

Maternal High-Fat Diet During Gestation or Suckling Differentially Affects Offspring Leptin Sensitivity and Obesity

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Maternal high-fat (HF) diet throughout gestation and suckling has long-term consequences on the offspring's metabolic phenotype. Here we determine the relative contribution of pre- or postnatal maternal HF diet on offspring's metabolic phenotype. Pregnant Sprague-Dawley rats were maintained on normal chow or HF diet throughout gestation and suckling. All litters were cross-fostered to chow or HF dams on postnatal day (PND)1, resulting in four groups. Body weight, body composition, and glucose tolerance were measured at weaning and in adulthood. Leptin sensitivity was assessed by signal transducer and activator of transcription (STAT)3 activation on PND10 and PND21. Pups cross-fostered to HF dams gained more body weight than chow pups by PND7 and persisted until weaning. Postnatal HF pups had greater adiposity, higher plasma leptin concentration, impaired glucose tolerance, and reduced phosphorylated STAT3 in response to leptin in the arcuate nucleus at weaning. After weaning, male offspring cross-fostered to HF dams were hyperphagic and maintained greater body weight than postnatal chow pups. Postnatal HF diet during suckling continued to impair glucose tolerance in male and female offspring in adulthood. Maternal HF diet during suckling has a greater influence in determining offspring's metabolic phenotype than prenatal HF diet exposure and could provide insight regarding optimal perinatal nutrition for mothers and children. *Diabetes* 61:2833–2841, 2012

Obesity has become a worldwide health problem that often leads to many related disorders, including cardiovascular disease, hypertension, type 2 diabetes, some cancers, sleep apnea, and arthritis (1–4). The prevalence of childhood obesity is also increasing, suggesting that the obesity epidemic will continue to worsen (5–7). Genetic and environmental factors both affect the development of obesity (2,8,9). Increasing evidence suggests that the early-life environment can also influence the development of obesity (10). Maternal undernutrition and overnutrition during the gestation and suckling periods have been shown to affect the metabolic phenotype of offspring (11–13). A significant issue in Western society is overnutrition as a result of the consumption of

modern diets that contain high amounts of fat. Many studies have now shown that maternal high-fat (HF) diet through gestation and suckling has a long-term effect on offspring's metabolism (14,15).

Leptin, an adipose tissue–secreted hormone, influences energy homeostasis and food intake by acting at a variety of brain sites (16–18). In the arcuate nucleus (ARC), leptin binds to the long isoform of the Ob receptor and activates the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway, leading to phosphorylation of the transcription factor STAT3 (19). Phosphorylated STAT3 (pSTAT3) modulates the expression of neuropeptides that control food intake and energy balance such as neuropeptide Y and proopiomelanocortin (20). Leptin also plays a critical neurotrophic role during the development of the hypothalamus in that neural projection pathways from the ARC are disrupted in leptin-deficient mice (21). The hypothalamus undergoes robust growth that begins in early gestation and continues through postnatal life (22). Hypothalamic neurogenesis occurs in rodents during midgestation, whereas the neural projections between different nuclei develop during early postnatal life (23). A change in environment during these critical developmental periods, for example, maternal HF diet consumption during gestation and suckling, may disrupt hypothalamic development and have long-term metabolic consequences for offspring.

Our previous studies showed that maternal HF diet throughout gestation and suckling resulted in rat offspring with increased body weight, adiposity, leptin concentrations, and impaired glucose tolerance at weaning, as well as greater susceptibility to diet-induced obesity in adulthood (24). Maternal HF diet consumption increases plasma leptin concentration in offspring as early as postnatal day (PND)7, suggesting the possibility of significant effects on the pups' hypothalamic development and leptin sensitivity. However, maternal HF diet through gestation and suckling does not distinguish between prenatal and postnatal effects (14,15). In this experiment, we used a cross-fostering paradigm to determine whether maternal consumption of a HF diet during the prenatal or postnatal period is more critical in offspring's metabolic programming. We measured offspring's body weight, body composition, and tested glucose tolerance at weaning and in adulthood and assessed leptin sensitivity on PND10 and 21.

RESEARCH DESIGN AND METHODS

All animal procedures were approved by the Johns Hopkins University School of Medicine Institutional Animal Care and Use Committee. Pregnant female Sprague-Dawley rats (Charles River, Kingston, NY) were received on gestation day 2 (GD2). Animals were individually housed in conventional tub cages with access to food and water ad libitum. The room was maintained on a 12-h light/dark cycle, with light onset at 0600 h.

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Pregnant rats were divided into two groups according to their diet throughout gestation and lactation: standard chow (CHOW) diet (LabDiet, 5001, 13.5% kcal from fat; $n = 16$) or HF diet (Research Diets, D12492, 60% kcal from fat, $n = 17$). All dams were started on their respective diets upon arrival on GD2. Dams' body weight and food intake were measured daily throughout gestation.

The day of parturition is PND0. On PND1, litters were culled to 10 pups each (5 males and 5 females). Only litters with ≥ 10 pups were included in the study to ensure standardized nutrition during suckling. All litters were cross-fostered to a CHOW or HF dam, resulting in four groups according to the dams' prenatal and postnatal diet: CHOW-CHOW ($n = 8$), CHOW-HF ($n = 8$), HF-CHOW ($n = 8$), and HF-HF ($n = 9$).

Pups and dams were weighed once a week on PND1, 7, 14, and 21. Dams' food intake was measured daily throughout the suckling period. On PND21, one male and one female pup per litter was killed by decapitation, and blood was collected, centrifuged at 4°C to collect plasma, and stored at -80°C for hormone analysis. Carcasses were reserved for later body composition analysis by nuclear magnetic resonance (NMR) imaging. The remaining pups were weaned on PND21 and housed in groups of two to three by sex and group. All pups were weaned to the standard chow diet.

Milk composition. On PND10 and 21, dams were removed from their home cages and were deeply anesthetized with ketamine and xylazine (4:3 mixture; 0.1 mL/100 g body weight i.p.) and injected with 4 IU i.p. oxytocin (Sigma, St. Louis, MO). Milk was collected 15 minutes later into glass capillary tubes (Drummond Scientific, Broomall, PA). Samples were stored in microcentrifuge tubes and frozen at -80°C for later analysis. After milk collection, dams were killed by decapitation. Total lipid content of milk was determined through sequential ether-extractions in a Rose-Gottlieb procedure modified for micro-amounts of fat (25). Similar to previous studies measuring milk leptin and insulin content, commercially available radioimmunoassay kits were used according to the manufacturer's protocol (Millipore, Billerica, MA) (26).

