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Maternal and Postnatal Overnutrition Differentially Impact Appetite Regulators and Fuel Metabolism

Hui Chen, David Simar, Karen Lambert, Jacques Mercier, and Margaret J. Morris

Departments of Pharmacology (H.C., M.J.M.) and Health and Exercise Science (D.S.), School of Medical Sciences, University of New South Wales, New South Wales 2052, Australia; and Institut National de la Santé et de la Recherche Médicale ERI25 (K.L., J.M.), Muscle et Pathologies, Service de Physiologie Clinique, Centre Hospitalier Universitaire de Montpellier, 34295 Montpellier Cedex 5, France

Maternal obesity is increasing, and it is known that the intrauterine experience programs fetal and newborn metabolism. However, the relative contributions of pre- or postnatal factors are unknown. We hypothesized that maternal overnutrition caused by long-term maternal obesity would exert a stronger detrimental impact than postnatal overnutrition on offspring metabolic homeostasis, with additional postnatal overnutrition exaggerating these alterations. Female Sprague Dawley rats were exposed to chow or high-fat cafeteria diet for 5 wk before mating and throughout gestation and lactation. On postnatal d 1, litters were adjusted to three per litter to induce postnatal overnutrition (*vs.* 12 in control). Hypothalamic appetite regulators neuropeptide Y and proopiomelanocortin, glucose transporter 4, and lipid metabolic markers were measured. At postnatal d 20, male pups born of obese dams, or those overnourished postnatally, were 42% heavier than controls; combining both interventions led to 80% greater body weight. Maternal obesity increased pup adiposity and led to glucose intolerance in offspring; these were exaggerated by additional postnatal overnutrition during lactation. Maternal obesity was also linked to hyperlipidemia in offspring and reduced hypothalamic neuropeptide Y and increased proopiomelanocortin mRNA expression. Postnatal overnutrition of offspring from obese dams amplified these hypothalamic changes. Both maternal and postnatal overnutrition reduced muscle glucose transporter 4. Adipose carnitine palmitoyl-transferase-1 and adipose triglyceride lipase mRNA was up-regulated only by postnatal overnutrition. Maternal overnutrition appears to alter central appetite circuits and promotes early-onset obesity; postnatal overnutrition interacted to cause peripheral lipid and glucose metabolic disorders, supporting the critical message to reduce early-life adverse nutritional impact. (*Endocrinology* 149: 5348–5356, 2008)

THE CENTRAL NEURAL pathways involved in appetite regulation and energy metabolism are well conserved across species. Appetite is regulated by a complex and redundant but highly reliable network. The hypothalamus is considered to be the main integrator and processor of peripheral metabolic information. Two groups of hypothalamic arcuate neurons, one expressing the potent appetite stimulator, neuropeptide Y (NPY), and the appetite suppressor proopiomelanocortin (POMC, precursor of α-MSH), interact with each other to match caloric intake to energy expenditure. Physiologically, NPY concentrations are elevated before a meal and decreased significantly after the initiation of eating, whereas POMC-derived α-MSH counteracts NPY to inhibit feeding (1). Hypothalamic mammalian target of rapamycin (mTOR) has been recently identified to be involved in brain fuel sensing (glucose), whereas insulin-sensitive glucose transporter (GLUT) 4 is important in cellular glucose uptake (2). mTOR is localized in NPY- and POMC-expressing neurons, suggesting glucose sensing by the hypothalamus can adjust feeding behavior via changing the expression of these appetite regulators (3). Leptin, an adipose-derived hormone, together with insulin, directly accesses the hypothalamus, to reduce NPY and activate POMC expression to inhibit feeding and increase energy expenditure via the long form of the leptin receptor (Ob-Rb). Central leptin and insulin resistance is commonly observed in dietary obesity (4).

