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Improved Lactational Nutrition and Postnatal Growth Ameliorates Impairment of Glucose Tolerance by Uteroplacental Insufficiency in Male Rat Offspring

Andrew L. Siebel, Amy Mibus, Miles J. De Blasio, Kerryn T. Westcott, Margaret J. Morris, Larissa Prior, Julie A. Owens, and Mary E. Wlodek

Departments of Physiology (A.L.S., A.M., K.T.W., M.E.W.) and Pharmacology (L.P.), The University of Melbourne, Victoria 3010, Australia; School of Pediatrics and Reproductive Health (M.J.D.B., J.A.O.), Disciplines of Obstetrics and Gynecology, University of Adelaide, South Australia 5005, Australia; and Department of Pharmacology (M.J.M.), University of New South Wales, New South Wales 2052, Australia

Intrauterine growth restriction and accelerated postnatal growth predict increased risk of diabetes. Uteroplacental insufficiency in the rat restricts fetal growth but also impairs mammary development and postnatal growth. We used cross fostering to compare the influence of prenatal and postnatal nutritional restraint on adult glucose tolerance, insulin secretion, insulin sensitivity, and hypothalamic neuropeptide Y content in Wistar Kyoto rats at 6 months of age. Bilateral uterine vessel ligation (restricted) to induce uteroplacental insufficiency or sham surgery (control) was performed on d-18 gestation. Control, restricted, and reduced (reducing litter size of controls to match restricted) pups were cross fostered onto a control or restricted mother 1 d after birth. Restricted pups were born small compared with controls. Restricted males, but not females, remained lighter up to 6 months, regardless of postnatal environment. By 10 wk, restricted-on-restricted males ate more than controls. At 6 months restricted-on-restricted males had increased hypothalamic neuropeptide Y content compared with other groups, and together with reduced-on-restricted males had increased retroperitoneal fat weight (percent body weight) compared with control-on-controls. Restricted-on-restricted males had impaired glucose tolerance, reduced first-phase insulin secretion, but unaltered insulin sensitivity, compared with control-on-controls. In males, being born small and exposed to an impaired lactational environment adversely affects adult glucose tolerance and first-phase insulin secretion, but improving lactation partially ameliorates this condition. This study identifies early life as a target for intervention to prevent later diabetes after prenatal restraint. (*Endocrinology* 149: 3067–3076, 2008)

UTEROPLACENTAL INSUFFICIENCY is responsible for much of the intrauterine growth restriction observed in Western society and increases the predisposition to adult metabolic diseases (1–3). Similarly, in nonhuman species, fetal growth restriction induced by uteroplacental restriction can impair whole body glucose tolerance and insulin secretion (4, 5), cause insulin resistance (5, 6), and induce obesity in offspring (4, 7). Additional evidence suggests that early postnatal accelerated growth in infants who were born small also predicts a range of metabolic diseases (2, 8). Increased weight gain in childhood, especially in those of low birth weight, independently predicts an increased risk of cardiovascular disease and diabetes (9–11). However, other studies suggest that slowed postnatal growth, especially during the first year, can lead to insulin resistance (12). Although this suggests that factors affecting growth at different developmental stages both before and after birth can influence later metabolic health, their precise impact is unclear. Similarly, variability in the reported outcome of uteroplacental insufficiency for later glucose tolerance and insulin action in the rat may occur in part from differences in early postnatal handling of offspring (4, 13–15).

Uteroplacental insufficiency induced by bilateral uterine artery ligation in late gestation in the rat restricts fetal growth, and induces fasting hyperglycemia, early onset insulin secretory defects, hepatic insulin resistance, obesity, and impaired glucose tolerance in adult offspring (4, 6). However, in another study similar uteroplacental restriction impaired glucose tolerance and insulin secretion and sensitivity in female offspring only (13). More recently we found that uteroplacental insufficiency does not alter glucose tolerance, insulin secretion, or relative adiposity in male or female adult offspring (14). These variable outcomes may occur in part from differences in the postnatal environment to which prenatally growth-restricted offspring were exposed, with the greatest adverse impact in those offspring that were cross fostered onto control mothers (4, 13). Leaving placentally growth-restricted offspring with their mothers may prevent their exposure to excess nutrition postnatally. We have previously shown that uteroplacental insufficiency impairs mammary function, compromises milk quality and quantity, and reduces calcium transport into milk, further restraining postnatal growth (16, 17). Restricted dams with lower circulating progesterone experienced premature lac-

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Abbreviations: ARC, Arcuate nucleus; EDL, extensor digitorum longus; HOMA-R, homeostasis model assessment for insulin resistance; IAGTT, intra-arterial glucose tolerance test; IC, insulin challenge; NPY, neuropeptide Y; PVN, paraventricular nucleus.

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togenesis, producing less milk per pup with altered composition compared with controls, further slowing growth during lactation. Reducing the litter size of pups born of normal birth weight (reduced litter) was also associated with decreased pup growth, highlighting the importance of appropriate controls. These findings highlight the importance of separating the influence of the prenatal and postnatal nutritional environments on long-term outcomes, providing a rationale for our current study. Reductions in litter size in control mothers, a common experimental control used in many studies, actually impaired glucose tolerance and insulin sensitivity in adult offspring (4, 18, 19). Because a modest reduction in litter size impairs mammary development and function in the rat (17), this suggests that compromised early postnatal nutrition and growth can impact adversely on later metabolic health (4, 13). Overall, these findings suggest that the impact of prenatal growth restriction on later metabolic control may be modifiable by the postnatal environment, which may also have its own separate effects.

Prenatal restraint and its interaction with postnatal nutrition may program later diabetes via influences on body composition and adiposity. People who are light and thin at birth tend to have a more abdominal distribution of adiposity than normal weight babies, as well as reduced muscle mass and high body fat content in adolescence and adulthood (20–22). Catch-up growth and weight gain in childhood also predict later obesity (23). In the rat, uteroplacental insufficiency induces adult obesity in offspring in some (4, 7), but not all (14, 24) studies, with cross fostering onto control mothers most consistently associated with later obesity (4). Placental restriction in other species can also program later obesity in association with increased appetite, suggesting altered satiety control (7). In the adult, appetite homeostasis is primarily controlled by neurons in specific hypothalamic subregions of the brain and the key appetite stimulating or inhibiting peptides, which they synthesize (25, 26). Local expression of a major orexigenic peptide, neuropeptide Y (NPY), which is synthesized in cell bodies that lie in the arcuate nucleus (ARC), is increased in response to energy deficit, and activates several pathways to stimulate food intake and reduce energy expenditure (27). The hypothalamic circuits regulating appetite are immature at birth, developing in the early postnatal period, and are influenced by ambient leptin concentrations in the rodent at least (28). Hypothalamic NPY content has been increased by early postnatal nutritional restraint, induced by increasing litter size (29). However, it is less clear to what extent changes in hypothalamic regulators of appetite may persist long term.