Body composition and fat distribution by NMR. On PND10 and 21, carcasses of male and female pups were kept for body composition measurements. An established procedure was used to determine relative distribution of visceral and subcutaneous fat in rats and mice (27).

Glucose tolerance test and endocrine assays. On PND23 and at age 10 weeks, rats were food-deprived overnight for 16 h with only water available. A baseline blood sample (~200 μ L) was taken via a small tail nick for determination of plasma insulin. Baseline fasted blood glucose was determined at the same time by a handheld Freestyle glucose meter (TheraSense, Alameda, CA). An oral gavage of glucose (2.0 g/kg body weight, 20% glucose in sterile water solution) was administered. Blood samples were collected at 15, 30, 45, 60, and 120 min after glucose gavage to determine plasma insulin levels. Blood glucose was determined at each interval using the glucometer.

Plasma and milk hormone analysis. Plasma and milk hormone concentrations were determined by commercially available radioimmunoassay kits for leptin and insulin for rat (Millipore).

Leptin sensitivity. On PND10 and 21 male and female pups ($n = 2$ per sex per litter at each time point) received an intraperitoneal injection of recombinant rat leptin (3 mg/kg i.p.; A.F. Parlow, National Hormone and Peptide Program, Torrance, CA) or saline. Pups were killed 3 h later by decapitation. Blood was collected into a heparinized microcentrifuge tube, centrifuged at 4°C to collect plasma, and stored at -80°C until analysis for leptin concentration. Brains were removed and immediately frozen on powdered dry ice and stored in -80°C. For PND10 brains, the medial basal hypothalamus (MBH) was collected for protein isolation and Western blot. For PND21 brains, the ARC was isolated from 500- μ m-thick frozen coronal sections using a blunted 16-gauge stainless steel needle (inner diameter, 1.65 mm) based on the coordinates for PND10 and 21 rat brains described by Sherwood and Timiras (28).

Western blotting. MBH or ARC samples were homogenized in lysis buffer (Sigma) with protease inhibitor cocktail (Roche) and phosphatase inhibitor cocktail (Roche). After lysis on ice for 2 h, samples were centrifuged at 12,000 rpm at 4°C for 15 min. Protein concentration was determined using a protein assay kit (Thermo Scientific). Protein (30 μ g) was run on a 3-8% Tris acetate gel and transferred onto polyvinylidene fluoride membranes. Blots were blocked with 5% nonfat dry milk for 2 h. pSTAT3, STAT3, phospho-AMP-activated protein kinase (pAMPK), AMPK, phospho-acetyl CoA carboxylase (pACC), and ACC were determined using corresponding antibodies (Cell Signaling, Beverly, MA; Millipore). Targeted proteins were revealed using SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific) and exposed to film (GE Healthcare). The intensity of bands was quantified using Scion Image Software (Scion Corp., Frederick, MD). The ratio of the intensity of the phosphoprotein (e.g., pSTAT3, pAMPK, or pACC) to that of corresponding total protein (e.g., STAT3, AMPK, or ACC) was calculated to represent the level of phosphorylation. β -Actin (Sigma) was used as the loading control.

Statistical analysis. Data were analyzed by ANOVA, repeated-measures ANOVA, or Student *t* tests for independent samples, as appropriate, using

SPSS 13 software (SPSS Inc., Chicago, IL). Subsequent comparisons between groups used Newman-Keuls procedures. Data are presented as the mean \pm SEM.

RESULTS

Dams. There were 16 CHOW-fed dams and 17 HF-fed dams in this study. The CHOW-fed and HF-fed dams did not differ significantly in maternal body weight on GD21 (374.4 \pm 5.0 vs. 384.5 \pm 5.7 g) or after parturition on PND1 (295.3 \pm 4.5 vs. 307.3 \pm 4.5 g; Table 1). Average food intake during gestation was higher ($P < 0.001$) in HF dams (90.7 \pm 1.9 kcal) than in CHOW dams (72.8 \pm 1.5 kcal).

Endocrine parameters for a subset of dams ($n = 8$ per dietary group) are presented in Table 1. On PND10, HF dams had higher plasma leptin than CHOW dams ($P < 0.05$), although there was no difference in leptin or insulin content of maternal milk. By PND21, there was no significant difference in plasma leptin or insulin between CHOW and HF dams; however, HF dams had higher leptin and fat content in their milk ($P < 0.05$).

Neonatal offspring. There were no significant differences in litter size, male-to-female ratios, or birth weight of males or females between the dietary groups. There was an overall significant effect of the postnatal maternal HF diet, resulting in increased offspring body weight independent of prenatal maternal diet (Fig. 1A and B). By PND7, male and female pups that were fostered to HF-fed dams were significantly heavier than those fostered to CHOW-fed dams, and this effect persisted in both sexes through weaning on PND21.

Male and female pups from postnatal HF dams (CHOW-HF and HF-HF) had greater adiposity than postnatal CHOW pups (CHOW-CHOW and HF-CHOW) on PND10 (Fig. 1C and D). The increased adiposity was evident in the visceral and subcutaneous depots ($P < 0.05$). On PND21, males and females in both postnatal HF diet groups continued to have greater total and subcutaneous adiposity (Fig. 1E and F). By this time, however, only females in those groups also had significantly greater visceral adiposity ($P < 0.05$). Lean tissue percentage was lower on

TABLE 1
Maternal body weight ($n = 16-17$ per diet group), endocrine, and milk composition measures ($n = 8$ per diet group) during gestation and suckling

	CHOW	HF
GD21		
Body weight (g)	374.4 \pm 5.0	384.5 \pm 5.7
Plasma leptin (ng/mL)	2.8 \pm 0.8	3.9 \pm 0.8
Plasma insulin (ng/mL)	1.5 \pm 0.2	1.8 \pm 0.1
Blood glucose (mg/dL)	68.8 \pm 1.7	69.1 \pm 2.6
PND10		
Plasma leptin (ng/mL)	0.7 \pm 0.1	2.2 \pm 0.3*
Plasma insulin (ng/mL)	0.9 \pm 0.2	0.7 \pm 0.3
Milk leptin (ng/mL)	3.8 \pm 0.2	4.7 \pm 0.8
Milk insulin (ng/mL)	7.4 \pm 1.2	4.6 \pm 0.4
Milk fat (% w/v)	10.5 \pm 0.5	10.0 \pm 0.5
PND21		
Plasma leptin (ng/mL)	0.2 \pm 0.1	1.2 \pm 0.4
Plasma insulin (ng/mL)	0.5 \pm 0.1	0.4 \pm 0.1
Milk leptin (ng/mL)	2.0 \pm 0.3	4.2 \pm 0.5*
Milk insulin (ng/mL)	5.2 \pm 1.3	5.9 \pm 1.7
Milk fat (% w/v)	10.1 \pm 1.2	14.4 \pm 1.2*

* $P < 0.05$ vs. CHOW group.