Differentiation of these brain pathways occurs in the last week of gestation in the rat, with development continuing until weaning (5). Exposure to altered levels of nutrients during this critical window could unfavorably program appetite control and fuel sensing, contributing to hyperphagia and obesity later in life (6). Indeed, restricted intrauterine nutrition has been shown to increase the density of NPY-expressing neurons (7, 8), which was thought to be responsible for catch up growth and obesity-prone phenotype. Although the expression of an appetite suppressor, cocaine- and amphetamine-regulated transcript, was also increased, it did not counteract the effect of NPY. This suggests a potent role of NPY in the onset of obesity. Overnutrition between d 1 after birth and weaning by increasing milk availability also led to early onset obesity and changes in hypothalamic NPY

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Abbreviations: ATGL, Adipose triglyceride lipase; BAT, brown adipose tissue; BMI, body mass index; CN, chow-fed mother with normal litter; CPT, carnitine palmitoyl-transferase; CS, chow-fed mother with small litter; GLUT, glucose transporter; HFD, high-fat diet; HN, HFD-fed mother with normal litter; HOMA, homeostasis model assessment; HS, HFD-fed mother with small litter; IPGTT, ip glucose tolerance test; LSD, least significance difference; MC4R, melanocortin-4 receptor; mTOR, mammalian target of rapamycin; N-A, nasoanal; NPY, neuropeptide Y; Ob-Rb, long form of the leptin receptor; POMC, proopiomelanocortin; Rp, retroperitoneal; SOCS, suppressor of cytokine signaling; STAT, signal transduction involves activation of transcription; WAT, white adipose tissue.

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concentration (9–11). The common outcome of these two kinds of early life nutritional interruption was increased adiposity, hyperlipidemia, glucose intolerance, insulin resistance, and reduced energy expenditure.

However, maternal obesity is a more prominent cause of childhood obesity and metabolic disorders linked to impaired glucose and lipid handling in the current environment of global obesity epidemic. Alarmingly, 61.8% of adult women in the United States, 62.1% in the United Kingdom, 40% in Australia, and 38.6% in Canada are overweight or obese (12). Evidence suggests that children's body mass index (BMI) is associated with maternal BMI and their own adulthood BMI (13–15). However, it is unclear how maternal obesity affects the hypothalamic appetite regulation and fuel-sensing circuitry.

Both maternal and early postnatal overnutrition lead to early onset obesity and alter glucose metabolism and insulin sensitivity in rats; however, the respective contribution of these two factors and their combined effects are still unknown. In this study, the impact of overnutrition starting *in utero*, induced by maternal obesity, was contrasted with that commencing from d 1 of life induced by litter size reduction. The combined effect of these two interventions was also examined. Specifically, central appetite regulators, leptin receptor and downstream signals, and peripheral glucose and lipid metabolic profiles were studied. Female breeders were fed either a low-fat chow or an unlimited palatable cafeteria-style high-fat diet (HFD) from 5 wk before conception to induce maternal overnutrition. We hypothesized that maternal overnutrition due to long-term obesity will exert a stronger adverse impact on programing brain appetite regulators (NPY, POMC, and NPY Y1 receptor), fuel sensors (mTOR and GLUT4), onset of obesity, and glucose intolerance and hyperlipidemia in offspring than postnatal overnutrition alone but also that their combination will further exaggerate those alterations.