Therefore, we hypothesized that uteroplacental insufficiency would impair adult glucose tolerance, insulin secretion and sensitivity, as well as increase adiposity and hypothalamic NPY content in adult offspring, and this would be ameliorated with restoration of postnatal nutrition through cross fostering. We further hypothesized that exposure to impaired postnatal nutrition only, by cross fostering control pups onto restricted mothers with impaired mammary development or by modestly reducing litter size (17), would also impair glucose tolerance and insulin sensitivity in adult offspring.

Materials and Methods

Animals

All experiments were approved by The University of Melbourne Animal Ethics Committee before commencement. Wistar Kyoto rats (9–13 wk of age) were obtained from the Australian Resource Centre (Murdoch College, Western Australia, Australia), and were provided with a 12-h light, 12-h dark cycle and had access to food and water *ad libitum*.

On d-18 gestation, pregnant rats were randomly allocated to restricted or control (sham surgery) groups. The restricted group underwent bilateral uterine artery and vein ligation to induce uteroplacental insufficiency as described previously (16, 17, 24). At birth, half the litters from the control (sham surgery) group (litter size nine to 14 pups) had their litter size randomly reduced to five to match the restricted group (24). Pups from each of the three groups (control, reduced, and restricted) were cross fostered the day after birth onto a different control mother or restricted mother as previously described (24). This generated six experimental groups: (pup-on-mother) control-on-control, control-on-restricted, reduced-on-restricted, reduced-on-control, restricted-on-control, and restricted-on-restricted, with a similar number of male and female pups in each litter. All pups remained with their mothers and then were weaned at postnatal d 35 as in previous studies (24).

Growth measurements and food intake

Because individual pups were not identified until postnatal d 3, weights at d 1 were taken as the average of the entire litter for a particular sex. From postnatal d 14, individual offspring from at least seven different litters per group were studied. To analyze growth profiles, offspring were weighed on postnatal d 1, 14, 28, and 35, as well as 6, 8, 10, 14, 16, 18, and 22 wk and 6 months (n = 22–68 male and female offspring per group). Dimensions (crown rump length and hind limb length) using digital vernier calipers (accurate to 0.01 mm) and abdominal circumference were measured at 6 months of age (n = 19–40 male and female offspring per group). Food intake (5–8, 10, and 12 wk) was measured during the rapid growth phase in rats that were housed individually (n = 20–46 male and female offspring per group).

Intra-arterial glucose tolerance test (IAGTT) and insulin challenge (IC)

At 6 months of age, offspring (one male and one female offspring from six to 11 different mothers per group) were weighed and given a single ip dose of anesthetic [ketamine: 50 mg/kg body weight; Ilium Xylazil-20 (Troy Laboratories Pty. Ltd., Smithfield, New South Wales, Australia): 10 mg/kg body weight] and analgesic (temgesic: 0.05 mg/kg body weight), and the carotid artery was catheterized, with the catheter exteriorized between the shoulder blades. IAGTTs were performed 2 d after catheterization after an overnight fast as previously described (14). Animals remained conscious and unrestrained in their cage throughout the experiment. Glucose (25 g in 50 ml 50%; Pharmalab, Lane Cove, New South Wales, Australia; 0.5 g/kg body weight) was administered via the carotid artery catheter, followed immediately by 0.2 ml saline. Intra-arterial administration of glucose was chosen rather than ip because it provides a better measure of insulin secretion with an almost instantaneous first-phase response after the glucose bolus is given (30). Arterial blood samples were collected via the catheter 10 and 5 min before, and 1, 3, 5, 10, 20, 30, 40, 60, and 120 min after glucose injection. Blood removed was replaced with a similar volume of saline.

In a separate cohort of animals (n = 4–8 male and female offspring per group), an insulin tolerance test or IC was performed after an overnight fast. A sc injection of insulin (Actrapid; Novo Nordisk Pharmaceuticals, North Rocks, New South Wales, Australia; 1 U/kg body weight) was administered. Tail vein blood samples were collected 5 min before, and 20, 40, and 60 min after insulin injection. Plasma was stored at −20 C until further analysis.

Postmortem tissue and blood collection

Approximately 3–6 d after tolerance testing, nonfasted rats were anesthetized with an ip injection with ketamine (30 mg/kg body weight) and Ilium Xylazil-20 (225 mg/kg body weight). After decapitation,

brains were rapidly removed and dissected on ice into subregions of the hypothalamus containing the paraventricular nucleus (PVN), ARC, dorsal medulla, and preoptic area (27). Brain regions were stored at −80 C until extraction and determination of NPY content, as described previously (31). Hind limb soleus and extensor digitorum longus (EDL) muscles from the left leg, as well as omental, retroperitoneal, and dorsal fat were excised and weighed.

Plasma analyses

Plasma glucose was measured in duplicate by colorimetric enzymatic analysis on an automated centrifugal analyzer (COBAS Mira; Roche Diagnostics Corp., Indianapolis, IN). Plasma insulin was measured using a RIA kit (Linco Pty. Ltd., Australian Laboratory Services, Sydney Markets, New South Wales, Australia). Fasting plasma glucose or insulin was taken as the average of two time points (10 and 5 min before injection) for the IAGTT and 5 min before injection for the IC, respectively. First-phase insulin secretion was calculated as the incremental area under the insulin curve between 0 and 5 min after the intra-arterial injection of glucose. To assess glucose tolerance, glucose concentrations were measured after 10 min (IAGTT) because this time point best reflects the response to elevated secretion of insulin by each of the major determinants of glucose homeostasis, such as the pancreas, liver, and skeletal muscle (32). Homeostasis model assessment for insulin resistance (HOMA-R) was determined because it provides an indication of the effectiveness of fasting insulin levels to regulate blood glucose levels calculated using the following formula: fasting plasma insulin (μU/ml^{-1}) × fasting plasma glucose (mmol/liter^{-1}) ÷ 22.5. This HOMA-R measure has correlated highly with whole-body insulin resistance in humans (33) and rats (34).

Statistical analyses

Glucose tolerance, insulin secretion, and insulin sensitivity data from the IAGTT and IC were analyzed using two-way repeated measures ANOVA [time (within factor) × treatment group (between factor)]. All other measures were analyzed using one-way ANOVA, with Duncan's *post hoc* analysis used where appropriate. All data were normally distributed and presented as mean ± SE, with the level of significance set at $P < 0.05$.

Results

Litter size, body weight, and dimensions

Bilateral uterine vessel ligation reduced litter size (6.0 ± 0.22) compared with controls (9.3 ± 0.24) on d 3 after birth ($P < 0.05$). Prenatally growth-restricted male pups were lighter compared with control-on-control males from postnatal d 1 (restricted-on-restricted by 21% and restricted-on-control by 14%), up to d 35, regardless of postnatal environment ($P < 0.05$; Table 1). Restricted-on-control males were heavier ($P < 0.05$) than restricted-on-restricted males from postnatal d 14 up to 10 wk of age. However, both prenatally growth-restricted males, regardless of postnatal exposure, remained lighter than control-on-control males at 6 months ($P < 0.05$; Table 2). Reducing the litter size of mothers exposed to sham surgery and then cross fostering onto a mother with poor lactation (reduced-on-restricted) reduced weight of male pups ($P < 0.05$) compared with controls on postnatal d 3 (data not shown), and these offspring remained lighter than control-on-control males up to 6 months of age (−9%). Reduced-on-control and control-on-restricted male offspring grew similarly to control-on-control offspring from birth to 6 months. Prenatally growth-restricted male adults were shorter (−3%; $P < 0.05$) than control-on-control males at 6 months (Table 2). Control-on-control males also had longer (+3%; $P < 0.05$) hind limbs than restricted-on-control, but not restricted-on-restricted, males. Abdominal circumference in male adults was not different across groups (Table 2).

