Pregnancy in aged rats that were born small: cardiorenal and metabolic adaptations and second-generation fetal growth

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ABSTRACT **Uteroplacental insufficiency is associated with adult cardiorenal and metabolic diseases, particularly in males. Pregnancy is the greatest physiological challenge facing women, and those born small are at increased risk of gestational hypertension and diabetes and delivering smaller babies. Increased maternal age is associated with exacerbated pregnancy complications. We hypothesized that pregnancy in aged, growth-restricted females unmasks an underlying predisposition to cardiorenal and metabolic dysfunction and compromises fetal growth. Uteroplacental insufficiency was induced by bilateral uterine vessel ligation (restricted group) or sham surgery (control group) on d 18 of gestation in Wistar Kyoto rats. At 12 mo, growth-restricted F1 female offspring were mated with a normal male. F1 restricted females had elevated systolic blood pressure, before and during pregnancy (+10 mmHg) but normal renal and metabolic pregnancy adaptations. F2 fetal weight was not different between groups. In control and restricted females, advanced maternal age (12 *vs.* 4 mo) was associated with a reduction in the hypoglycemic response to pregnancy and reduced F2 fetal litter size and body weight. Aged rats born small exhibited mostly normal pregnancy adaptations, although they had elevated blood pressure. Advanced maternal age was associated with poorer fetal outcomes that were not exacerbated by low maternal birth weight.—Gallo, L. A., Tran, M., Moritz, K. M., Jefferies, A. J., Wlodek, M. E. Pregnancy in aged rats that were born small: cardiorenal and metabolic adaptations and second-generation fetal growth. *FASEB J.* 26, 4337–4347 (2012). www.fasebj.org**

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Low birth weight is associated with an increased predisposition to a number of adult diseases, including hypertension, renal insufficiency, and impaired glucose tolerance (1–4). Perturbed development of key organs, including reductions in nephron and cardiomyocyte number and pancreatic β-cell mass may be factors contributing to the common adult phenotypes described (5–9).

Uteroplacental insufficiency affects ~10% of pregnancies in the Western world and is the most common cause of reduced fetal growth (10). It is characterized by poor placental function, leading to compromised delivery of nutrients and oxygen to the fetus (11). Whereas maternal undernutrition models mimic conditions of the developing world (12–14), disease outcomes and mechanistic pathways may not be relevant when nutrition is abundant. We and others have used a rat model that mimics uteroplacental insufficiency, whereby the uterine vessels are bilaterally ligated during late gestation, resulting in offspring born 10–15% lighter than those exposed to sham surgery (8, 9, 15, 16). Male offspring generally present with more severe cardiovascular and metabolic outcomes than their female counterparts, including hypertension and impaired glucose intolerance at 6 mo of age (8, 15, 17, 18). Despite similar morphological deficits in nephron number and pancreatic β-cell mass, growth-restricted females do not become hypertensive (19) and exhibit normal metabolic control (17). In states of additional postnatal demand, however, the susceptibility to disease risk may become apparent in those who were small at birth.

Certainly, pregnancy is an intricate physiological state with profound cardiovascular, renal, and metabolic adaptations essential to support growth and development of the fetus. Small-birth-weight women, compared with those born of normal weight, are more likely to develop pregnancy-induced hypertension and gestational diabetes (20, 21) with potentially adverse consequences for fetal growth (22). We have recently reported that, when pregnant at 4 mo, equivalent to a young adult age in humans, growth-restricted female rats developed loss of glucose tolerance and glomerular

Abbreviations: E, embryonic day; IPGTT, intraperitoneal glucose tolerance test; P, postnatal day

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hypertrophy during late gestation, and second generation (F2) fetal weight was reduced by 5–6%. The risk of obstetric complications rises with increasing maternal age (23), and, together with current trends of delayed childbearing, it is of utmost relevance to investigate pregnancy outcomes in aged, premenopausal females who were small at birth.

We studied 12-mo-old growth-restricted rat offspring when they, in turn, become pregnant in the absence of any further uteroplacental insufficiency challenge. We hypothesized that the physiological challenge of pregnancy in aged, growth-restricted female rats would unveil and exacerbate adverse cardiovascular, renal, and metabolic phenotypes, slowing growth and development of the next generation. Given the potential stress associated with various physiological measurements (24), we also assessed fetal body and placenta weights in a cohort of pregnancies in which maternal physiological measurements were not made.

MATERIALS AND METHODS

Animal procedures

All experiments were approved by The University of Melbourne Animal Ethics Committee. Wistar Kyoto rats (9–13 wk of age) were housed in an environmentally controlled room (22°C) with a 12-h light-dark cycle and access to food and tap water *ad libitum*. Rats were mated, and surgery was performed on d 18 (6, 8, 15, 17–19, 25–28). In brief, F0 pregnant rats were randomly allocated to a sham treatment group (offspring termed control) or uteroplacental insufficiency group (offspring termed restricted). The restricted group underwent bilateral uterine vessel (artery and vein) ligation surgery. The F0 females delivered naturally at term on d 22 of gestation, and birth weights of F1 female offspring (control and restricted) were recorded. Uteroplacental insufficiency reduced total (male and female) litter size (6 *vs.* 9 control pups), but litter size was not equalized between the groups (n=10 litters/group). We have shown previously that reducing litter size from sham-operated dams impairs maternal mammary morphology, lactation, and subsequent postnatal growth and health of the offspring (8, 18, 26). Thus, we do not regard sham-exposed, culled litters as adequate controls. One female per litter was allocated to each group; there were no siblings within a group (n=10/group). Before mating, nonpregnant control and restricted females underwent physiological measurements (tail cuff blood pressure and 24-h metabolic cage). At 12 mo, these same females were mated, and the same physiological measurements plus a non-food-restricted intraperitoneal glucose tolerance test (IPGTT) were performed during late gestation (pregnant 1 group). At postmortem, on embryonic day (E) 20, successful pregnancies were defined as ≥3 live pups, and F2 fetal and placenta weights were recorded. An additional group of age-matched control and restricted animals (1 litter/group; n=10/group) underwent a non-food-restricted IPGTT at 12 mo of age (nonpregnant group). Given the potential stress associated with physiological measurements during late gestation, we generated a second cohort of pregnant females (pregnant 2 group) to determine the effect on fetal and placenta weights; the pregnant rats were not handled (except for animal husbandry purposes; 1 litter/group; n=4–8/group). To determine the effect of age on pregnancy outcome (pregnancy success rate, F2 litter size, body and placenta weight, and fetal/placenta ratio), 12-mo-old pregnant rats from the current study were compared with our previously published 4-mo-old pregnant cohort that were simultaneously generated.

