal environmental effects on the development of hypertension. A sensitive period may exist in various environmental categories. The critical period for salt taste sensitivity appears later in embryonic development (3–8 d) than does the critical period for the BP sensitivity (1–3 d). The sensitive period for maternal dietary Na restrictions may be before the day-8 embryonic stage, in order to reduce taste responses to NaCl in the offspring. Studies suggested that NaCl susceptibility may be prevented with a low NaCl diet from the 2-day prenatal period, and possibly also through early drug intervention, as demonstrated in the intrauterine treatments with angiotensin-converting enzyme inhibitors (ACE-I) suggested by earlier studies.[9,27] The preventive effects are passed on to the offspring, even if the unaltered genetic background still exists and no antihypertensive treatment is given. But the beginning of the treatment time is critical.[27] However, this study involved a short time span without long-term follow-up and did not examine the renal arteries. Recent studies suggest that while a brief exposure to ACE-I or the angiotensin 1 receptor blockers in young SHR temporarily reduces BP by producing a specific abnormality during the renal development with the upregulated renin–angiotensin system, this also results in malignant hypertension with hypertrophied renal arterioles and cerebral hemorrhage, and premature death.[28,29]

Another study indicated that the prenatal environment in SHR does not appear to affect the final BP development of offspring at maturity,[13] which is contradicted by the present study. Others have argued that the antihypertensive effect of shr pups on W-nursing may reflect an increase in milk intake during a critical period of growth and development,[11,14] while the maternal nutritional status of the animal may play an important role in determining its susceptibility to elevated BP and subsequent pathophysiology.[12] Based on those studies, we speculated that S-nursing behavior and the delivery of milk to donors cause some BW variations. This was seen in day-7 BW data, but the variation was diffused as they matured, as shown in day-120 data, and donor strains were the determinant factor in the outcome.

In day-0 BP comparisons, the W-uterine environment appeared to lower BP in shr donors but did not reach a significant level, which may be due to a larger variation ($22.5 \pm 2.1$ mmHg) (sS versus sW, $P$ = NS) compared with the S-uterine environment in wky donors ($22.4 \pm 1.5$ mmHg) (sS versus wS, $P < 0.05$) (Figure 1). For BP data at day-7, the S-uterine environment may be a factor that raises BP in wky donors slightly but significantly (wWW versus wSS and wSW), while the W-uterine environment lowered shr donors' BP significantly (sSS versus sWS and sWW; Figure 2b). The W-nursing factor in shr donors (sSW) showed a tendency to lower BP compared with the unaltered environment (sSS), but did not reach a statistically significant level with a large standard error. The results indicate that the maternal environment plays a critical role during the developmental stage. At day-120, the wky genetic and W-uterine factors were the consistent determining factors in lowering BPs, while double environmental factors showed unexplained results as additive (wSS) or subtractive (sWW) effects (Figure 3b). The present study did not extensively examine the maternal nutritional status. Nevertheless, the maternal physical conditions were set in the study protocol, and a healthy appearance with the same diet provided in this study should not yield issues in our current study results. Furthermore, we tried to avoid any bias in the study protocol.

From our established procedures in animal BP measurements, we found that arterial BP measurements were the best for reliable data collections, regardless of whether under conditions of anesthesia or ambulatory status.[4,6,8,10,21–23] In our BP measurements, we always calibrated the equipment used with a standard mercury sphygmomanometer to ensure the accuracy of the equipment. In our old studies, we also used the tail-cuff methods, which were not always reliable, before establishing our direct arterial BP measurement methods. Thus, we measured arterial BPs in all study stages in the current study.

In BP measurements, the rhythm-adjusted 24-h mean (MESOR), BP variability (amplitude) and peak timing of the day (acrophase) should be accounted in various individuals. If BP was measured at partial times, the entire BP fluctuation spectrum, based on the individual's built-in internal rhythmic patterns, would be missed. BP is lowered at night while resting and raised during the active hours.[1–5,30] Night-time non-dipping BP as a risk factor is

well studied.[30–42] Recent studies indicate that circadian hyperamplitude tension (CHAT), circadian BP overswing, represents a greater risk than MESOR-hypertension.[2,30,43] In 1974, Barter[44] acknowledged that a patient was diagnosed with different BPs by two physicians who saw him at different times of the day. By conventional standards, this patient was clearly normotensive every morning, but BP taken in the same patient everyday at 18:00 was convincingly hypertensive. It is essential to collect enough information to allow objective characterization of a periodic phenomenon to obtain MESOR (circadian mean), amplitude (variability) and an estimate of acrophase (measure of timing).[1–4,30,43] Circadian overswinging (CHAT) and deficient (non-dipping) variability were associated with higher risks of cerebral ischemic events and nephropathy than in MESOR-hypertension.[30,43] It is also clinically significant among normotensives to detect cardiovascular disorders in a preclinical stage.