Female restricted-on-restricted pups were lighter than control-on-controls on postnatal d 1 (−17%; $P < 0.05$; Table 1). Furthermore, restricted-on-restricted and restricted-on-control female pups were lighter than control-on-restricted and reduced-on-control female pups on postnatal d 1 (−15 to 20%; $P < 0.05$). On postnatal d 14, restricted-on-control and

TABLE 1. Effect of prenatal and postnatal growth restriction and cross fostering on body weight from birth to 6 months of age

Pup-on-mother	Control-on-control	Control-on-restricted	Reduced-on-restricted	Reduced-on-control	Restricted-on-control	Restricted-on-restricted
Male offspring						
d 1	3.93 ± 0.72^{b}	4.04 ± 0.63^{b}	3.94 ± 0.45^{b}	4.26 ± 0.79^{c}	3.61 ± 0.69^{a}	3.58 ± 0.40^{a}
d 14	22.09 ± 2.06^{d}	21.22 ± 2.36^{c}	19.46 ± 3.10^{b}	22.07 ± 2.47^{d}	19.57 ± 2.43^{b}	16.50 ± 4.30^{a}
d 28	58.68 ± 5.48^{cd}	57.16 ± 4.98^{c}	53.75 ± 5.84^{b}	59.57 ± 5.66^{d}	54.93 ± 8.47^{b}	46.80 ± 8.47^{a}
d 35	87.73 ± 7.93^{c}	86.40 ± 7.19^{c}	82.25 ± 8.78^{b}	87.52 ± 6.50^{c}	81.51 ± 7.64^{b}	74.47 ± 11.70^{a}
wk 6	121.2 ± 13.3^{c}	119.5 ± 13.6^{c}	113.9 ± 8.8^{b}	121.2 ± 12.6^{c}	112.6 ± 12.7^{b}	101.8 ± 17.9^{a}
wk 8	199.0 ± 19.5^{e}	191.4 ± 19.9^{cd}	187.1 ± 20.5^{bc}	196.5 ± 16.6^{de}	183.5 ± 17.3^{b}	177.0 ± 29.8^{a}
wk 10	248.3 ± 20.5^{c}	243.0 ± 26.1^{bc}	240.0 ± 19.8^{b}	247.9 ± 15.3^{c}	239.2 ± 21.7^{b}	222.3 ± 25.7^{a}
wk 14	313.4 ± 25.1^{c}	301.2 ± 28.2^{b}	296.8 ± 27.5^{b}	313.0 ± 19.2^{c}	282.3 ± 22.6^{a}	283.9 ± 28.4^{a}
wk 16	326.9 ± 36.7^{bc}	323.3 ± 29.4^{bc}	317.0 ± 29.4^{ab}	331.2 ± 20.5^{c}	306.3 ± 28.2^{a}	304.0 ± 29.5^{a}
wk 18	350.0 ± 31.7^{d}	332.7 ± 34.5^{bc}	336.2 ± 27.5^{c}	334.2 ± 29.7^{cd}	320.2 ± 32.0^{a}	324.5 ± 27.7^{ab}
wk 22	371.1 ± 32.0^{b}	351.4 ± 36.6^{a}	354.9 ± 36.7^{a}	368.6 ± 23.1^{b}	342.3 ± 36.4^{a}	345.3 ± 33.3^{a}
Female offspring						
d 1	3.61 ± 0.81^{b}	3.82 ± 0.55^{c}	3.89 ± 0.43^{c}	4.11 ± 0.73^{d}	3.49 ± 0.47^{ab}	3.42 ± 0.49^{a}
d 14	21.71 ± 1.78^{d}	20.77 ± 2.56^{c}	19.32 ± 3.12^{b}	20.91 ± 2.96^{c}	19.24 ± 4.06^{b}	18.40 ± 2.82^{a}
d 28	55.10 ± 4.35^{c}	54.17 ± 5.58^{c}	51.60 ± 5.31^{b}	53.72 ± 6.59^{c}	51.38 ± 9.32^{b}	48.30 ± 8.15^{a}
d 35	79.29 ± 5.89^{e}	78.03 ± 6.70^{de}	74.87 ± 7.08^{bc}	76.43 ± 7.40^{cd}	72.82 ± 9.32^{ab}	71.75 ± 7.62^{a}
wk 6	103.1 ± 11.3^{c}	102.7 ± 9.9^{c}	98.04 ± 6.84^{b}	102.1 ± 10.5^{c}	97.29 ± 9.84^{b}	92.60 ± 8.78^{a}
wk 8	151.7 ± 14.8^{b}	151.5 ± 13.1^{b}	144.9 ± 11.6^{a}	154.2 ± 15.8^{b}	144.8 ± 13.4^{a}	146.2 ± 13.6^{a}
wk 10	186.3 ± 16.9^{bc}	188.3 ± 17.0^{c}	183.5 ± 11.2^{bc}	181.7 ± 14.6^{ab}	182.3 ± 14.4^{ab}	177.4 ± 12.5^{a}
wk 14	223.8 ± 15.4^{bc}	223.7 ± 21.9^{bc}	214.4 ± 15.7^{a}	230.4 ± 27.4^{c}	213.3 ± 17.0^{a}	218.2 ± 17.1^{ab}
wk 16	227.0 ± 33.3^{ab}	227.3 ± 20.3^{ab}	221.7 ± 10.3^{a}	236.5 ± 27.2^{b}	223.8 ± 14.2^{a}	228.4 ± 24.9^{ab}
wk 18	236.1 ± 14.8^{ab}	239.9 ± 30.7^{b}	227.8 ± 11.0^{a}	239.1 ± 28.3^{b}	229.3 ± 17.7^{a}	232.4 ± 19.0^{ab}
wk 22	245.4 ± 15.0^{ab}	243.8 ± 29.1^{ab}	235.8 ± 13.8^{a}	247.4 ± 29.1^{b}	235.8 ± 17.3^{a}	241.5 ± 22.0^{ab}

Body weight (in grams) measured for male and female offspring. All data are expressed as mean ± SE (n = 22–68). Significant differences ($P < 0.05$) across the groups are indicated by *different letters*: ^{a} is different from ^{b}, but not different from ^{ab}.