Body weight, blood pressure, and IPGTT

F1 body weights from control and restricted females were measured at postnatal day (P) 1 and P7 and at 4 and 9 mo, at mating (12 mo), and during pregnancy at E14 and E20 (postmortem). Systolic blood pressure was measured in the morning by tail cuff before mating (nonpregnant) and at E17 of pregnancy at 12 mo (pregnant 1) in animals that were acclimatized to the restraint procedure (8, 19, 25, 27). Then a non-food-restricted IPGTT was performed in the pregnant (E18) animals and also in age-matched nonpregnant groups to prevent any fetal compromise associated with food withdrawal (6, 17, 18, 25). Tail vein blood samples were collected before and after an intraperitoneal bolus injection of glucose (1 g/kg body weight; Pharmalab, Lane Cove, NSW, Australia) in conscious animals, and plasma was stored at −20°C.

Plasma insulin concentrations were measured in duplicate using a rat insulin radioimmunoassay kit (Millipore; Abacus ALS, Brisbane, QLD, Australia). Plasma glucose concentrations were measured in duplicate using a scaled-down version of the enzymatic fluorometric analysis. Nonfasting basal plasma glucose and insulin levels were taken as the average of 2 time points (10 and 5 min before glucose injection). Area under the glucose curve and area under the insulin curve were calculated as the total area under the curve from basal to 120 min using the trapezoidal model (25). The first-phase insulin response was calculated as the area under the insulin curve from 0 to 5 min, and the second-phase insulin response was calculated as the area under the insulin curve from 5 to 60 min.

Food and water intake and renal function

Before mating (nonpregnant) and at E19 of pregnancy (pregnant 1), animals were weighed and placed individually in metabolic cages for 24-h measurements of food and water intake and urine production (19, 25). Rats were acclimatized to the metabolic cages by placing them in the cages for short daylight periods on two separate occasions and once overnight. Measurements of sodium, chloride, creatinine, total protein, uric acid (Cobas Integra 400; Roche Diagnostics, Burgess Hill, UK) and osmolality (Advanced Model 2020 Osmometer; Advanced Instruments, Norwood, MA, USA) were performed on urine samples. Plasma samples were collected postmortem from pregnant animals and analyzed for creatinine and sodium to determine creatinine clearance ([urinary creatinine (μM)×24-h urine production (ml)]/[plasma creatinine (μM)×1440 min]) and fractional sodium excretion, respectively.

Postmortem tissue collection

At E20, animals (pregnant 1 and pregnant 2) were anesthetized with an intraperitoneal injection of ketamine (100 mg/kg body weight) and Ilium Xylazil-20 (30 mg/kg body weight). Heart, kidneys, pancreas, adrenal glands, and uterus were excised from successful pregnancies only and weighed. Fetal body (F2 generation) and placenta weights were measured and are presented as an average per litter separated by sex (25).

TABLE 1. *Body and organ weights in control and restricted successful pregnancies (pregnant 1 and pregnant 2)*

Parameter	Pregnant 1		Pregnant 2	
	Control	Restricted	Control	Restricted
F0 litter size	8.7 ± 0.7	5.9 ± 0.6*	7.6 ± 0.7	6.0 ± 0.7*
F1 body weight (g)				
P1	4.2 ± 0.1	3.4 ± 0.2*	4.3 ± 0.1	3.4 ± 0.1*
P7	10.3 ± 0.5	8.2 ± 0.9*	10.6 ± 0.3	9.7 ± 0.4*
4 mo	218 ± 3	209 ± 10*	233 ± 5	210 ± 6*
9 mo	256 ± 2	240 ± 9*	266 ± 5	248 ± 6*
Mating	267 ± 2	250 ± 9*	282 ± 5	255 ± 6*
E14	310 ± 3	286 ± 12*	—	—
E20	328 ± 3	306 ± 10*	343 ± 5	317 ± 11*
Pregnancy weight gain	61 ± 1	56 ± 3	61 ± 3	62 ± 7
Organ weight (% body weight)				
Heart	0.319 ± 0.004	0.321 ± 0.005	0.297 ± 0.005	0.310 ± 0.006#
Left ventricle	0.230 ± 0.002	0.231 ± 0.004	0.218 ± 0.005	0.224 ± 0.007#
Kidney	0.511 ± 0.008	0.502 ± 0.010	0.501 ± 0.008	0.515 ± 0.003
Pancreas	0.212 ± 0.009	0.208 ± 0.008	0.215 ± 0.014	0.222 ± 0.003
Adrenal glands	0.017 ± 0.001	0.017 ± 0.001	0.016 ± 0.001	0.017 ± 0.001
Uterus	0.601 ± 0.026	0.589 ± 0.036	0.621 ± 0.034	0.683 ± 0.032

Measurements were performed in pregnant (E20) animals exposed (pregnant 1) and not exposed (pregnant 2) to physiological measurements (mean ± SE; n = 4–9/group). —, no measure because animals were not handled during pregnancy. *$P < 0.05$ *vs.* control (main effect). #$P < 0.05$ *vs.* pregnant 1 (main effect).

Statistical analyses

Values are expressed as measn ± SE with n representing the number of offspring from separate mothers per group. Homogeneity of variance was tested before any statistical analyses and found to be normally distributed. Data were analyzed using a 2-way ANOVA to determine the effects of uteroplacental insufficiency (control and restricted) and exposure to environmental stress (pregnant 1 and pregnant 2) (see **Table 1**), uteroplacental insufficiency and pregnancy (nonpregnant and pregnant; see **Fig. 2**), and uteroplacental insufficiency and F2 sex (male and female; see **Fig. 3**). Two-way ANOVA with repeated measures was performed to determine the effects of uteroplacental insufficiency and pregnancy (before mating nonpregnant and pregnant; see **Table 2**) and pregnancy success (successful and unsuccessful restricted) and pregnancy (before mating nonpregnant and pregnant) (see **Table 3**). A 3-way ANOVA was used to determine the effects of uteroplacental insufficiency, exposure to environmental stress, and age (4 and 12 mo pregnant; see **Table 4**). If significant interactions were observed from the factorial ANOVAs, individual group means were compared using Student's unpaired or paired t test, where appropriate.