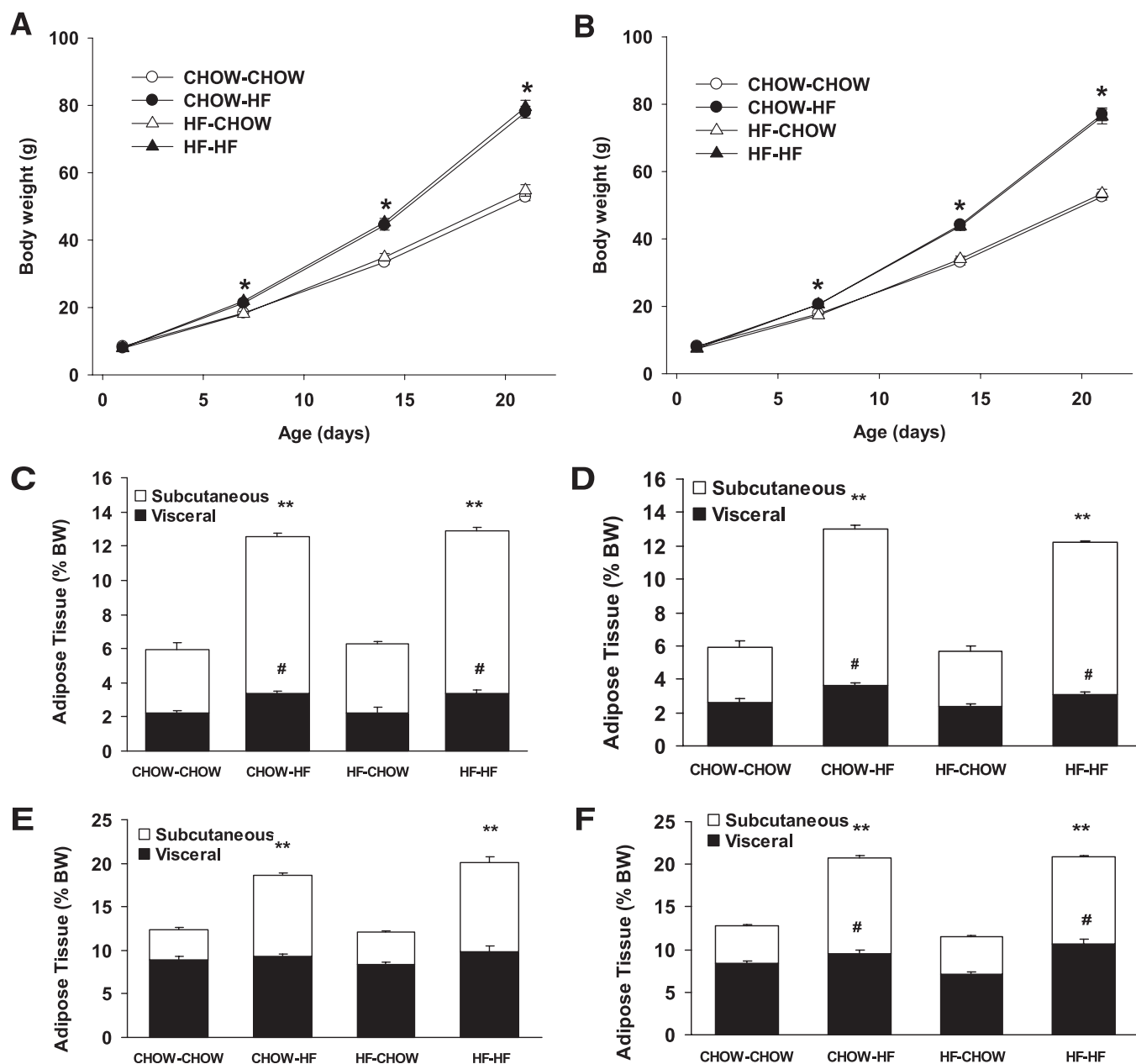


FIG. 1. Body weight and body composition of offspring before weaning. Male (A) and female (B) offspring were weighed on PND1, 7, 14, and 21. Fat tissue as a percentage of body weight (BW) was measured in male (C and E) and female (D and F) offspring on PND10 (C and D) and 21 (E and F). $n = 8$ litter per group. *Main effect of postnatal HF diet, $P < 0.05$ vs. postnatal CHOW diet. #Main effect of postnatal HF diet on visceral fat percentage, $P < 0.05$ vs. postnatal CHOW diet. **Main effect of postnatal HF diet on total and subcutaneous fat percentage, $P < 0.05$ vs. postnatal CHOW diet.

PND10 and 21 in male ($P < 0.01$) and female ($P < 0.01$) pups cross-fostered to HF-fed dams compared with those fostered to CHOW-fed dams (Supplementary Fig. 1). Consistent with increased adiposity, postnatal HF diet exposure also resulted in significantly higher plasma leptin levels in male ($P < 0.01$) and female ($P < 0.01$) pups on PND10 and 21 compared with pups of postnatal CHOW-diet dams (Table 2).

Glucose tolerance test. At weaning, HF-CHOW males had significantly lower baseline blood glucose and plasma insulin compared with the CHOW-CHOW males ($P < 0.05$; Fig. 2A and C). Postnatal HF-diet male pups had significantly higher glucose levels at 45, 60, and 120 min ($P < 0.01$). The overall glucose area under the curve (AUC; Fig. 2A,

inset) was higher in male pups cross-fostered to HF-diet dams ($P < 0.05$). Male pups in the HF-CHOW group also had a higher glucose AUC ($P < 0.05$), suggesting that the prenatal HF diet had influenced glucose tolerance, despite a lack of difference in body weight (Fig. 2A). Plasma insulin was higher at 45, 60, and 120 min in CHOW-HF and HF-HF pups ($P < 0.01$; Fig. 2C). Insulin AUC was also higher in these two groups ($P < 0.01$; Fig. 2C, *inset*).

Glucose tolerance data for females pups are presented in Figs. 2B and D. Female pups cross-fostered to dams fed the HF diet had higher glucose and insulin levels at 45, 60, and 120 min ($P < 0.05$) and higher glucose and insulin AUC ($P < 0.05$).