TABLE 2. Effect of prenatal and postnatal growth restriction and cross fostering on body weight and dimensions in adult offspring

Pup-on-Mother	Body weight (g)	Crown rump length (mm)	Hind limb length (mm)	Abdominal circumference (mm)
Male offspring				
Control-on-control	386.1 ± 7.0^{b}	210.0 ± 2.0^{b}	55.7 ± 0.4^{b}	163.3 ± 3.0
Control-on-restricted	368.9 ± 2.3^{ab}	205.8 ± 1.5^{ab}	55.7 ± 0.3^{b}	162.9 ± 2.9
Reduced-on-restricted	351.8 ± 9.5^{a}	208.3 ± 1.7^{ab}	55.4 ± 0.4^{ab}	161.4 ± 3.3
Reduced-on-control	368.4 ± 7.0^{ab}	206.3 ± 1.4^{ab}	55.3 ± 0.4^{ab}	166.8 ± 3.4
Restricted-on-control	355.4 ± 7.1^{a}	203.7 ± 1.6^{a}	54.0 ± 0.4^{a}	161.1 ± 2.7
Restricted-on-restricted	353.9 ± 6.9^{a}	203.8 ± 1.5^{a}	54.3 ± 0.5^{ab}	163.8 ± 2.8
Female offspring				
Control-on-control	239.3 ± 4.1	180.8 ± 1.4^{abc}	49.3 ± 0.4	140.1 ± 2.3^{b}
Control-on-restricted	233.1 ± 6.5	183.2 ± 1.1^{bc}	50.3 ± 0.3	131.4 ± 4.2^{a}
Reduced-on-restricted	226.9 ± 4.7	184.4 ± 1.6^{bc}	50.2 ± 0.4	138.4 ± 3.2^{ab}
Reduced-on-control	238.3 ± 3.7	185.6 ± 0.9^{c}	49.8 ± 0.4	142.8 ± 2.2^{b}
Restricted-on-control	233.9 ± 5.9	179.7 ± 1.4^{ab}	49.5 ± 0.6	145.0 ± 2.3^{b}
Restricted-on-restricted	229.0 ± 4.6	177.9 ± 1.6^{a}	49.4 ± 0.3	139.5 ± 2.2^{ab}

Body weight (g) and dimensions (mm) measured at 6 months of age for male and female offspring. All data are expressed as mean ± SE (n = 19–40). Significant differences ($P < 0.05$) across the groups are indicated by *different letters*: ^{a} is different from ^{b}, but not different from ^{ab}.

reduced-on-restricted female pups were approximately 9% lighter ($P < 0.05$) than control-on-control females (Table 1). By d 35, restricted-on-restricted female pups were 9% lighter ($P < 0.05$) than control-on-control pups, whereas reduced-on-restricted pups were only 5% lighter. There was no difference in female body weight or hind limb length across groups at 6 months (Table 2). At 6 months, crown rump length was not different from control-on-control for any of the female offspring. Control female pups cross fostered onto a restricted mother had a lower (−6 to 9%; $P < 0.05$) abdominal circumference at 6 months of age when compared with control-on-control, reduced-on-control, and restricted-on-control (Table 2).

Food intake

At 5 wk, male reduced-on-restricted pups ate more (+23%; $P < 0.05$), and restricted-on-restricted males ate less (−17%; $P < 0.05$) than control-on-controls (Table 3). At 6 wk, control-on-control male offspring ate less than all other males. By wk 8, restricted-on-control ate more (+9%; $P < 0.05$) than restricted-on-restricted males (Table 3). At 10 wk, control-on-restricted and restricted-on-restricted male offspring ate more (+12 and 16%, respectively; $P < 0.05$) than control-on-controls. Female reduced-on-restricted pups ate more at wk 5 (+19%) and wk 8 (+10%; $P < 0.05$) compared with control-on-controls (Table 3). Whereas, restricted female pups fostered onto a different restricted mother ate less (−16%; $P < 0.05$) than control-on-controls at 5 wk. By wk 10, reduced-on-restricted female offspring ate less ($P < 0.05$) than control-on-controls (Table 3). At 12 wk of age, female control-on-restricted pups ate less (−11%; $P < 0.05$) than control-on-control.

Plasma glucose and insulin, glucose tolerance assessment, HOMA-R, and insulin sensitivity

Fasting plasma glucose was not altered by prenatal or lactational restraint, cross fostering, or sex (Table 4). All animals showed a typical response to a glucose load, with an instantaneous increase in plasma glucose 1 min after the bolus, with circulating glucose concentrations returning to basal after 60–120 min. In the male offspring, there were clear differences in glucose concentrations between groups 1 min after the glucose load, with those subjected to uteroplacental insufficiency followed by a poor lactational environment (restricted-on-restricted) having increased concentrations compared with controls. Furthermore, plasma glucose concentrations at 10 min after the commencement of the IAGTT were increased (+28%; $P < 0.05$) in reduced-on-restricted

TABLE 3. Effect of prenatal and postnatal growth restriction and cross fostering on food intake in postnatal offspring

Pup-on-mother	Control-on-control	Control-on-restricted	Reduced-on-restricted	Reduced-on-control	Restricted-on-control	Restricted-on-restricted
Male offspring						
wk 5	0.17 ± 0.010^{b}	0.15 ± 0.016^{ab}	0.22 ± 0.024^{c}	0.16 ± 0.006^{ab}	0.16 ± 0.008^{b}	0.14 ± 0.007^{a}
wk 6	0.12 ± 0.002^{a}	0.14 ± 0.006^{bc}	0.13 ± 0.013^{b}	0.13 ± 0.004^{bc}	0.14 ± 0.006^{c}	0.13 ± 0.003^{bc}
wk 7	0.12 ± 0.004^{ab}	0.12 ± 0.006^{ab}	0.11 ± 0.010^{a}	0.12 ± 0.004^{ab}	0.11 ± 0.006^{a}	0.13 ± 0.004^{b}
wk 8	0.11 ± 0.004^{ab}	0.11 ± 0.004^{ab}	0.12 ± 0.006^{bc}	0.11 ± 0.003^{abc}	0.12 ± 0.003^{c}	0.11 ± 0.004^{a}
wk 10	0.08 ± 0.002^{ab}	0.09 ± 0.004^{cd}	0.08 ± 0.003^{a}	0.09 ± 0.002^{bc}	0.08 ± 0.002^{a}	0.10 ± 0.005^{d}
wk 12	0.08 ± 0.002^{a}	0.07 ± 0.002^{a}	0.07 ± 0.002^{a}	0.08 ± 0.004^{a}	0.08 ± 0.002^{a}	0.08 ± 0.002^{a}
Female offspring						
wk 5	0.17 ± 0.012^{b}	0.17 ± 0.016^{b}	0.21 ± 0.024^{c}	0.17 ± 0.006^{b}	0.17 ± 0.010^{b}	0.14 ± 0.008^{a}
wk 6	0.13 ± 0.004^{a}	0.14 ± 0.006^{a}	0.13 ± 0.012^{a}	0.13 ± 0.003^{a}	0.14 ± 0.005^{a}	0.13 ± 0.004^{a}
wk 7	0.13 ± 0.003^{a}	0.13 ± 0.009^{a}	0.13 ± 0.007^{a}	0.13 ± 0.008^{a}	0.12 ± 0.004^{a}	0.12 ± 0.004^{a}
wk 8	0.11 ± 0.005^{ab}	0.11 ± 0.007^{abc}	0.12 ± 0.006^{c}	0.12 ± 0.005^{bc}	0.11 ± 0.003^{ab}	0.10 ± 0.004^{b}
wk 10	0.10 ± 0.003^{b}	0.10 ± 0.003^{b}	0.09 ± 0.004^{a}	0.10 ± 0.002^{ab}	0.09 ± 0.002^{ab}	0.10 ± 0.003^{b}
wk 12	0.09 ± 0.002^{b}	0.08 ± 0.002^{a}	0.09 ± 0.003^{b}	0.09 ± 0.003^{b}	0.09 ± 0.004^{b}	0.08 ± 0.003^{ab}

Food intake (percent body weight) measured in postnatal male and female offspring. All data are expressed as mean ± SE (n = 20–46). Significant differences ($P < 0.05$) across the groups are indicated by *different letters*: ^{a} is different from ^{b}, but not different from ^{ab}.