RESULTS

Body and organ weights

Uteroplacental insufficiency in F0 females reduced F1 female weight (−21 to −32%) at P1 ($P<0.050$; Table 1). Restricted females remained lighter at P7 and at 4 and 9 mo ($P<0.05$; Table 1). Restricted females remained smaller than controls at mating and throughout pregnancy at E14 and E20 ($P<0.05$), and there were no differences between pregnant 1 and pregnant 2 (Table 1). Weight gain during pregnancy was not different between groups (Table 1). Total heart and left ventricular weights (relative to body weight) were lighter in pregnant 2 rats ($P<0.05$) with no differences between control and restricted groups (Table 1). There were no differences in relative kidney, pancreas, adrenal gland, and uterus weight between groups (Table 1).

Cardiorenal and metabolic function

Systolic blood pressure was elevated in restricted females by ~10 mmHg compared with that in controls, before and during pregnancy (E17; group: $P<0.05$; **Fig. 1**). Pregnancy was associated with, on average, a 14-mmHg reduction in systolic blood pressure in control and restricted groups compared with prepregnant values (pregnancy: $P<0.05$; Fig. 1).

Restricted females consumed more food for their body weight compared with controls before and during preg-

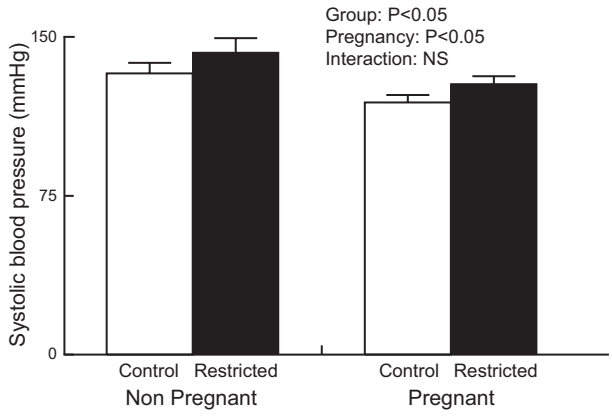

Figure 1. Systolic blood pressure in control and restricted successful pregnancies (pregnant 1). Measurements were performed before mating (nonpregnant) and during late (E17) pregnancy (mean±SE; n=6–8/group).

nancy (E19–E20; +12%; $P<0.05$; Table 2). Pregnant females drank more water (+20%) and had increased urine flow rate as well as reduced urine osmolality and excretion of sodium and chloride compared with nonpregnant values ($P<0.05$; Table 2). Total protein, uric acid, creatinine clearance, and fractional sodium excretion were not different between groups (Table 2).

There were no differences in basal or glucose-stimulated insulin and glucose levels between control and restricted groups (Fig. 2). Pregnant females had elevated fed basal plasma insulin (>2-fold; Fig. 2*A*) and reduced fed basal glucose (−26% (Fig. 2*B*) concentrations compared with those of nonpregnant animals (E18; $P<0.05$). Pregnancy was also associated with elevated plasma insulin secretion in response to a glucose load ($P<0.05$; Fig. 2*C*, *E*), but there were no differences in plasma glucose profile (Fig. 2*D*). Area under the glucose curve was reduced by 24% in pregnant *vs.* nonpregnant females ($P<0.05$; Fig. 2*F*), whereas our previously published 4-mo-old cohort showed a 70% reduction (25).

Unsuccessful pregnancy characteristics

Maternal body weight at P1, at mating, and at E20 was not different between successful and unsuccessful restricted pregnancies (Table 3). Unsuccessful pregnancies in restricted females, however, were associated with reduced maternal weight gain compared with that in their counterparts with successful pregnancies (−10 g; $P<0.05$; Table 3). Systolic blood pressure was not different between successful and unsuccessful pregnancies but was reduced, on average, by 10 mmHg in pregnant *vs.* nonpregnant states ($P<0.05$; Table 3). The second-phase insulin response was elevated in restricted pregnancies that were not successful (+27%; $P<0.05$), whereas glucose area under the curve and first-phase insulin response were not different between groups (Table 3). Food and water intake and 24-h renal excretion were not different between successful and unsuccessful restricted groups, but there was a tendency for increased sodium (+27%) and total protein (>2-fold) excretion in unsuccessful restricted animals before and during pregnancy ($P=0.06$; Table 3). Urine flow rate was elevated, whereas urine osmolality and sodium excretion were reduced in pregnant *vs.* nonpregnant groups ($P<0.05$; Table 3). Uric acid excretion was not different between groups (Table 3).

F2 fetal body and organ weights

Total (male and female) F2 litter size was not different between control and restricted groups (E20; 5.1±0.5 *vs.* 4.9±0.5 pregnant 1; 4.9±0.5 *vs.* 5.8±0.8 pregnant 2, respectively). F2 sex ratios were also not different between control and restricted litters (12-mo pregnant columns in Table 4). Male and female F2 fetal weight was not different between control and restricted or between pregnant 1 and pregnant 2 groups (Fig. 3*A*, *B*). Placenta weight and fetal/placenta ratio were also unchanged between groups (Fig. 3*C–F*).

Pregnancy outcome associated with maternal age

Pregnancy success rate was determined as the percentage of pregnancies with ≥3 live pups at E20 in animals that were identified (by weight) as being pregnant at E14. Increased maternal age (12 *vs.* 4 mo) was associated with a reduction in pregnancy success rate, particularly in restricted females (85.5 *vs.* 95.0% in controls; 53.5 *vs.* 95.0% in restricted females; Table 4). Exposure to physiological measurements during late gestation (pregnant 1 group) did not compromise the pregnancy success rate compared with that in the pregnant 2 group (Table 4).