Materials and Methods

Maternal obesity

Virgin outbred female Sprague Dawley rats (aged 8 wk, n = 20) were obtained from Animal Resource Centre Pty. Ltd. (Perth, Australia). Animals were housed at 20 ± 2 C with two to three in each cage and maintained on a 12-h light, 12-h dark cycle (lights on at 0600 h). Rats were assigned to two groups of equal average body weight. The control group was exposed to standard laboratory chow (11 kJ/g, 14% energy as fat, 21% energy as protein, 65% energy as carbohydrate; Gordon's Specialty Stockfeeds, New South Wales, Australia), whereas the HFD group was presented with a palatable HFD (average 15.33 kJ/g, 34% energy as fat, 19% energy as protein, 47% energy as carbohydrate), consisting of pelleted high-fat chow (made of powdered standard laboratory chow, sweetened condensed milk, skim milk powder, and saturated animal fat), cakes, and biscuits (four different food types per day) of known caloric content. Fresh food was provided at 1700 h daily. Body weight and food intake of mothers was measured once a week. For the measurement of energy intake, preweighed food was put inside a container into a clean cage and the remainder collected 24 h later. An overnight fasting blood sample was collected from the tail vein before mating for the measurement of plasma hormones. Females were exposed to chow or HFD for 5 wk before mating with male rats (aged 10 wk) from the same source. Female rats were monitored throughout the 3-wk gestation period and housed individually before delivery. Mothers continued on the same diet until pups reached 20 d of age. The current study was approved by the Animal Ethics Committee of the University of New South Wales.

Postnatal litter size adjustment

On d 1 after birth, some litters were adjusted to a size of three (mixed gender) animals of similar body weight to induce early postnatal overnutrition by reducing the competition for milk. Whereas the majority of pups stayed with their mother, pups were distributed between the dams in each of the diet groups that littered on the same day to derive litter sizes of three and 12. Thus, across both litter sizes, each mother received pups from another mother. Fostering of pups did not lead to any adverse effects. In the control group, litter size was kept at 12 pups (male to female ratio = 1:1) per litter for normal postnatal nutrition as described previously (9). This yielded four experimental groups: chow-fed mother with normal litter (CN), chow-fed mother with small litter (CS), HFD-fed mother with normal litter (HN), and HFD-fed mother with small litter (HS). Body weight of pups was monitored every 3 d.

Sample collection

At 20 d, male pups and their mothers were deeply anesthetized (ketamine/xylazine 180/32 mg/kg, ip). Pups were taken away from mothers immediately before they were killed. After measurement of nasoanal (N-A) length, blood was collected by cardiac puncture, and blood glucose level was measured by a glucose meter (Accu-Chek; Roche, Nutley, NJ). Plasma was stored at −20 C for hormone measurements. Then pups were killed by decapitation. The brain was placed ventral side up, and coronal cuts were made at the optic chiasm and the rostral border of the hypothalamus. The hypothalamus (minus preoptic area) was then separated by making two incisions at the lateral edge of the hypothalamic sulcus and an incision above the third ventricle. The hypothalamus was snap frozen in liquid nitrogen and stored at −80 C for determination of mRNA expression of genes of interest. Body fat of pups (brown adipose tissue (BAT), epididymal white adipose tissue (WAT), retroperitoneal (Rp) WAT, and mesenteric WAT) was dissected and weighed, as were various organs (heart, liver, kidney, and pancreas) and skeletal muscles (tibialis). RpWAT, BAT, and skeletal muscles were kept to provide further markers of peripheral metabolism. Tibia length was measured as a marker of growth.

Intraperitoneal glucose tolerance test (IPGTT)

At the age of 20 d (normal weaning age), IPGTT was performed in a subgroup of littermates of the pups killed in the current study. Animals were fasted for 5 h by separating them from mothers and weighed. A blood sample was collected from the tail tip to establish baseline glucose level at T_0 by glucose meter (Accu-Chek; Roche). Then the rats were administered 2 g glucose/kg body weight by ip injection. Blood samples were taken at 15, 30, 60, and 90 min to monitor glucose levels.

Plasma triglyceride, leptin, and insulin measurements

Plasma triglyceride concentration was measured using glycerol standard (equivalent to 0–8.46 mM triglyceride; Sigma, St. Louis, MO) and triglyceride reagent (Roche). Briefly, samples and standards were incubated with triglyceride reagent at 37 C for 20 min and read on a microplate reader (680XR; Bio-Rad Laboratories, Hercules, CA) at 490 nm. Plasma leptin and insulin concentrations were measured using commercially available RIA kits (Linco, St. Charles, MO). The detection limits for leptin and insulin were 0.5 and 0.1 ng/ml, respectively. The insulin resistance index of mothers was estimated by homeostasis model assessment (HOMA): fasting plasma insulin (nanograms per milliliter) × fasting plasma glucose (millimoles/liter)/(22.5 × 0.0417): the greater the HOMA value, the greater the level of insulin resistance (16, 17). This was not calculated for pups because they were killed in the nonfasting state.