TABLE 4. Effect of prenatal and postnatal growth restriction and cross fostering on fasting glucose and insulin, including an index of insulin sensitivity in adult offspring

Pup-on-mother	Fasting plasma glucose (mmol/liter)	Fasting plasma insulin (ng/ml)	HOMA-R
Male offspring			
Control-on-control	5.77 ± 0.21	1.28 ± 0.24^{ab}	8.37 ± 1.76^{ab}
Control-on-restricted	5.81 ± 0.19	1.82 ± 0.17^{b}	11.33 ± 1.22^{b}
Reduced-on-restricted	5.64 ± 0.27	1.32 ± 0.33^{ab}	8.14 ± 2.31^{ab}
Reduced-on-control	5.52 ± 0.19	0.91 ± 0.12^{a}	5.26 ± 0.78^{a}
Restricted-on-control	5.76 ± 0.20	1.03 ± 0.17^{a}	6.09 ± 0.95^{a}
Restricted-on-restricted	5.26 ± 0.18	1.09 ± 0.16^{a}	6.54 ± 1.15^{a}
Female offspring			
Control-on-control	5.15 ± 0.18	1.07 ± 0.24	6.31 ± 1.58
Control-on-restricted	5.69 ± 0.18	1.23 ± 0.23	8.08 ± 1.64
Reduced-on-restricted	5.64 ± 0.22	1.23 ± 0.21	7.55 ± 1.41
Reduced-on-control	5.50 ± 0.20	1.09 ± 0.14	6.54 ± 0.99
Restricted-on-control	5.49 ± 0.20	1.40 ± 0.19	7.91 ± 1.30
Restricted-on-restricted	5.66 ± 0.11	1.13 ± 0.13	6.74 ± 0.79

Metabolic parameters measured after the IAGTT at 6 months of age in male and female offspring. All data are expressed as mean ± SE (n = 7–10). Significant differences ($P < 0.05$) across the groups are indicated by *different letters*: ^{a} is different from ^{b}, but not different from ^{ab}.

males when compared with control-on-controls, with a trend to increase (+19%; $P = 0.067$) in restricted-on-restricted (Fig. 1B). In response to an IAGTT, the area under the glucose curve was increased (+43%; $P < 0.05$; Fig. 1A) in restricted-on-restricted male offspring compared with control-on-controls, consistent with impaired glucose tolerance. Plasma insulin profiles in response to the IAGTT were similar between groups, with a distinct first-phase insulin secretion and returning to basal after 120 min in both male and female offspring. However, first-phase insulin secretion in response to the glucose load was impaired in restricted-on-restricted male offspring when compared with control-on-controls (−45%; $P < 0.05$; Fig. 1C). The insulin secretory response to glucose, expressed as the area under the insulin curve to area under the glucose curve ratio, was reduced (−62%, Fig. 1D) in restricted-on-restricted male offspring compared with control-on-controls. Fasting hyperinsulinemia, along with increased HOMA-R, was observed in control-on-restricted male offspring when compared with reduced-on-control, restricted-on-control, and restricted-on-restricted males ($P < 0.05$; Table 4), suggestive of insulin resistance. However, insulin sensitivity, as assessed by area under the glucose curve in response to an IC, was not altered by prenatal or lactational restraint, cross fostering, or sex (Table 5). Female growth-restricted offspring showed no signs of impaired glucose tolerance, altered first-phase insulin secretion, or insulin tolerance in relation to control-on-control females (Fig. 1, E–H, and Table 5).

Body composition–fat depots and hind limb muscle weights

When expressed relative to body weight, male retroperitoneal fat weight was heavier in reduced-on-restricted (+39%; $P < 0.05$) and restricted-on-restricted (+50%; $P < 0.05$) offspring than control-on-controls (Table 6), with no differences found in any of the cross-foster groups in relative retroperitoneal fat weight in females. Relative omental and dorsal fat in males was increased ($P < 0.05$) in restricted-on-control offspring compared with restricted-on-restricted, whereas dorsal fat was reduced (−20%; $P < 0.05$) in restricted-on-restricted offspring compared with control-on-control males (Table 6). Relative omental fat weight in females was increased (+24%; $P < 0.05$) in reduced-on-restricted compared with reduced-on-control female offspring. There were no differences in perirenal fat, a surrogate for visceral fat in the rat, across the cross-foster groups for either males or females (data not shown). Male EDL muscle weight as a percentage of body weight was lighter (−7%; $P < 0.05$) in restricted-on-control offspring compared with control-on-controls, with no differences in females (Table 6). Relative soleus muscle weight in males was heavier (+16%; $P < 0.05$) in reduced-on-control offspring compared with control-on-controls, with no differences in any females when compared with control-on-controls.

Relative hypothalamic NPY content

Total hypothalamic NPY content was increased (+6 to 11%; $P < 0.05$) in the nonfasted restricted-on-restricted male offspring compared with all other groups (Fig. 2A). Total NPY content was also increased (+2 to 4%; $P < 0.05$) in the reduced-on-restricted males, compared with the control-on-control and restricted-on-control males. NPY content was increased ($P < 0.05$) in the dorsal medulla (data not shown) and preoptic area (data not shown) in the restricted-on-restricted males compared with control-on-controls. Cross fostering a restricted pup onto a control mother prevented this increase in NPY content within these subregions observed in the restricted-on-restricted males. There were no differences in NPY content observed between restricted-on-restricted, restricted-on-control, and control-on-control male offspring within the PVN (Fig. 2B) or ARC (Fig. 2C). Total hypothalamic NPY content was increased (+9 to 11%; $P < 0.05$) in the restricted-on-restricted and restricted-on-control female offspring compared with control-on-restricted only (Fig. 2D). There were no differences in NPY content within the PVN of any females when compared with control-on-controls (Fig. 2E), whereas restricted-on-restricted females had increased (+16%; $P < 0.05$) NPY content in the ARC compared with control-on-restricted offspring only (Fig. 2F).