F2 litter size was reduced in 12- *vs.* 4-mo-old pregnancies (−50%; $P<0.05$) with no effect of maternal birth weight or exposure to physiological measurements (Table 4). Body weight in F2 male and female fetuses was reduced in 12- *vs.* 4-mo-old dams (−12%; $P<0.05$), although there was a trend for lower body weight in restricted *vs.* control male fetuses from pregnant 1 rats ($P = 0.07$; −5%; Table 4). Placenta weight was increased, on average, by 40% in 12- *vs.* 4-mo-old pregnant females and reduced in all groups exposed to

TABLE 2. *Twenty-four-hour renal excretion in control and restricted successful pregnancies (pregnant 1)*

Parameter	Nonpregnant (before mating)		Pregnant	
	Control	Restricted	Control	Restricted
Food intake [$g\cdot(24\ h)^{-1}\cdot kg^{-1}$]	67 ± 3	73 ± 4*	69 ± 1	79 ± 3*
Water intake [$ml\cdot(24\ h)^{-1}\cdot kg^{-1}$]	143 ± 9	151 ± 7	168 ± 9	185 ± 12#
Urinary excretion				
Urine flow rate [$L\cdot(24\ h)^{-1}\cdot kg^{-1}$]	0.070 ± 0.005	0.071 ± 0.010	0.104 ± 0.008	0.117 ± 0.015#
Osmolality [$mosmol\cdot(kg\ H_2O)^{-1}$]	688 ± 37	623 ± 92	423 ± 42	435 ± 66#
Sodium excretion [$mM\cdot(24\ h)^{-1}\cdot kg^{-1}$]	3.4 ± 0.2	3.4 ± 0.3	2.7 ± 0.2	2.8 ± 0.3#
Chloride excretion [$mM\cdot(24\ h)^{-1}\cdot kg^{-1}$]	5.8 ± 0.3	6.3 ± 0.7	4.7 ± 0.4	4.8 ± 0.4#
Total protein excretion [$mg\cdot L^{-1}\cdot(24\ h)^{-1}\cdot kg^{-1}$]	13.9 ± 2.6	15.6 ± 4.1	13.4 ± 2.5	12.2 ± 2.0
Uric acid excretion [$mM\cdot(24\ h)^{-1}\cdot kg^{-1}$]	43 ± 10	35 ± 13	47 ± 12	46 ± 16
Creatinine clearance ($ml\cdot min^{-1}\cdot kg^{-1}$)	—	—	3.1 ± 0.3	2.9 ± 0.1
Fractional sodium excretion (%)	—	—	0.379 ± 0.024	0.414 ± 0.088

Measurements were performed before mating (nonpregnant) and during late (E19–E20) pregnancy (mean ± SE; n = 7–9/group). —, no measure. *$P < 0.05$ *vs.* control (main effect). #$P < 0.05$ *vs* nonpregnant (main effect).