An increase in BP attributable to the 'white coat response' is frequently associated with manual BP recordings performed in community-based practice. In recognizing this limitation of manual office BP, some guidelines recommend that home BP and 24-h ambulatory BP (ABP) monitoring need to obtain an accurate measure of a patient's BP status.[45] Cardiovascular risk showed a direct and independent association with the observed ABP and an inverse association with the degree of BP reduction. Home BP, but not office BP, was predictive of cardiovascular events and systolic home BP was the sole predictor of total mortality.[46,47] White-coat hypertension versus ambulatory hypertension and dippers versus non-dippers are two classifications based on arbitrary operational risk categories. A blunted or absent BP reduction from day to night was associated with a worse outcome regardless of the average value of ABP during the 24 h. Overall, the studies indicate that ABP monitoring is particularly valuable to refining cardiovascular risk stratification in untreated subjects with office hypertension and in those with resistant hypertension.[30,43] Many studies showed that ABP provides data closely linked to individuals' true daily BP levels, and night-time BP is recognized as being the most sensitive predictor of outcome.[30–33] BP declines in the night-time during sleep, with a modest rise preawakening to regain daytime BP levels in the normal individual.[2,3,30,34,35] In ABP profiles from healthy school children aged 5–18 y, the peak–trough difference (amplitude) was 25.9 mmHg, with the peaks (acrophase) occurring at early afternoon hours during the 24-h ambulatory monitoring.[36] An actively regulated central mechanism is involved in BP resetting of the baroreflex during night-time sleep and extrinsically induced non-dipping sustained BP will be the result of an actively lowered baroreflex set point.[37] Awakening BP variability was positively correlated with awaking activity.[38] The Black population experience higher levels of systolic and diastolic BP, both at night and during the day. The differences were significantly greater at night than during the day.[39] ABP measurement is the only technique that permits close examination of the circadian profile and identification of patterns of an elevated nocturnal BP (nocturnal hypertension), which may be associated with cardiovascular adverse risk.[34]

Dipping/non-dipping and the autonomic failure have been largely ignored in clinical practice. In hypertensive patients (or some normotensive patients with cardiovascular disease), BP may not decline (non-dipping) or may have a marked nocturnal fall (extreme dipping or overswing), creating a risk of ischemic stroke and ischemic myocardial infarction.[30,33–35,40–43] Muscle sympathetic nerve traffic was significantly higher in non-dipper and extreme dipper hypertensives than in normotensive controls.[34] The reverse dipping state is characterized by a sympathetic activation of greater magnitude than that seen in the other conditions displaying abnormalities in the night-time BP pattern. Thus, sympathetic activation represents a mechanism potentially responsible for the day–night BP difference.[34] Nocturia, which may reflect impaired renal tubular function, is associated with non-dipping in patients with chronic kidney disease and appears to be mediated by increased nocturnal activity.[44] Patients with resistant hypertension present a high prevalence of the non-dipper BP pattern, which is partly related to the absence of 24-h therapeutic coverage, treated with single morning doses. In patients with resistant hypertension, pharmacological therapy should take into account when to treat with each patient's activity-cycle to improve control and to avoid the non-dipper pattern. Patients with true resistant hypertension based on controlled ABP monitoring, subjects receiving one drug at bedtime showed a significant reduction in the 24-h mean of systolic and diastolic BP. This reduction was much more prominent during night-time. Accordingly, the diurnal/nocturnal BP ratio was significantly increased and the prevalence on non-dipping reduced in patients taking one drug at bedtime.[48] Apparently the normal 24-h ABP can be nocturnal hypertension.[31,44,49] The misleading unacceptable swing values by the office or hospital delivery spot check can be corrected by personalized health care at home to access and identify acceptable everyday physiological variability as normotensive or hypertensive for treatment of hypertensive patients. The same dose of a drug can be harmful or beneficial depending on the time it is taken. Opposite results with different rhythms can be obtained at different times of the day, and the hypertensive treatment may unknowingly induce a circadian overswing. Furthermore, around-the-clock BP and heart rate monitoring can detect vascular variety disorders. The detection of individual vascular variability disorders can lead to the modification of existing measures and better care at much less cost, as well as the prevention of cardiovascular and other conditions, such as non-dipping or overswinging, when it is still in a preclinical stage. Therefore, this present study is designed to collect the complete 24-h BP variability data to present the complete observation of the circadian stage BP changes on the genetic and environmental factor modifications. Although ambulatory monitoring studies are time consuming, we attempted to closely monitor around-the-clock variability for the actual fluctuations in our present study (Figures 3b and c).

In summary, the current study indicates that prenatal environmental factors are powerful modulators of the offsprings' BP, and that the factors are enhanced as they move into the early stage of ontogenesis. The

pronormotensive and/or hypertensogenic actions start in the oviductal-uterine–nursing origin, and genetics and environmental factors are intertwined to contribute to the total BP variations. The oviduct is therefore likely an important locus-generating factor that modulates the expression of hypertension in this model. The hypertensive shr strain showed significantly lowered BPs when provided with an WKY-uterine environment and/or by WKY-nursing mothers, indicating that environment can modify genetic influences; yet the lowered shr MESORs by WKY environments remain above MESORs encountered in wky-donors.

Future studies should consider additional environmental factors affecting genetic hypertension, including the effects of gametogenesis and all stage chemical compositions during the entire pregnancy, and genetics and epigenetic controlling mechanisms.

#### ACKNOWLEDGEMENTS

The authors thank Drs Franz Halberg and Charles Christians for their invaluable review, comments and advice. The authors also thank John S Lee for his critical review and comments, and editorial assistance. This work was supported by NIH Grant RO1 HL-35643 and a grant by the Minnesota Medical Foundation, Minneapolis, Minnesota.

This work is dedicated to Dr Silvia H Azar. Unfortunately, she became ill and could not see this work published before her departure.

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*(Received March 3, 2010, Accepted July 1, 2010)*