TABLE 2
Plasma leptin concentration (ng/mL) in PND10 and PND21 offspring

	C-C	C-H	H-C	H-H
<i>n</i>	8	8	7	8
PND10				
Male	4.5 ± 0.9	27.7 ± 2.0*	4.6 ± 1.0	24.7 ± 5.8*
Female	3.7 ± 0.8	12.1 ± 4.6*	2.3 ± 0.8	17.7 ± 2.7*
PND21				
Male	1.1 ± 0.2	9.9 ± 1.5*	1.3 ± 0.8	11.1 ± 1.1*
Female	2.3 ± 0.3	16.0 ± 0.7*	2.7 ± 0.6	11.3 ± 0.1*

C-C, CHOW-CHOW; C-H, CHOW-HF; H-C, HF-CHOW; H-H, HF-HF. *Main effect of postnatal HF diet, $P < 0.05$ vs. postnatal CHOW diet.

Leptin sensitivity

PND10. Leptin injection significantly increased the pSTAT3 level in the MBH in the four diet groups ($P < 0.01$; Fig. 3). In male pups, however, leptin induced significantly less pSTAT3 in the MBH of CHOW-HF, HF-CHOW, and HF-HF

pups compared with CHOW-CHOW pups ($P < 0.05$; Fig. 3A), indicating that prenatal and postnatal HF diet both reduced leptin-induced activation of STAT3 on PND10. In females, only pups exposed to the prenatal HF diet had lower pSTAT3 level after the leptin challenge compared with the CHOW-CHOW group ($P < 0.05$; Fig. 3B), suggesting that prenatal HF diet reduces leptin sensitivity in the hypothalamus on PND10 while postnatal HF diet had no effect.

PND21. Baseline pSTAT3 level was indistinguishable among males in the four diet groups (Fig. 4A and C). In contrast, female pups cross-fostered to HF-fed dams had higher pSTAT3 at baseline than the pups cross-fostered to CHOW-fed dams ($P < 0.05$; Fig. 4B and D). Leptin significantly increased the pSTAT3 level in the ARC in males and females in all four groups ($P < 0.01$). Male and female pups cross-fostered to HF-diet dams had lower pSTAT3 after the leptin challenge compared with the CHOW-CHOW control group ($P < 0.05$; Fig. 4C and D), indicating lower leptin sensitivity in the ARC at weaning. Leptin did not affect pAMPK or pACC levels in the ARC in males or females on PND21 (Supplementary Fig. 2).

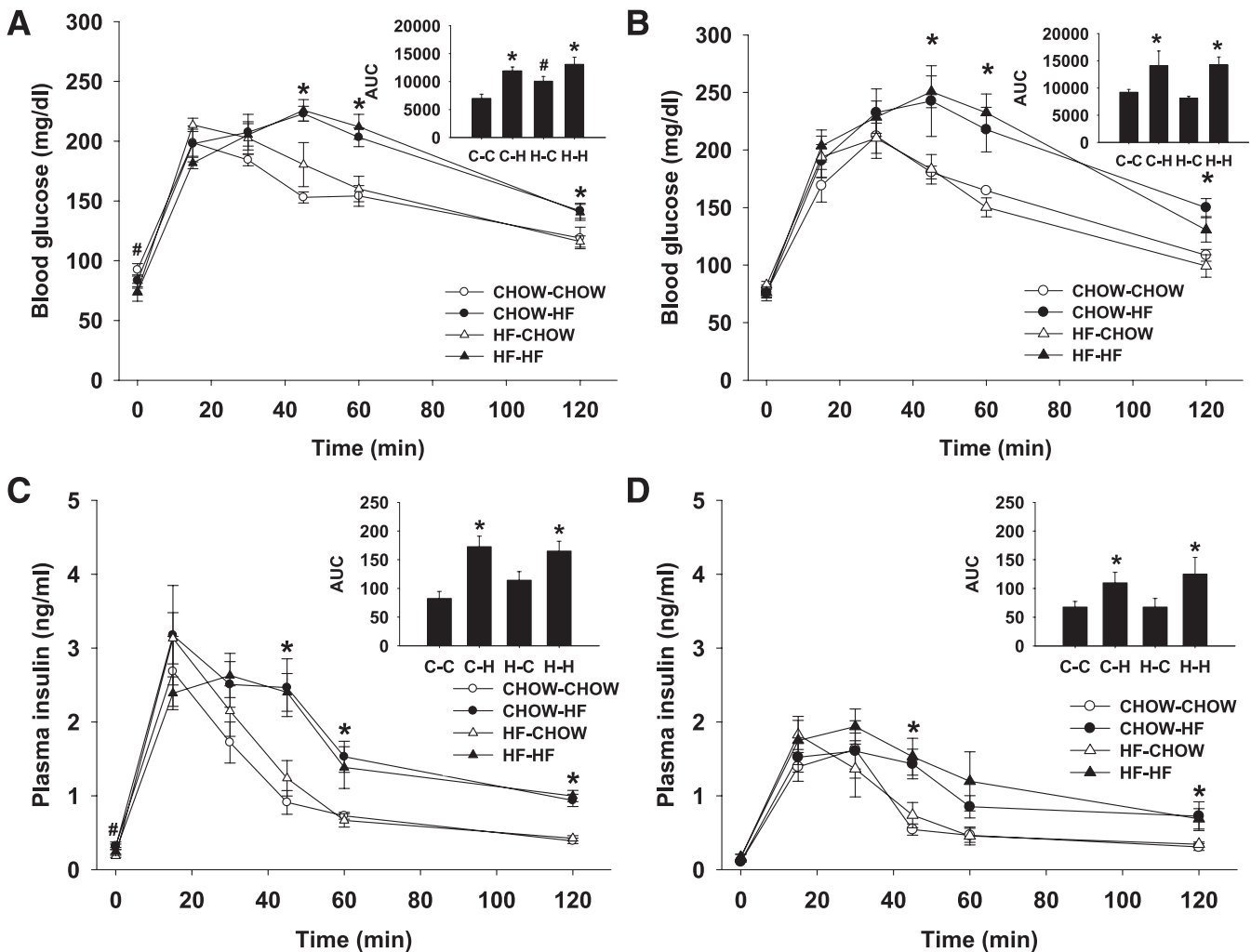


FIG. 2. Glucose tolerance test (2.0 g/kg, oral gavage) results for male (A and C) and female (B and D) offspring on PND23. Blood glucose (A and B) and plasma insulin (C and D) were determined for 2 h after oral administration of glucose. The integrated AUC (inset) was determined for glucose and insulin using the trapezoidal method. CHOW-CHOW (C-C), $n = 4$; CHOW-HF (C-H), $n = 4$; HF-CHOW (H-C), $n = 5$; HF-HF (H-H), $n = 5$. *Main effect of postnatal HF diet, $P < 0.05$ vs. postnatal CHOW diet. # $P < 0.05$, HF-CHOW vs. CHOW-CHOW.