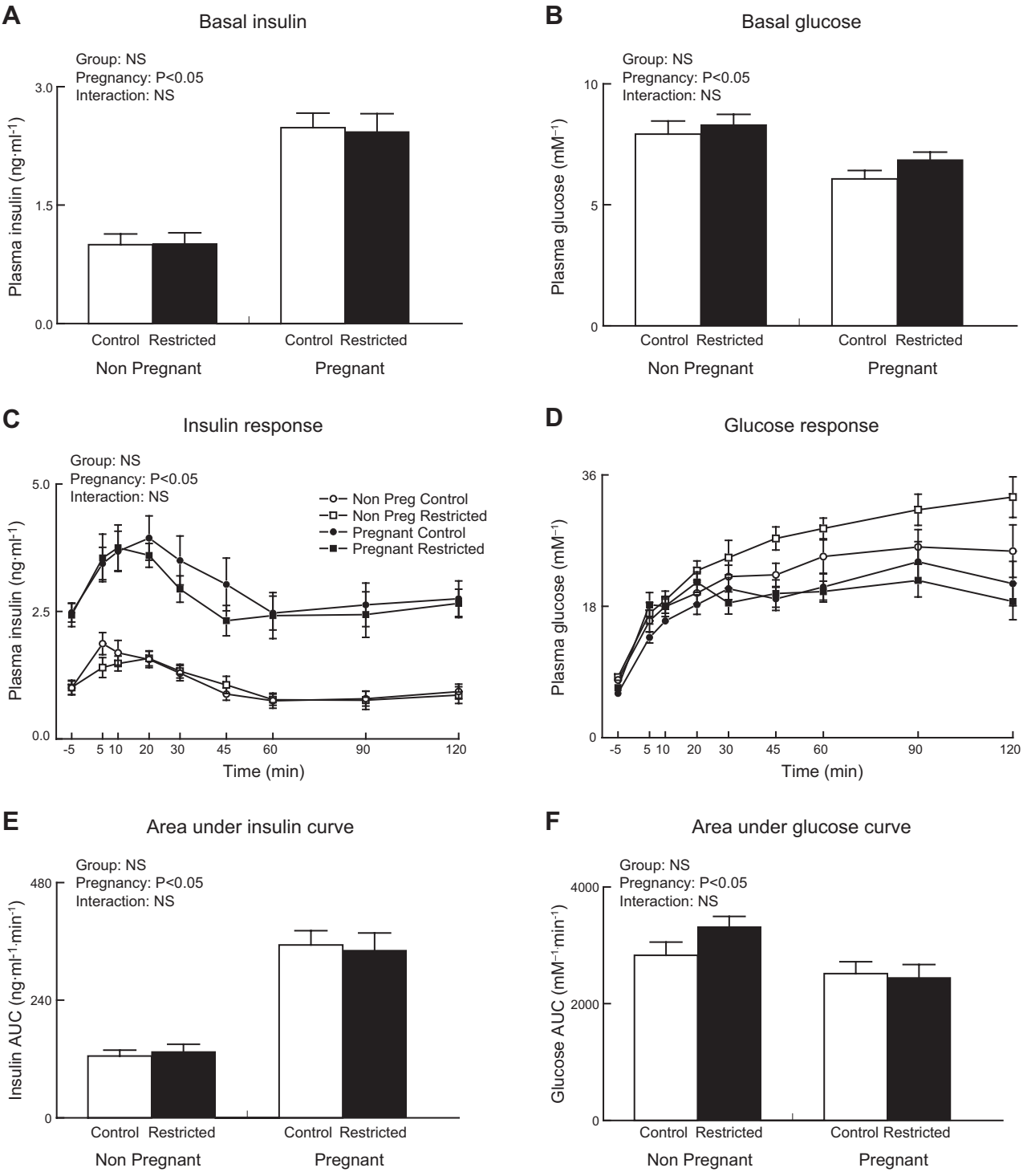

Figure 2. Plasma insulin and glucose before and in response to an IPGTT in control and restricted nonpregnant and successful pregnancies (pregnant 1). Measurements were performed in age-matched nonpregnant and pregnant (E18) animals (mean±SE; n=10/group). *A*) Basal insulin. *B*) Basal glucose. *C*) Insulin response. *D*) Glucose response. *E*) Area under the insulin curve. *F*) Area under the glucose curve. No significant differences were observed for panel *D*.

physiological measurements (pregnant 1 *vs.* pregnant 2, −8%; $P<0.05$; Table 4). The fetal/placenta weight ratio was reduced in male and female fetuses from 12-mo-old and increased in those from pregnant 1 rats compared with their respective counterparts ($P<0.05$; Table 4). However, a significant interaction between age and stress revealed that only in the 4-mo-old cohort did stress exposure increase this ratio in male fetuses ($P<0.05$; Table 4). In female fetuses, physiological measurements during late gestation were associated with increased fetal/placenta weight ratio in control ($P=0.08$) and restricted animals ($P<0.05$) from the 4-mo-old cohort and in control animals only from the 12-mo-cohort ($P<0.05$; Table 4).

TABLE 3. *Body weights and functional measurements in successful and unsuccessful restricted pregnancies (pregnant 1)*

Parameter	Nonpregnant (before mating) restricted		Pregnant restricted	
	Successful	Unsuccessful	Successful	Unsuccessful
F1 body weight (g)				
P1	—	—	3.4 ± 0.2	3.6 ± 0.1
Mating	—	—	250 ± 9	250 ± 8
E20	—	—	306 ± 10	296 ± 6
Pregnancy weight gain	—	—	56 ± 3	46 ± 3*
Functional measurements				
Blood pressure (mmHg)	143 ± 7	137 ± 4	128 ± 4	131 ± 5#
Glucose area under the curve ($mM \cdot min^{-1}$)	—	—	2447 ± 225	2624 ± 358
First-phase insulin response ($ng \cdot ml^{-1} \cdot min^{-1}$)	—	—	30 ± 2	31 ± 2
Second-phase insulin response ($ng \cdot ml^{-1} \cdot min^{-1}$)	—	—	163 ± 13	207 ± 8*
Food intake [$g \cdot (24\ h)^{-1} \cdot kg^{-1}$]	73 ± 4	72 ± 4	79 ± 3	69 ± 7
Water intake [$ml \cdot (24\ h)^{-1} \cdot kg^{-1}$]	151 ± 7	162 ± 11	185 ± 12§	168 ± 11
Urine flow rate [$L \cdot (24\ h)^{-1} \cdot kg^{-1}$]	0.071 ± 0.010	0.085 ± 0.005	0.117 ± 0.015	0.118 ± 0.016#
Osmolality [$mosmol \cdot (kg\ H_2O)^{-1}$]	623 ± 92	595 ± 60	435 ± 66	367 ± 45#
Sodium excretion [$mM \cdot (24\ h)^{-1} \cdot kg^{-1}$]	3.4 ± 0.3	4.4 ± 0.5	2.8 ± 0.3	3.5 ± 0.4
Chloride excretion [$mM \cdot (24\ h)^{-1} \cdot kg^{-1}$]	6.3 ± 0.7	6.7 ± 0.8	4.8 ± 0.4	5.9 ± 1.5
Total protein excretion [$mg \cdot L^{-1} \cdot (24\ h)^{-1} \cdot kg^{-1}$]	15.6 ± 4.1	18.9 ± 6.4	12.2 ± 2.0	37.4 ± 14.1
Uric acid excretion [$mM \cdot (24\ h)^{-1} \cdot kg^{-1}$]	35 ± 13	56 ± 17	46 ± 16	45 ± 22

Measurements were performed before mating (nonpregnant) and during late pregnancy (mean ± SE; $n = 6–7$/group). —, no measure. *$P < 0.05$ *vs.* successful. #$P < 0.05$ *vs.* nonpregnant (main effect). §$P < 0.05$ *vs.* nonpregnant successful (after significant interaction).

DISCUSSION

We demonstrate that 12-mo-old growth-restricted females exhibited renal and metabolic pregnancy adaptations comparable to those of normal-birth-weight controls despite previous evidence for glomerular hypertrophy and loss of glucose tolerance during pregnancy at 4 mo of age (25). Our novel finding of elevated systolic blood pressure, both before and during late pregnancy, was not associated with any fetal compromise. Of great interest, compared with results in pregnant control and growth-restricted offspring at 4 mo of age (25), the hypoglycemic response to pregnancy was reduced and next-generation fetal outcomes were severely compromised. Low maternal birth weight had no additional consequences for these fetal parameters but was associated with a compromised pregnancy success rate. Maternal exposure to physiological measurements during late gestation at 12 mo of age had no additional adverse effects for fetal outcomes.

Maternal growth trajectory, feeding responses, and organ weights

Late gestation uteroplacental insufficiency resulted in female offspring born 21–32% smaller and remaining lighter throughout the entire postnatal study period and during pregnancy (12 mo of age). The lack of accelerated catch-up growth is consistent with some (27, 29) but not all (9, 25, 28) published observations. Differences may be due to disparities in the degree of growth restriction at birth, presently more severe than the 10–15% reduction in other studies (9, 25, 28). Certainly, vast differences in postnatal growth are reflected in human infants born small for gestational age and, this fact, together with our findings, reflect the complexity of prenatal influences on later growth.