Discussion

This study has shown that exposure to uteroplacental insufficiency followed by cross fostering onto a mother with

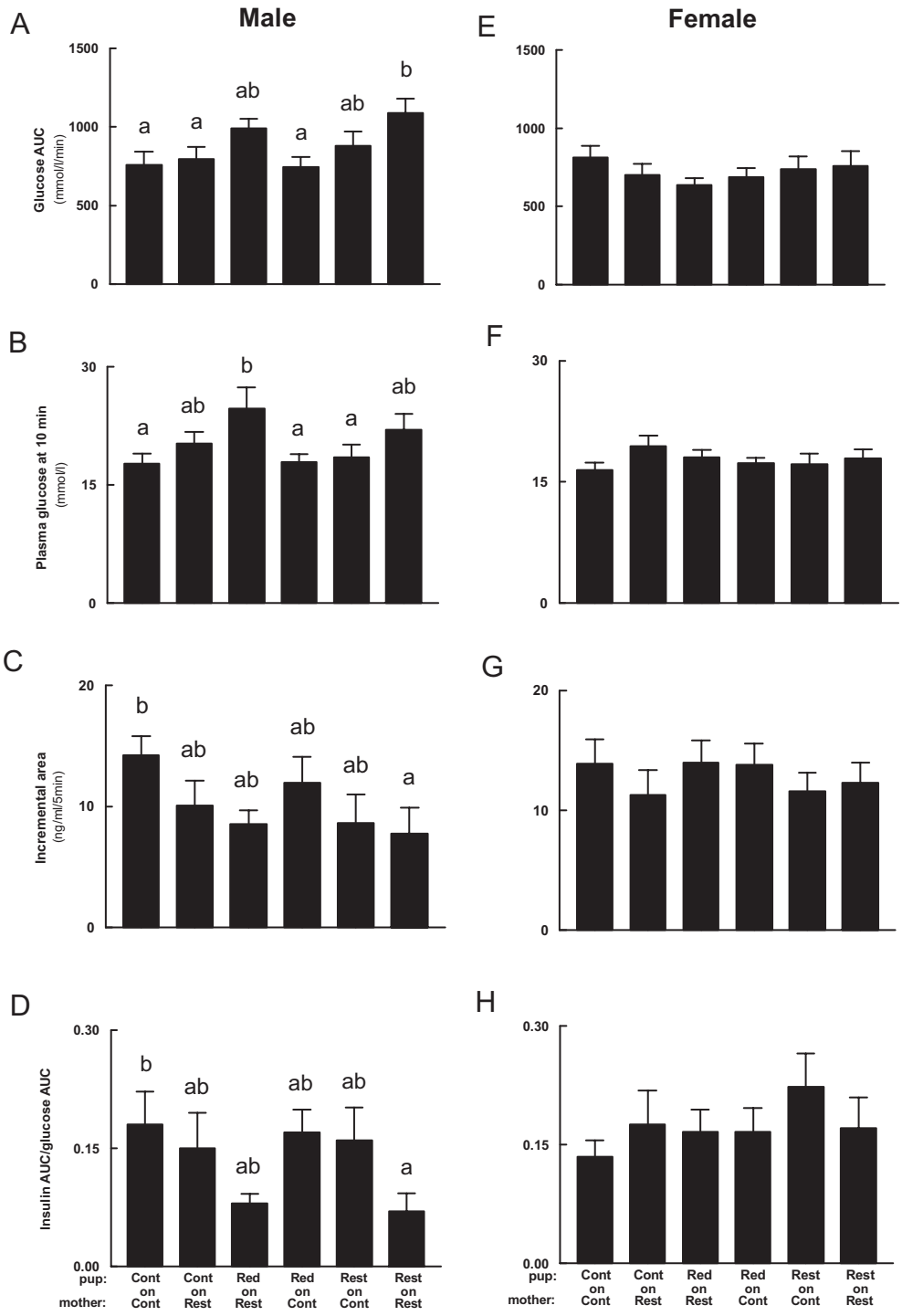

FIG. 1. The effect of prenatal and postnatal growth restriction and cross fostering on glucose tolerance and insulin secretion in adult male (A–D) and female (E–H) offspring. Values are mean ± SE with seven to 10 for all groups. Significant differences ($P < 0.05$) across the groups are indicated by *different letters*: "a" is different from "b," but not different from "ab." AUC, Area under the curve; Cont, control; Red, reduced litter; Rest, restricted.

compromised mammary development and function impairs glucose tolerance and insulin secretion, but not insulin sensitivity, in adult male rat offspring. The novel finding from this study is that this impaired glucose tolerance and altered first-phase insulin secretion observed in the adult male offspring exposed to perinatal restraint (restricted-on-restricted) can be ameliorated by cross fostering as a pup onto a mother providing an improved lactational environment (restricted-on-control). This implicates the lactational environment itself as a critical developmental stage for programming later disease as well as modifying the impact of prenatal challenges. This also shows that amelioration of the long-term effects of uteroplacental restriction on metabolic control is possible by restoration of early postnatal nutrition. We have previously found that uteroplacental insufficiency or reducing litter size after birth, to induce prenatal or postnatal growth restriction, without cross fostering, did not impair glucose tolerance or insulin secretion in male or female adult offspring (14). In the current study, early postnatal restriction or lactational restraint induced via cross

TABLE 5. Effect of prenatal and postnatal growth restriction and cross fostering on insulin sensitivity in adult offspring

Pup-on-mother	Fasting plasma glucose (mmol/liter)	Glucose AUC (mmol/liter·min)
Male offspring		
Control-on-control	6.05 ± 0.174	190.6 ± 23.46
Control-on-restricted	5.66 ± 0.274	157.4 ± 13.70
Reduced-on-restricted	5.79 ± 0.284	160.2 ± 16.73
Reduced-on-control	5.46 ± 0.196	149.9 ± 9.89
Restricted-on-control	5.72 ± 0.259	153.0 ± 15.96
Restricted-on-restricted	5.67 ± 0.178	152.2 ± 11.87
Female offspring		
Control-on-control	5.63 ± 0.377	159.9 ± 20.62
Control-on-restricted	5.18 ± 0.417	145.9 ± 12.26
Reduced-on-restricted	5.59 ± 0.418	137.8 ± 27.04
Reduced-on-control	5.54 ± 0.450	145.1 ± 17.53
Restricted-on-control	5.53 ± 0.400	154.2 ± 10.66
Restricted-on-restricted	4.98 ± 0.260	131.0 ± 10.09

Metabolic parameters measured after the IC at 6 months of age in male and female offspring. All data are expressed as mean ± SE (n = 7–10). There are no significant differences among groups. AUC, Area under the curve.

fostering a control pup onto a restricted mother also had no adverse effect on glucose tolerance or insulin secretion. In addition, prenatal and postnatal restraint in combination with cross fostering impacted adversely on metabolic control and insulin action in male, but not female, offspring. This suggests that cross fostering itself may be a stressor that interacts with prenatal restraint to impair glucose tolerance and insulin secretion in adult male offspring. In some other rat studies investigating the effect of uteroplacental insufficiency on glucose homeostasis, it is not stated whether both sexes were used (4, 13). Increased susceptibility of male offspring to metabolic programming and sex differences in outcomes have been reported in other species after prenatal growth restriction by placental or nutritional methods (13, 35, 36). Similarly, males were more adversely affected in our most recent study examining the relationship between nephron number and blood pressure in adult offspring after cross fostering at birth (24).