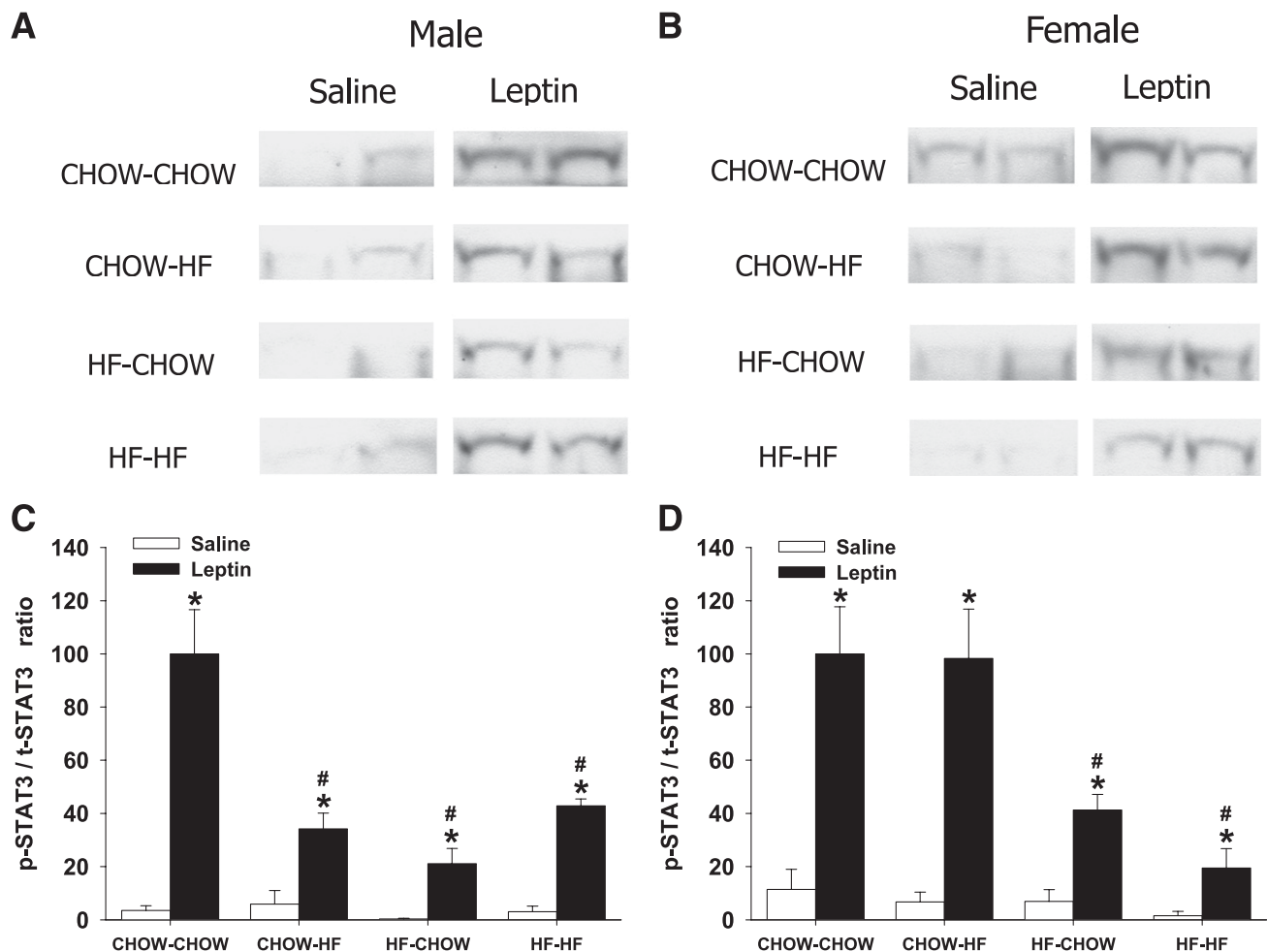


FIG. 3. Western blot of pSTAT3 in MBH in male (A) and female (B) offspring on PND10. The pSTAT3-to-total (t)STAT3 ratio (compared with leptin-injected pups in CHOW-CHOW group) was calculated in male (C) and female (D) offspring. The pups were injected with saline ($n = 3$) or leptin ($n = 5$) in each group. * $P < 0.05$ vs. saline group. # $P < 0.05$ vs. CHOW-CHOW group.

Adult offspring. Male offspring in CHOW-HF and HF-HF groups continued to have greater body weight compared with postnatal CHOW groups in adulthood ($P < 0.01$; Fig. 5A). At age 9 weeks, food intake was significantly greater in postnatal HF groups. However, when adjusted for body weight, there were no longer any differences among the groups of males. In contrast, female body weights (Fig. 5B) and food intake were indistinguishable among groups after age 8 weeks. Male offspring cross-fostered to HF-diet dams postnatally had a higher percentage of subcutaneous fat compared with the CHOW-CHOW group at 17 weeks ($P < 0.05$; Fig. 5C), but body composition was not different among females at 12 weeks (Fig. 5D).

There were no differences among the groups in glucose clearance in the glucose tolerance test in 10-week-old males (Fig. 6A). Male offspring in the CHOW-HF group had higher insulin AUC compared with the CHOW-CHOW group ($P < 0.05$; Fig. 6C). Among the females, offspring from postnatal HF dams had no significant differences in glucose AUC (Fig. 6B) but had higher insulin AUC ($P < 0.05$; Fig. 6D), indicating that they required more insulin to clear the glucose load.

DISCUSSION

The intrauterine and early postnatal environments are critical in the development of offspring. Previous work has

documented that maternal HF diet throughout gestation and suckling can have long-term metabolic consequences at weaning and in adulthood (14,15,24,29). In this study, we used a cross-fostering procedure to determine whether prenatal or postnatal HF-diet exposure had a greater influence on offspring metabolic phenotype. Our data suggest that the maternal HF diet during the suckling period is more critical in determining metabolic consequences for offspring such as leptin resistance.