Note that despite remaining smaller throughout adult life, restricted females consumed, on average and corrected for body weight, 12% more food than controls in both the nonpregnant and pregnant state. This hyperphagic behavior contradicts our previous work (25, 27) but corroborates the work of Vickers *et al.* (30) who showed fasting plasma insulin and leptin resistance in prenatally undernourished rats born 35% smaller and remaining lighter throughout adulthood. Programming of feeding responses has been linked with alterations in hypothalamic structures (31, 32) and the contrasting findings highlight the sensitivity of this system to the type of model and severity of growth restriction.

Interestingly, heart weight (relative to body weight), but not that of other organs, was greater in pregnant 1 than in pregnant 2 rats and was attributed to increased left ventricular weight. This result was independent of maternal birth weight and suggests that exposure to physiological measurements during late gestation place additional strain on the heart, which may have implications for long-term maternal health.

Metabolic profile

Previously, we reported reduced fed basal plasma insulin levels and pancreatic β-cell mass in nonpregnant growth-restricted females, which was resolved by late pregnancy at 4 mo of age (25). Insulin response to a glucose load was comparable to that of controls, but despite this, growth-restricted pregnant females developed some loss of glucose tolerance. A number of studies have shown that aging is associated with a

TABLE 4. *Pregnancy outcome at 4 and 12 mo of age (pregnant 1 and pregnant 2)*

Parameter	Pregnant 1				Pregnant 2			
	4 mo pregnant		12 mo pregnant		4 mo pregnant		12 mo pregnant	
	Control	Restricted	Control	Restricted	Control	Restricted	Control	Restricted
Pregnancy success (%)	100	100	82	50	90	90	89	57
Litter size								
Total	10.2 ± 0.5	10.6 ± 0.8	5.1 ± 0.5	4.9 ± 0.5$^{\#}$	11.1 ± 0.6	9.1 ± 0.8	4.9 ± 0.5	5.8 ± 0.8$^{\#}$
Male	5.0 ± 0.5	4.9 ± 0.7	2.6 ± 0.4	2.7 ± 0.4$^{\#}$	6.1 ± 0.6	4.3 ± 0.5	2.6 ± 0.3	3.3 ± 0.6$^{\#}$
Female	5.1 ± 0.4	5.7 ± 0.9	2.6 ± 0.6	2.1 ± 0.4$^{\#}$	5.0 ± 0.5	4.8 ± 0.4	2.3 ± 0.5	2.5 ± 0.6$^{\#}$
Body weight (g)								
Male	1.91 ± 0.04	1.82 ± 0.03	1.71 ± 0.07	1.63 ± 0.04$^{\#}$	1.90 ± 0.03	1.88 ± 0.02	1.62 ± 0.06	1.77 ± 0.06$^{\#}$
Female	1.86 ± 0.04	1.77 ± 0.02	1.59 ± 0.04	1.55 ± 0.08$^{\#}$	1.84 ± 0.03	1.82 ± 0.03	1.59 ± 0.06	1.62 ± 0.06$^{\#}$
Placenta weight (g)								
Male	0.283 ± 0.006	0.271 ± 0.008	0.411 ± 0.024	0.415 ± 0.013$^{\#}$	0.313 ± 0.007	0.326 ± 0.006	0.405 ± 0.020	0.445 ± 0.019$^{*,\#}$
Female	0.283 ± 0.007	0.270 ± 0.007	0.381 ± 0.014	0.410 ± 0.013$^{\#}$	0.305 ± 0.010	0.319 ± 0.005	0.422 ± 0.017	0.418 ± 0.011$^{*,\#}$
Fetal/placenta weight								
Male	6.81 ± 0.16	6.81 ± 0.22	4.31 ± 0.33	3.99 ± 0.16$^{\#}$	5.95 ± 0.16§	5.80 ± 0.13§	4.08 ± 0.18	4.03 ± 0.15$^{*,\#}$
Female	6.66 ± 0.14	6.68 ± 0.19	4.22 ± 0.08	3.91 ± 0.20$^{\#}$	5.85 ± 0.39	5.74 ± 0.05§	3.81 ± 0.13§	3.90 ± 0.12$^{*,\#}$

Measurements were performed at E20 of pregnancy (mean ± SE; n = 4–9/group). $^{*}P < 0.05$ *vs.* pregnant 1 (main effect). $^{\#}P < 0.05$ *vs.* 4-mo pregnant (main effect). $^{\S}P < 0.05$ *vs.* pregnant 1 counterpart (after significant interaction between age and stress).

decline in glucose tolerance and insulin secretion and sensitivity (33). Of particular interest, Blondeau *et al.* (34) showed an age-related inability to increase pancreatic insulin content in late pregnant, prenatally undernourished rats, and, thus, we hypothesized that aging would exacerbate the glucose intolerance observed at 4 mo of age (25). Here, however, we report normal basal plasma insulin, glucose-stimulated insulin release, and glucose tolerance in 12-mo-old growth-restricted pregnant females compared with pregnant controls. Others have shown that pregnancy does not aggravate the age-related decline in glucose metabolism in normal rats (35), and we now confirm that low maternal birth weight has no additional consequences.

Blood pressure and kidney function

Systolic blood pressure, measured before mating and at E17 of pregnancy, was increased by 10 mmHg in growth-restricted females compared with controls. This is the first time that we report elevated blood pressure in female rat offspring exposed to late gestation uteroplacental insufficiency. Previous studies by our group using similar methodology consistently reported the development of hypertension in growth-restricted male offspring (8, 15), but female blood pressure was not different from that of controls across various cohorts: cross-fostered females at 18 mo (19) and during late pregnancy at 4 mo (25) and even at 8-9 mo after a pregnancy at 12-13 mo of age (27). This is an intriguing finding and may be attributable, in part, to the greater severity of growth restriction in the current study. Whereas Ojeda *et al.* (36) reported protective effects of estrogen in mitigating the development of hypertension in small-birth-weight females, we recently showed that plasma estradiol levels were in fact 25% greater in 13-mo-old growth-restricted females than in controls (27). This finding suggests that mechanisms other than an age-related decline in estradiol levels are involved and requires further investigation. Pregnancy itself was associated with, on average, a 14-mmHg reduction in blood pressure in both control and restricted females, consistent with the known hemodynamic responses to pregnancy (37). Notably, pregnancy does not exacerbate the elevated blood pressure in 12-mo-old restricted females.