Quantitative real-time PCR

Total RNA was isolated from the hypothalamus, BAT, RpWAT, and skeletal muscles (30–100 mg) by homogenizing tissue in 1 ml TriZol reagent (Invitrogen Australia Pty. Ltd., Melbourne, Victoria, Australia) according to the manufacturer's instructions. Then RNA was separated by chloroform and precipitated by isopropanol. Total RNA concentration was determined spectrophotometrically at 260 and 280 nm. The purified RNA (1 ng) was used as a template to generate first-strand cDNA synthesis using random primer and Moloney murine leukemia

TABLE 1. Effect of HFD feeding on body parameters in breeders

	Chow fed breeder (n = 11)	HFD fed breeder (n = 9)
BW (g) (before diet)	185.2 ± 4.3	183. 6 ± 5.3
BW (g) (before mating)	231.2 ± 6.7	285.0 ± 11.1[a]
BW (g) (after delivery)	296.2 ± 10.6	358.0 ± 12.3[a]
Energy intake (kJ/24 h) (before mating)	209.8 ± 6.8	494.8 ± 18.6[a]
Plasma triglyceride (mM)	0.33 ± 0.05	1.34 ± 0.36[b]
Fasting blood glucose (mM)	5.65 ± 0.22	6.58 ± 0.52
Fasting plasma insulin (ng/ml)	1.05 ± 0.15	1.91 ± 0.38[b]
HOMA	6.46 ± 1.05	13.71 ± 2.91[a]
Fasting plasma leptin (ng/ml)	4.21 ± 0.60	7.62 ± 1.50[a]

Results are expressed as mean ± SEM. Data were analyzed by Student's unpaired *t* test. BW, Body weight.
[a] $P < 0.01$, significantly different from chow fed breeders.
[b] $P < 0.05$, significantly different from chow fed breeders.

virus reverse transcriptase, ribonuclease H Minus, point mutant kit (Promega, Madison, WI). TaqMan probe/primers (Applied Biosystems, Foster City, CA) that were preoptimized and validated by the manufacturer were used for quantitative real-time PCR (Realplex 2; Eppendorf, AG, Hamburg, Germany). Markers of appetite regulation [NPY, POMC, Y1 receptor, melanocortin-4 receptor (MC4R)], glucose sensor and transporter (mTOR and GLUT4), Ob-Rb and signaling, signal transduction involves activation of transcription (STAT) 3, and suppressor of cytokine signaling (SOCS) 3) were measured in the hypothalamus. Markers of lipid oxidation [carnitine palmitoyl-transferase (CPT)-1, adipose triglyceride lipase (ATGL)], and insulin resistance (TNFα) were measured in the RpWAT. The probes for target genes were labeled with FAM and those for 18s housekeeping genes were labeled with VIC. Gene expression was quantified in a single multiplexing reaction, in which our gene of interest was standardized to 18s rRNA labeled with FAM. An individual sample from the control group was arbitrarily assigned as a calibrator against which all other samples are expressed as fold difference.

Western blotting

Proteins were isolated from muscles as previously described (18). Protein concentration was determined by the bicinchoninic acid assay (Interchim; Pierce, Montlucon, France) using BSA as standard. Proteins (40 μg) and prestained molecular mass markers (magic standard; Invitrogen, Groningen, The Netherlands) were separated with the Novex system (Invitrogen) and transferred to polyvinylidene difluoride membranes. Membranes were incubated overnight at room temperature in Tris buffer containing nonfat dried milk and were then further incubated with the primary antibody (GLUT4; Santa Cruz Biotechnology, Santa Cruz, CA) for 90 min and then with the corresponding horseradish peroxidase-conjugated secondary antibody (BI2407; BioSys, Compiegne, France) for 45 min. Protein expression was detected by enhanced chemiluminescence (Biomax MR films; Kodak, Rochester, NY). Films were developed and fixed using a hyperprocessor, RNP 1700 (Amersham, Les Ulis, France). Protein band density was determined by scanning (AGFA Duo Scan T1200) with Scion Image software (Frederick, MD). Results are expressed in arbitrary units.