Other studies have used a HF-diet period before conception to induce maternal obesity, including 5 or 6 weeks before mating and throughout gestation and suckling (15). Maternal obesity alone has significant effects on oocyte development, maturation, and embryo development (30,31), any of which could have adverse effects on offspring independent of diet during gestation and suckling. Maternal obesity could also produce diabetic conditions, before or during pregnancy, which could predispose the offspring to metabolic side effects (32). Consistent with previous studies using HF diet only during gestation, those dams fed the HF diet consumed more calories during gestation and lactation, but body weight did not differ significantly between the dietary groups throughout the experiment, perhaps suggesting that the HF-fed dams may have increased their energy expenditure during this time (33). Plasma insulin and blood glucose levels on GD21 were similar in CHOW- and HF-diet-fed dams, suggesting HF dams had not

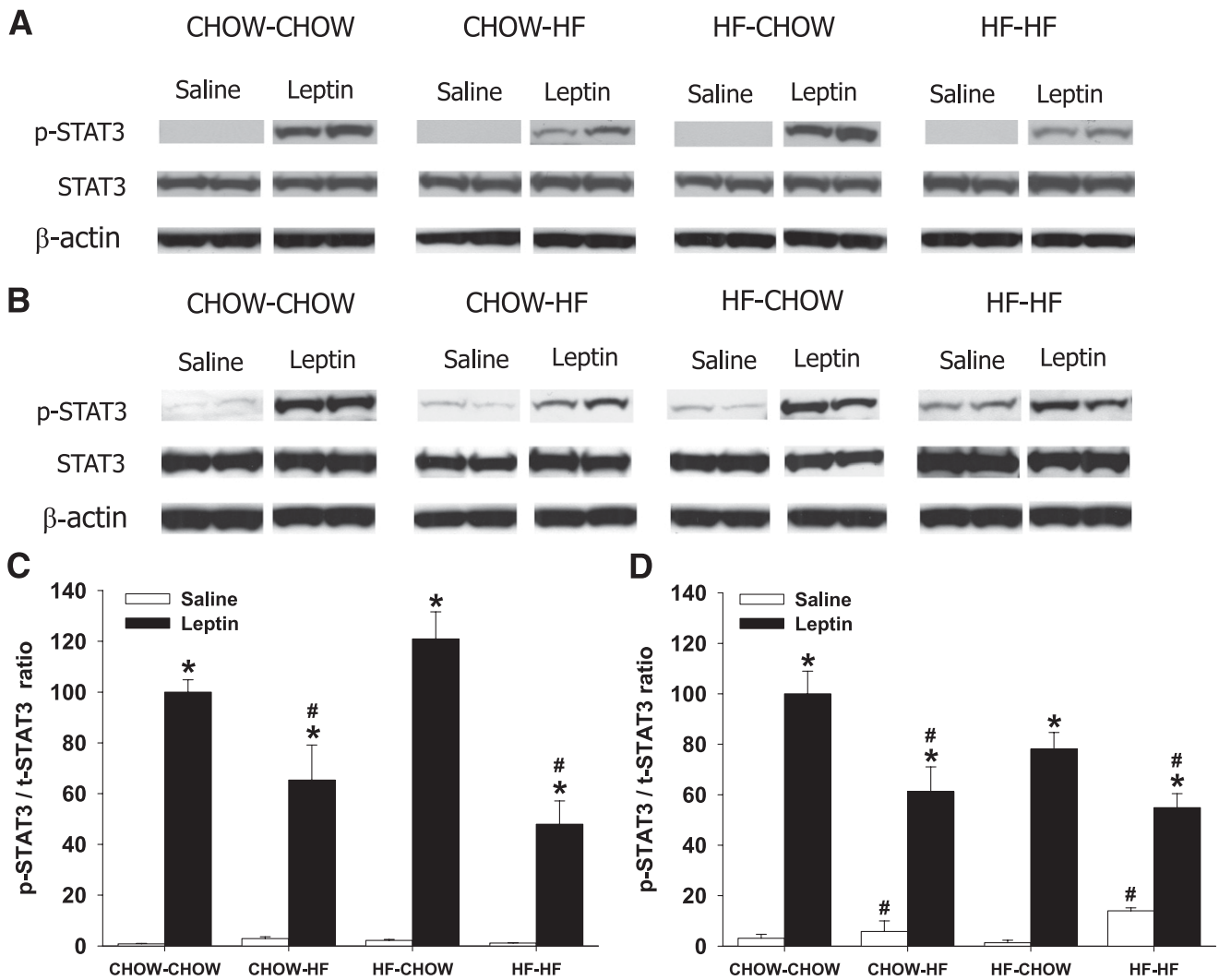


FIG. 4. Western blot of pSTAT3 and total (t)STAT3 in ARC in male (A) and female (B) offspring on PND21. The pSTAT3-to-tSTAT3 ratio (compared with leptin injected pups in CHOW-CHOW group) was calculated in male (C) and female (D) offspring. The pups were injected with saline ($n = 3$) or leptin ($n = 5$) in each group. * $P < 0.05$ vs. saline group. # $P < 0.05$ vs. CHOW-CHOW group.

developed gestational diabetes, although this remains to be tested directly.

Maternal HF diet, prenatally or postnatally, resulted in a significant attenuation of pSTAT3 activation in male offspring, whereas exposure only to the prenatal maternal HF diet resulted in decreased pSTAT3 activation in females. This sex difference was lost by PND21, when male and female pups cross-fostered to HF diet dams postnatally were less sensitive to leptin compared with the CHOW-CHOW group. It is unclear why there were sex differences in the response to leptin at PND10 but not at PND21. We and others have reported sex differences in offspring response to maternal HF diet in adulthood (15). It is intriguing to postulate that those differences in metabolic programming in males and females have origins during the early postnatal period, even before puberty, and may be related to deficits in hypothalamic development secondary to impaired leptin signaling during the neonatal period.

An unexpected outcome was the finding that the prenatal HF diet alone (HF-CHOW) group had no effect, with the exception of PND10 leptin sensitivity, compared with the CHOW-CHOW control group, suggesting three

possibilities as follows: 1) prenatal HF diet exposure alone was not sufficient to impair leptin sensitivity and that the exposure to maternal HF diet during the postnatal period is required for this phenomena, 2) the prenatal HF diet imparts increased susceptibility to metabolic abnormalities that is precipitated when provided with a HF diet after weaning, or 3) cross-fostering the prenatal HF pups to a CHOW-fed dam postnatally corrects for any impairment in leptin sensitivity that may have developed during the prenatal period. More detailed study of the time course of the effects of maternal diet on leptin signaling during the early postnatal period may distinguish among these possibilities.