Recently, we reported a 33% nephron deficit in 4-mo-old growth-restricted females associated with concomitant glomerular hypertrophy during pregnancy (25). This was not associated with overt renal dysfunction; sodium excretion only was reduced compared with that in pregnant controls (25). It was suggested that optimal renal function was preserved but that increased maternal age may challenge the functional renal reserve during pregnancy. We currently report, however, similar pregnancy-induced changes in urinary flow rate (+58%) and sodium and chloride excretions (−20%) between control and restricted groups at 12 mo of age. Certainly, this pregnancy-induced renal hyperfiltration may predispose susceptible females to kidney damage and subsequent proteinuria. Despite

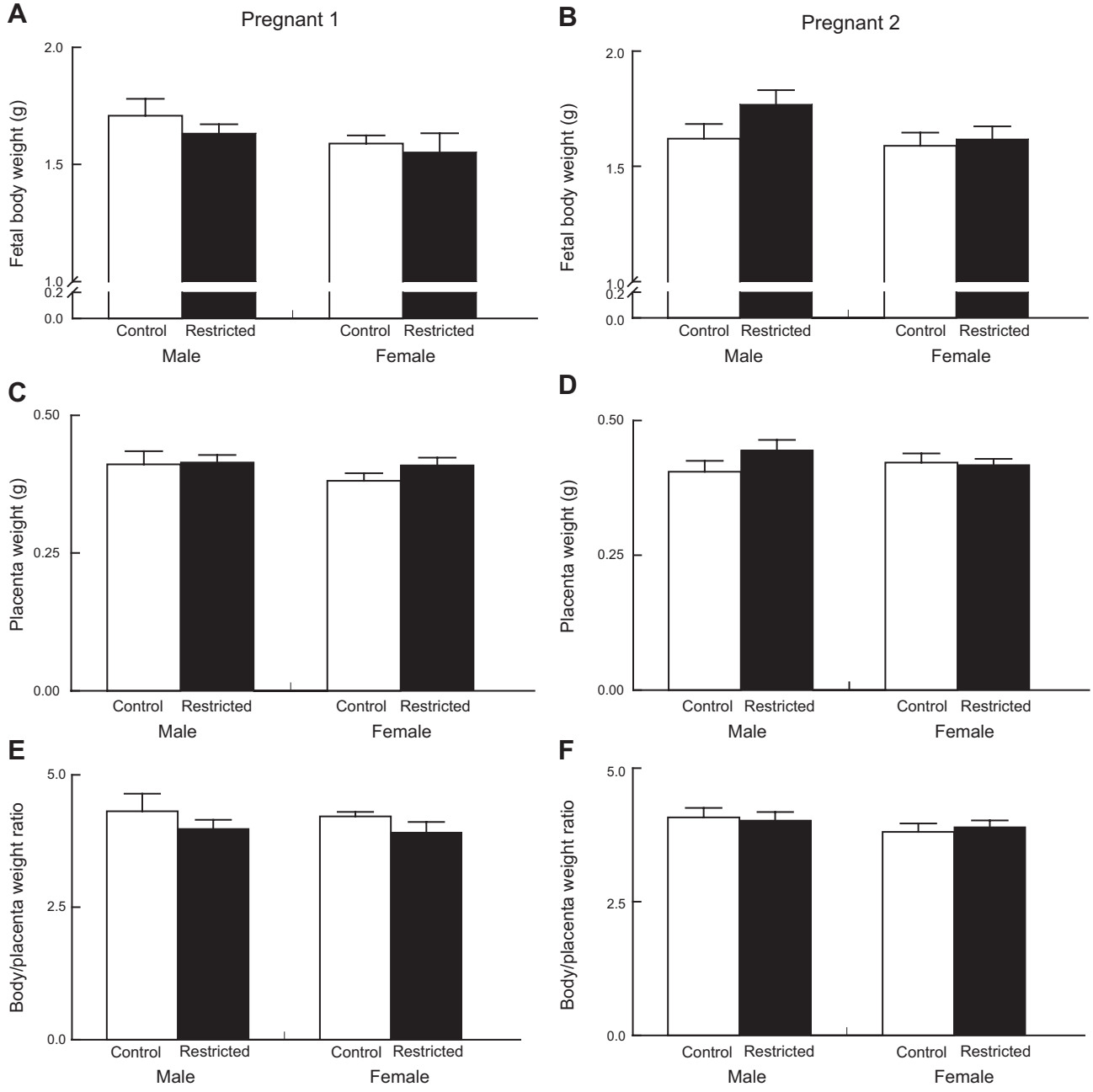

Figure 3. F2 fetal body and placenta weights in control and restricted successful pregnancies (pregnant 1 and pregnant 2). Measurements were performed at E20 of pregnancy (mean±SE; n=4–9/group). *A, B*) Fetal body weight (*A*, pregnant 1; *B*, pregnant 2). *C, D*) Placenta weight (*C*, pregnant 1; *D*, pregnant 2). *E, F*) Body/placenta weight ratio (*E*, pregnant 1; *F*, pregnant 2). No significant differences were observed.

elevated blood pressure in restricted females, and a likely reduction in nephron number with glomerular hypertrophy (25), there were no differences in total protein and uric acid excretion, creatinine clearance, and fractional sodium excretion between groups. We propose that obesity and/or high-salt/fat diets in the presence of hypertension in restricted females may place an additional challenge on renal function that is exacerbated with pregnancy. Moreover, elevated blood pressure in restricted females, irrespective of pregnancy, may have long-term consequences for renal function that warrant future follow-up.

F2 fetal parameters

Much research has focused on the transgenerational effects of poor prenatal conditions, particularly with respect to birth weight and future health outcomes. Several studies from the Dutch hunger winter have provided evidence for perpetuated disease outcomes including cardiovascular and metabolic diseases arising from women who were malnourished *in utero* (38, 39). In addition, we have recently shown that male and female fetuses from 4-mo-old growth-restricted mothers were 5–6% smaller from those from controls (25).