One study has shown that at 30 wk of age, diabetes is evident in all sham-operated male rats, but not female rats (37). Castration significantly reduced the incidence of diabetes in male rats, whereas administration of testosterone restored it. Ovariectomy increased the incidence of diabetes in females to 30%, whereas estrogen replacement protected females against glucose intolerance. *In vivo* insulin-stimulated glucose uptake as measured by a euglycemic clamp was reduced in sham-operated males, castrated males with testosterone replacement, and castrated females without estrogen replacement, as compared with sham-operated females and castrated females with testosterone. These results demonstrate that glucose intolerance is closely related to insulin insensitivity, and that sex hormones are directly or indirectly responsible for this condition (37). Further study is required into the influence of sex hormones on, and the mechanistic pathways that may alter, glucose tolerance and insulin secretion in a sex-specific manner. Nevertheless, it appears that the males are more vulnerable to early life nutrient restriction overall than females.

The adult phenotype observed in the current study after uteroplacental insufficiency differs somewhat from that reported by others (4), with impaired glucose tolerance and first-phase insulin secretion deficiency, in the absence of insulin resistance and site-specific increases in adiposity in our adult male Wistar Kyoto rats. In previous studies using Sprague Dawley rats, uteroplacental insufficiency followed by reducing litter size and cross fostering offspring onto unoperated mothers resulted in severe impairment of glucose tolerance and insulin secretion as early as 7 wk of age, with clear insulin resistance at 7–10 wk and later obesity (4). However, in the current study, when prenatally growth-restricted offspring were cross fostered onto a control mother, adult glucose tolerance was substantially restored. These differences may occur from strain differences in responses to bilateral uterine vessel ligation, cross fostering, and reducing litter size, as well as in metabolic control and insulin action.

In previous studies, the extent of uteroplacental restriction was greater, with a 20% reduction in weight at birth (4, 18) compared with 10–15% in the current study, although litter size was greater and was reduced to eight at birth, in contrast to our typical average litter size of approximately nine (16). Significantly, in those previous studies (4, 18), prenatally

TABLE 6. Effect of prenatal and postnatal growth restriction and cross fostering on adiposity and skeletal muscle in adult offspring

Pup-on-mother	Retroperitoneal fat (% body weight)	Omental fat (% body weight)	Dorsal fat (% body weight)	EDL muscle (% body weight)	Soleus muscle (% body weight)
Male offspring					
Control-on-control	0.11 ± 0.01^{a}	0.94 ± 0.05^{ab}	1.31 ± 0.06^{bc}	0.053 ± 0.001^{b}	0.036 ± 0.001^{ab}
Control-on-restricted	0.15 ± 0.01^{ab}	0.95 ± 0.05^{ab}	1.34 ± 0.09^{bc}	0.052 ± 0.001^{ab}	0.038 ± 0.001^{bc}
Reduced-on-restricted	0.18 ± 0.01^{bc}	0.98 ± 0.05^{ab}	1.28 ± 0.09^{bc}	0.050 ± 0.001^{ab}	0.033 ± 0.001^{ab}
Reduced-on-control	0.14 ± 0.01^{ab}	0.86 ± 0.05^{a}	1.13 ± 0.08^{ab}	0.052 ± 0.001^{ab}	0.043 ± 0.004^{c}
Restricted-on-control	0.14 ± 0.01^{ab}	1.03 ± 0.05^{b}	1.36 ± 0.10^{c}	0.049 ± 0.002^{a}	0.036 ± 0.001^{ab}
Restricted-on-restricted	0.22 ± 0.03^{c}	0.84 ± 0.04^{a}	1.04 ± 0.07^{a}	0.051 ± 0.001^{ab}	0.032 ± 0.001^{ab}
Female offspring					
Control-on-control	2.03 ± 0.09	1.04 ± 0.04^{ab}	1.40 ± 0.06	0.056 ± 0.001	0.043 ± 0.001^{ab}
Control-on-restricted	1.88 ± 0.09	1.02 ± 0.05^{ab}	1.37 ± 0.06	0.056 ± 0.001	0.040 ± 0.001^{ab}
Reduced-on-restricted	1.80 ± 0.09	1.23 ± 0.08^{b}	1.51 ± 0.08	0.054 ± 0.001	0.039 ± 0.001^{ab}
Reduced-on-control	1.85 ± 0.13	0.93 ± 0.06^{a}	1.30 ± 0.10	0.059 ± 0.005	0.044 ± 0.003^{b}
Restricted-on-control	1.84 ± 0.10	1.08 ± 0.05^{ab}	1.40 ± 0.07	0.056 ± 0.002	0.044 ± 0.003^{b}
Restricted-on-restricted	1.72 ± 0.10	1.08 ± 0.05^{ab}	1.47 ± 0.09	0.053 ± 0.001	0.038 ± 0.001^{a}

Fat depot and hind limb muscle weights (percent body weight) at 6 months of age in male and female offspring. All data are expressed as mean ± SE (n = 20–37). Significant differences ($P < 0.05$) across the groups are indicated by *different letters*: ^{a} is different from ^{b}, but not different from ^{ab}.