Two of the downstream targets in the leptin-signaling cascade are AMPK and ACC. AMPK and ACC in the hypothalamus are dephosphorylated in response to leptin activation of STAT3 signaling, and the resulting decreases in pAMPK and pACC in the hypothalamus inhibit food intake in adult rats (34–37). There was no change in total or pAMPK or pACC after the leptin challenge in all four groups compared with baseline levels. The pSTAT3 response to leptin is clear in rodent neonates as early as

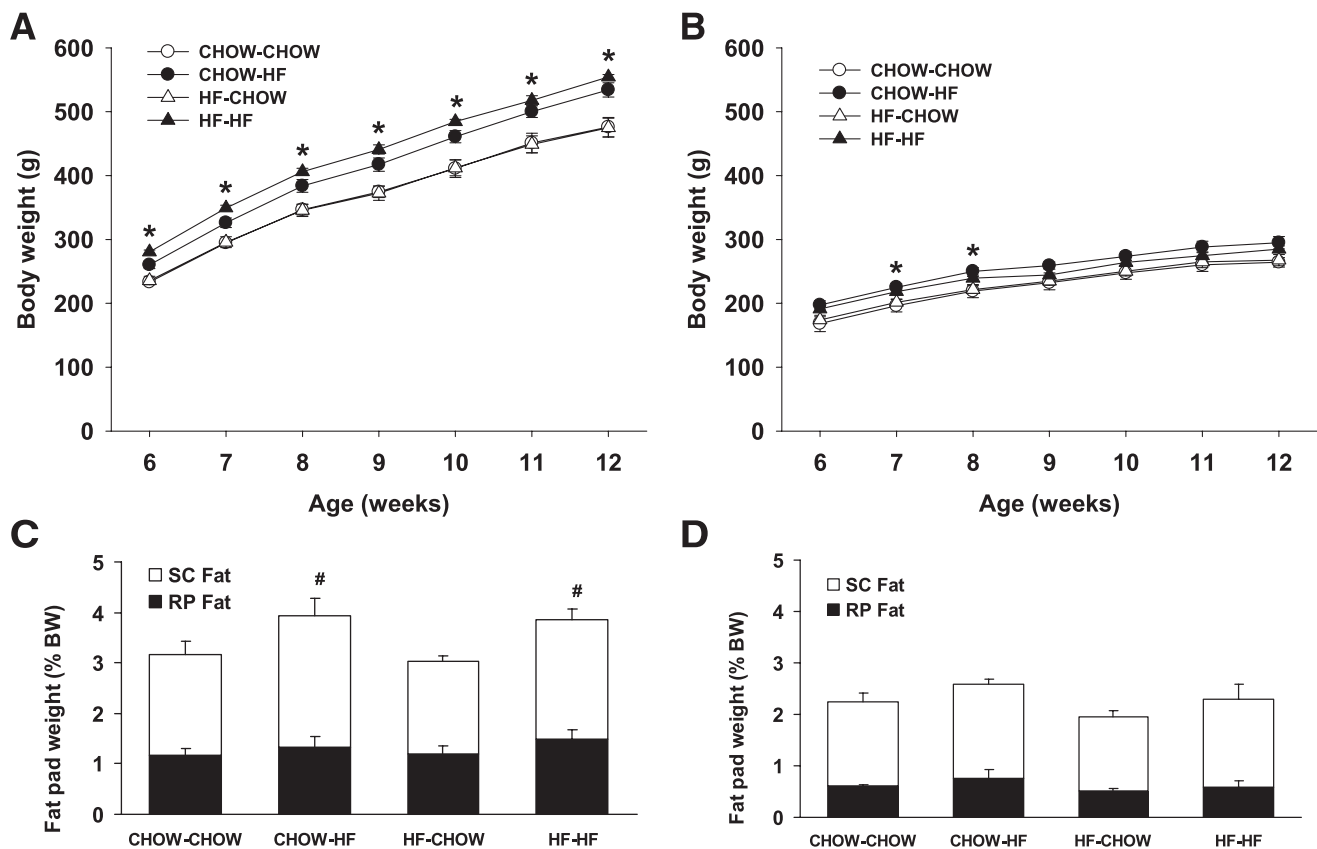


FIG. 5. Body weight in adult male (A) and female (B) offspring at age 12 weeks. Fat for males (C) and females (D) is expressed as the weight of the dorsosubcutaneous and inguinal (SC) and retroperitoneal (RP) fat pad as a percentage of body weight (BW). $n = 8$ per group. *Main effect of postnatal HF diet, $P < 0.05$ vs. postnatal CHOW diet. #Main effect of postnatal HF diet on subcutaneous fat, $P < 0.05$ vs. postnatal CHOW diet.

PND1 (38). Behaviorally, mouse pups do not respond to peripheral leptin on PND17 but do display a decrease in food intake by PND28 (39). Thus, although we observe a significant activation of the STAT3 pathway, it appears that the downstream mediators, such as AMPK and ACC, may not normally be functional until after weaning. This signaling pathway may mature later in development to allow neonates to maximize their food intake during a period of rapid growth (40). This notion may be further supported by and is consistent with leptin's primary role as a critical trophic factor for neurodevelopment rather than an adiposity signal that controls food intake during the early postnatal period.

The precise timing of when pathways that control food intake become functional and influence behavior remains to be determined. It is clear, however, that pups suckled by dams fed a HF diet have a deficit in their pSTAT3 response to peripheral leptin, at least up to PND21, and this may be the reason those offspring are hyperphagic and remain heavier through adulthood compared with CHOW offspring. In addition, we administered leptin peripherally, and it is possible that there is a deficit in the transport of leptin across the blood-brain barrier (BBB) as a result of obesity or maternal HF diet, pre- or postnatally. Diet-induced obese rats develop deficits in leptin transport across the BBB, although whether this is due to obesity, age, or a combination of both is unknown (41).

The field of early-life metabolic programming has made significant progress in establishing the offspring phenotypes resulting from changes in perinatal diet. However, the question of what mechanisms are responsible for these

outcomes remains. There were no differences in leptin content of maternal milk on PND10, but there was greater leptin and fat content of milk of dams fed a HF diet on PND21. Leptin can be transmitted to offspring via maternal milk; however, the obese and leptin-resistant phenotype was evident by PND10, indicating that milk leptin may have an influence during the later postnatal period but is not responsible for the early metabolic phenotype. Alternative possibilities include other hormones that were not measured, such as ghrelin, which has recently been implicated as a trophic factor in hypothalamic development (42) and fatty acid composition of maternal milk (26). In addition, we previously reported that neonatal offspring of HF-fed dams consume more milk in an independent ingestion test (25). Greater caloric consumption could also have a significant influence on pups' development of obesity, as demonstrated by studies using small litters to increase milk availability and consumption in rat and mouse pups (43,44). These possibilities all represent future directions for our studies.