This was not evident in offspring from restricted mothers who were not exposed to physiological measurements during late pregnancy, suggesting an additive adverse effect of low maternal birth weight and environmental stress on fetal weight. In this study, we performed the same physiological measurements in 12-mo-old rats (pregnant 1) but report similar F2 fetal weight between control and restricted dams. There were also no differences in placenta weight or fetal/placenta ratio, but when measurements were analyzed together with the 4-mo pregnant cohort (Table 4), placenta weight was reduced in those exposed to physiological measurements compared with pregnant 2 rats. Differences in fetal weight between the two studies may be explained by the overall maternal age-related reduction in F2 fetal weight, perhaps masking the more subtle effects of combined low maternal birth weight and exposure to physiological measurements. Notably, however, studies have reported raised systolic blood pressure, reduced nephron number, endothelial dysfunction, and altered glucose and insulin metabolism in normal-birth-weight F2 rat offspring from mothers exposed to protein restriction during their own development (12–14). This finding suggests that fetal growth is not a sole predictor of transgenerational disease outcomes, nor can it always be correlated with prenatal events (22).

Effects of maternal age on pregnancy outcome

Advanced maternal age was associated with poorer pregnancy outcomes compared with those in our previously published study in 4-mo-old pregnant rats (25). The pregnancy success rate was substantially reduced from 90–100% at 4 mo to 50–89% at 12 mo. Notably, the large variation at 12 mo was attributed to the effects of low maternal birth weight, with only a 50–57% pregnancy success rate in the restricted dams *vs.* 82–89% success rate in the controls (discussed further below). Notably, however, low maternal birth weight did not exacerbate other F2 outcomes. From the successful pregnancies at 12 mo of age, F2 litter size was halved compared with that in younger rats. Others have reported that despite unchanged litter size at E11 between 12- and 40-wk-old pregnant mice, the number of viable pups was reduced by 60% at delivery in the older animals (40). This finding indicates that an increased rate of intrauterine fetal death rather than a reduced ability to conceive is the major cause of reduced litter size with advanced maternal age. Studies in humans have reported positive correlations between maternal age and rates of spontaneous abortion (41, 42). Increased incidence of aneuploidy due to deterioration in ova quality is the most likely causal factor (43), although maternal endocrine and anatomic factors can play a role. There is evidence for impaired reactivity and blunted remodeling in the uterine arteries of aged pregnant rodents (40, 44) that may certainly compromise uteroplacental blood flow. However, studies investigating the role of advanced recipient age on the success of donor egg therapy revealed that risks of spontaneous abortion and chromosomal defects were consistent with donor age (45) and only from the late 40s does recipient age have negative consequences for pregnancy outcomes (46).

In the current study, fetal body weight was 12% lighter that that of 4-mo-old rats, but placenta weight was 40% heavier. Previously, others have shown in 9-mo-old rats that both fetal and placenta weights were reduced compared with those of offspring from 3-mo-old females (35). Reasons for the placenta weight disparity are not known, but our findings corroborate those from a large population-based human study reporting heavier placentas from older mothers (47). Further, when average placenta weight was corrected for maternal body weight, 12-mo-old rats retained a 23% greater ratio compared with the 4-mo-old cohort (data not shown). Others have shown that gestational diabetes was associated with heavier placentas in humans (48) and given the small reduction in maternal blood glucose levels, enhanced placental growth might reflect attempted compensation for reduced glucose transport. In addition, reduced uteroplacental blood flow is likely to stimulate an angiogenic growth response that contributes to increased placenta weight. With respect to fetal weight, others have reported increased rates of small-for-gestational-age infants with increasing maternal age (49). Interestingly, the negative effect of advanced maternal age on fetal weight appears to arise in late pregnancy, because fetal growth in the first trimester was in fact greater in older mothers (50). This finding might indicate a greater contribution from maternal responses to pregnancy (*i.e.*, uteroplacental insufficiency *vs.* a primary embryo effect) in reducing fetal weight with higher maternal ages.

To interpret the reduced rates of pregnancy success associated with low maternal birth weight, we compared various body and physiological characteristics between unsuccessful and successful restricted pregnancies. Body weight in restricted females whose pregnancies were unsuccessful was not different at birth, at mating, or postmortem. These growth-restricted females, however, gained 10 g less weight than their counterparts with successful pregnancies, which may be attributed to the fewer viable pups. The second-phase insulin response was elevated by 27% in unsuccessful *vs.* successful pregnancies, suggesting some degree of peripheral insulin resistance. Despite this, glucose tolerance was normal. Given that maternal insulin does not cross the placenta, it is unlikely that this hyperinsulinemia, in the absence of impaired glucose tolerance, contributed to adverse fetal outcomes in unsuccessful pregnancies. During pregnancy, blood pressure decreased to normal values compared with premating levels. Food and water intake were unchanged between groups, and renal excretions did not reveal any differences that might be associated with reduced pregnancy success rates in restricted females. The overarching, mostly comparable, maternal physiology between successful and unsuccessful pregnancies in late gestation suggests embryo-

specific alterations, such as reduced ovum quality with age in growth-restricted females. However, we cannot discount the potential adverse contributions from early pregnancy adaptations, uterine blood flow, and/or placental function that were not assessed in the current study. Nevertheless, these data highlight the transgenerational effects of perturbed development *in utero* on pregnancy success rate and provide impetus for follow-up.

CONCLUSIONS

Female rats exposed to late gestation uteroplacental insufficiency exhibited renal and metabolic pregnancy adaptations that were mostly comparable to those of normal-birth-weight controls at 12 mo of age. We report, however, for the first time, increased systolic blood pressure in nonpregnant females born small that persisted but was not exacerbated during pregnancy. Despite this, F2 fetal weight was not different between groups. Previously, we reported that growth-restricted females developed some loss of glucose tolerance during late pregnancy at 4 mo, which was associated with transgenerational fetal growth restriction. This study indicates that aging does not exacerbate the gestational metabolic pathology or F2 fetal weight reduction for female rats that were born small, although pregnancy success rate was compromised. Regardless of maternal birth weight, advanced maternal age was associated with profound reductions in the hypoglycemic response to pregnancy and poor F2 outcomes including reductions in litter size and body weight. Although these data suggest adverse fetal consequences with delayed child-bearing, we conclude that pregnancy does not exacerbate disease outcome in aged females that were born small. FJ

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