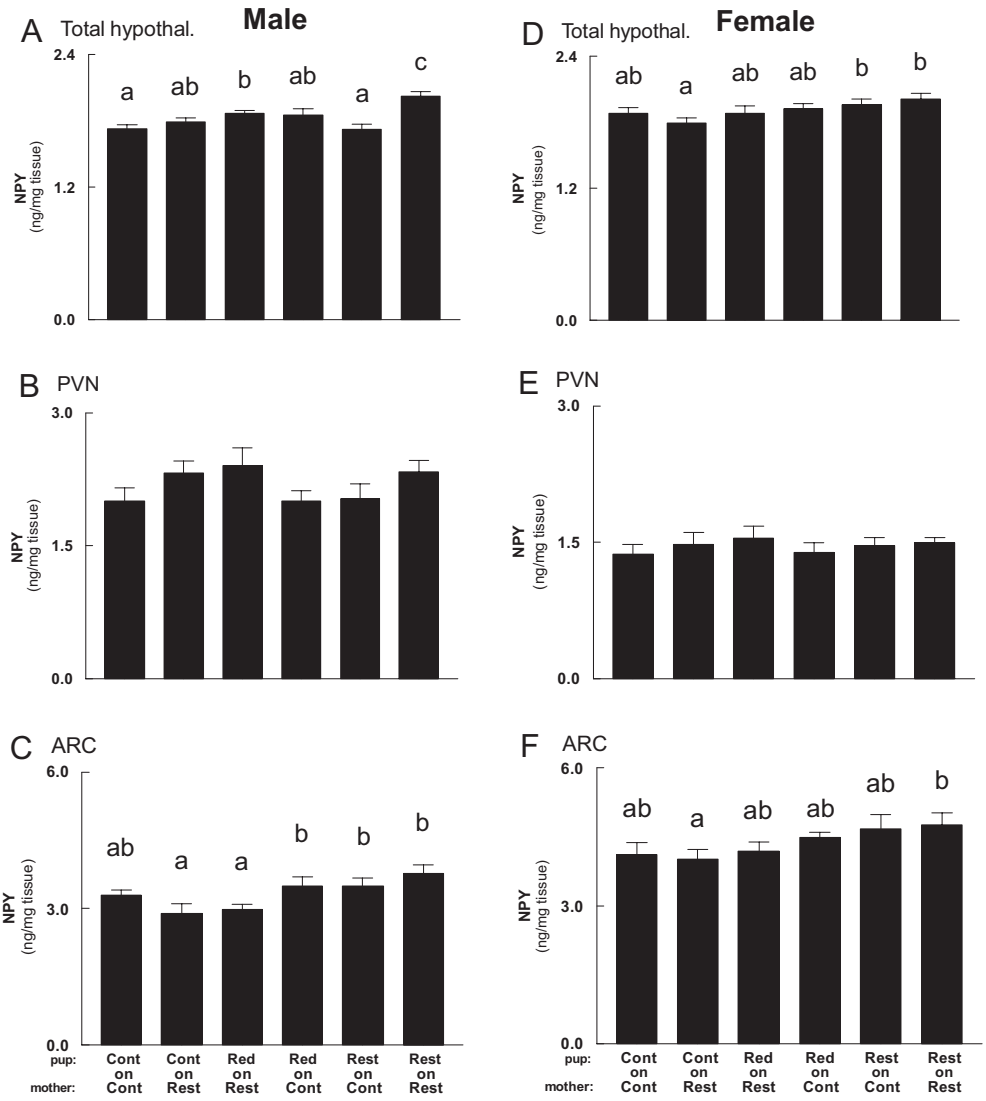

FIG. 2. The effect of prenatal and postnatal growth restriction and cross fostering on NPY content (ng/mg tissue) in adult male (A–C) and female (D–F) offspring. Values are mean ± SE with 15–18 for all groups. Significant differences ($P < 0.05$) across the groups are indicated by *different letters*: "a" is different from "b," but not different from "ab." Cont, Control; hypothal, hypothalamus; Red, reduced litter; Rest, restricted.

growth-restricted rats caught up in weight to controls by 7 wk of age with concomitant mild glucose intolerance. By 10 wk, growth-restricted rats weighed significantly more than controls and developed hyperinsulinemia, suggestive of insulin resistance, and by 6 months were obese with acute first-phase insulin secretion virtually absent (4). In contrast, in the current study, prenatally growth-restricted offspring remained lighter throughout postnatal life, regardless of postnatal nutrition, although they showed some evidence of increased adiposity. Thus, prenatal and postnatal growth restriction increased relative retroperitoneal fat in adult male offspring, whereas prenatal restriction followed by a normal lactational environment increased relative omental and dorsal fat, suggesting that increased early postnatal nutrition can exacerbate the onset of overall obesity after prenatal restriction.

Cross fostering control pups onto mothers with compromised mammary development and function impaired insulin sensitivity as assessed by HOMA-R in adult male offspring, whereas no impact was observed with other exposures before or after birth. In our previous study in which cross fostering was not used (14), female offspring, in which litter size was modestly reduced, also exhibited insulin resistance in terms of an increased HOMA-R compared with control and prenatally growth-restricted offspring. This finding suggests that insulin sensitivity is particularly sensitive to nutritional perturbations and other challenges in the immediate postnatal period in the rat. Although there is a paucity of information in the rat and other species, placental restriction in the sheep is associated with rapid catch-up growth during the first month of life, resulting in visceral obesity and insulin resistance (5, 7). Increased weight gain in children of low birth weight between 2 and 7 yr of age predicts an increased risk of cardiovascular disease, obesity, and diabetes (9–11, 38). Whereas other studies suggest that slowed postnatal growth, especially during the first year based on weight at 1 yr, can lead to later insulin resistance (12). In the current study, control male offspring fostered onto mothers with impaired lactation showed reduced weight at d 14 but caught up in weight by 1 month, a growth profile that may have been important in the later onset of insulin resistance. Further studies are needed to evaluate the

effects of reduced nutrition followed by restored nutrition at birth or at later stages of early postnatal life on later insulin action and metabolic control.

It is clear that altered growth profiles in late gestation and early postnatal life can severely impact on adult metabolic health. This critical period is important for the development and later function of the endocrine pancreas, particularly β-cells, which are the primary determinants of glucose tolerance. Initially, pancreatic β-cells are poorly responsive to glucose, and it is not until late gestation in the rat that these β-cells are replaced, after a wave of apoptosis, with new islet cells that are sensitive to glucose with acute first-phase insulin release (39). Therefore, nutritional perturbations, such as uteroplacental insufficiency and maternal undernutrition during gestation and lactation, can adversely affect endocrine pancreas development, resulting in reduced β-cell mass at a later age (40, 41) and impaired glucose tolerance and first-phase insulin secretion (4, 42). The impaired glucose tolerance and insulin secretion deficiency in our adult male rats with prenatal and postnatal nutritional restraint may be due to an irreversible pancreatic deficit and impaired intrinsic cell function, specifically reduced β-cell mass, programmed around birth. The importance of the immediate postnatal environment for later metabolic function is confirmed in another study that found that exposure to the long-acting glucagon-like peptide-1 analog Exendin-4 in early postnatal life can normalize β-cell proliferation rate, reversing the adverse consequences of fetal programming, therefore preventing the development of diabetes in adulthood (43).

The current study has also shown that uteroplacental insufficiency in the rat increases food intake of offspring postnatally, similar to that observed after placental restriction in the sheep (7). Furthermore, we have shown for the first time that prenatal and lactational restraint increase total hypothalamic NPY content and that of the ARC in nonfasted adult male offspring, suggesting programming of longer term elevated expression of appetite stimulatory pathways. Whether this increase in central NPY content was present earlier to drive the increase in food intake observed in these males at 10 wk of age is yet to be determined.

In summary, we have shown that male offspring exposed to uteroplacental insufficiency followed by a poor lactational environment (restricted-on-restricted) have impaired glucose tolerance and insulin secretion, but not insulin resistance as adults. Importantly, we have shown for the first time that these defects in metabolic control and insulin secretion in the adult male exposed to prenatal growth restriction can be ameliorated by providing an improved lactational environment. These findings support the hypothesis that a compromised early life environment can program a later impairment in insulin action and glucose homeostasis, particularly in males. Defining the underlying mechanisms responsible may provide insight into what early life interventions might lessen these adverse consequences for longer term health. Identification of critical periods after birth that influence adult health, rather than periods of sensitivity during pregnancy, would offer a greater likelihood that practical public health interventions may be developed in this emerging field.

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Address all correspondence and requests for reprints to: Dr. Andrew Siebel, Department of Physiology, University of Melbourne, Parkville, 3010, Australia. E-mail: asiebel@unimelb.edu.au.

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