Postnatal HF pups had greater circulating leptin, independent of their prenatal dams' diet, as early as PND10 in this study and may contribute to alterations in hypothalamic development and leptin resistance. Others have shown that elevating neonatal leptin levels with exogenous leptin administration to offspring of ad libitum, chow-fed dams results in an increase in the risk of obesity compared with saline treatment (45–47). In the converse situation, Vickers et al. (48,49) showed that neonatal leptin treatment to restore plasma leptin levels to normal reversed developmental programming in offspring of undernourished dams. Thus,

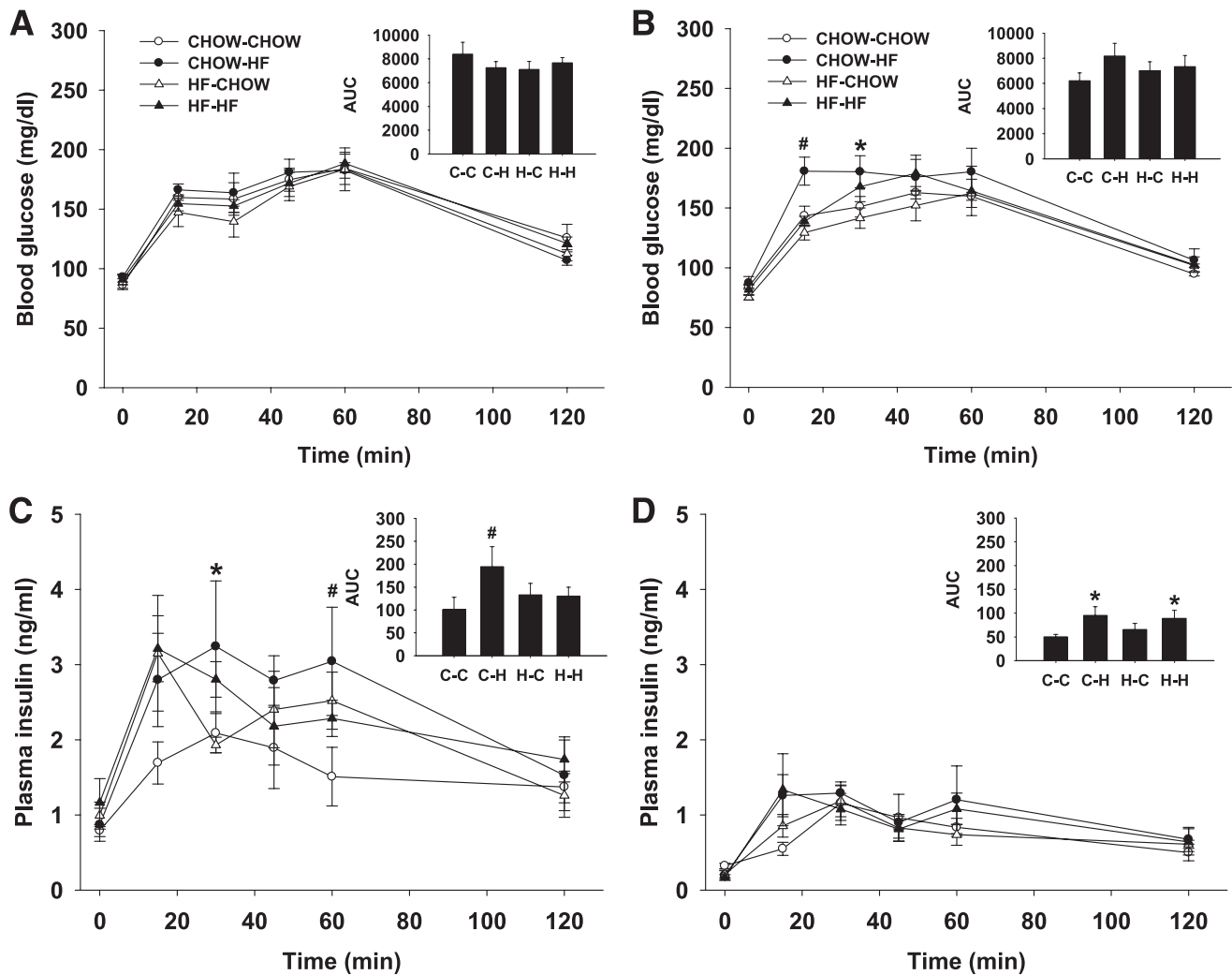


FIG. 6. Glucose tolerance test in males (A and C) and females (B and D) at age 10 weeks. Blood glucose (A and B) and plasma insulin (C and D) were determined for 2 h after oral administration of glucose. The integrated AUC (inset) was determined for glucose and insulin using the trapezoidal method. *n* = 5 per group. *Main effect of postnatal HF diet, *P* < 0.05 vs. postnatal CHOW diet; #*P* < 0.05 vs. CHOW-CHOW group. C-C, CHOW-CHOW; C-H, CHOW-HF; H-C, HF-CHOW; H-H, HF-HF.

manipulation of neonatal leptin levels has significant effects on offspring metabolic phenotype.

This then calls into question whether hypothalamic development proceeds normally in offspring from dams fed a HF diet, prenatally, postnatally, or both. In rodents, hypothalamic neuronal proliferation occurs primarily during midgestation, but the development of neural projections from these neurons to their downstream target sites is initiated during the early postnatal period (23,50). Leptin has been identified as a critical trophic factor that influences the development of the hypothalamic projections, which continues during the early postnatal period in rats (22). Alterations in the pattern of leptin secretion (premature peak, excess, or deficiency) during neonatal life have significant adverse effects on hypothalamic development and metabolic phenotype (21,45). We previously found that pups born to HF-fed dams had higher plasma leptin levels than those from CHOW-fed dams beginning during the first postnatal week, and this persisted throughout the suckling period (24), suggesting that hypothalamic development may be altered in offspring suckled by HF-fed dams.

In summary, our studies demonstrate that a perinatal HF diet influences offspring metabolic phenotype in a time- and

sex-dependent manner. The next step is to elucidate the mechanisms responsible for adverse consequences of HF-diet exposure early in life. Additional important studies will be those directed at determining the mechanisms involved in correction of HF diet-related deficits by manipulation of postnatal diet (i.e., CHOW diet) or behavior (e.g., exercise) and in further refining the critical windows for development of systems regulating energy homeostasis and associated metabolic processes.

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manuscript. R.H.P. and C.E.T. contributed significantly to experiments. J.Y. and T.H.M. contributed to experimental design and writing of the manuscript. K.L.K.T. contributed to experimental design, experiments, data analysis and interpretation, and writing of the manuscript. K.L.K.T. is the guarantor of this work and, as such, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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