Statistical methods

Results are expressed as mean ± SEM. Data on female breeders were analyzed by Student's unpaired *t* test. Body weight of pups over time was analyzed using ANOVA with repeated measures, followed by *post hoc* Fisher's least significance difference (LSD) test. Differences in fat and organ weights, blood and plasma hormone concentrations, mRNA, and protein expression in all tissues were analyzed using two-way ANOVA followed by a *post hoc* LSD test. $P < 0.05$ was considered significant.

Results

Effect of HFD feeding on breeders

To induce long-term dietary obesity in breeders, palatable cafeteria HFD was provided for 5 wk before mating, whereas the control group was fed chow for the same period. The body weights of breeders were well matched at the beginning of the experiment (Table 1). Over the premating period, caloric intake of HFD-fed rats was more than double that of the chow-fed rats. After 5 wk, the HFD-fed group was 23.4% heavier than the chow-fed group ($P < 0.01$), with greater weight gain during pregnancy ($P < 0.01$, Table 1). Plasma triglyceride levels were 4 times higher in the HFD-fed mothers ($P < 0.05$). The adiposity marker, plasma leptin, was also doubled by dietary intervention ($P < 0.05$). Plasma insulin levels and the HOMA insulin resistance index were significantly higher in HFD-fed mothers ($P < 0.05$, 0.01 respectively).

Effect of maternal and postnatal overnutrition on pups

Growth and adiposity. Whereas body weights of all animals were similar at birth (Table 2), both pre- and postnatal over-

TABLE 2. Effects of maternal diet and postnatal litter size adjustment on body weight, length, organ mass, and adiposity at 20 d of age

	CN (n = 12)	CS (n = 13)	HN (n = 12)	HS (n = 12)
BW at 1 d (g)	7.22 ± 0.16	7.10 ± 0.20	7.03 ± 0.09	7.38 ± 0.35
BW at 20 d (g)[a,b]	33.15 ± 0.27	47.42 ± 1.86[c]	47.43 ± 1.44[d]	61.28 ± 2.50[c,d]
N-A length (cm)[a,b]	10.52 ± 0.07	11.91 ± 0.17[c]	11.79 ± 0.11[d]	12.74 ± 0.15[c,d]
Tibia (cm)[a,b]	2.13 ± 0.04	2.25 ± 0.04[e]	2.18 ± 0.04	2.50 ± 0.05[c,f]
Liver (mg)[a,b]	1181.2 ± 25.3	1763.3 ± 90.0[c]	1869.1 ± 74.1[d]	2467.8 ± 96.9[c,f]
Kidney (mg)[a,b]	200.8 ± 3.6	280.3 ± 10.3[c]	249.9 ± 6.6[d]	308.7 ± 9.8[c,f]
Heart (mg)[a,b]	170.0 ± 3.6	245.5 ± 6.6[c]	247.9 ± 9.7[d]	324.6 ± 13.0[c,d]
Pancreas (mg)[a,b]	62.4 ± 4.6	110.28 ± 7.6[c]	141.4 ± 4.9[d]	179.1 ± 12.2[c,d]
BAT (mg)[a,b]	102.7 ± 6.2	160.3 ± 11.4[c]	136.5 ± 7.3[f]	256.2 ± 7.6[c,d]
RpWAT (mg)[a,b]	27.7 ± 2.3	85.3 ± 11.8[e]	140.5 ± 13.9[d]	299.9 ± 28.9[c,d]
Epididymal WAT (mg)[a,b]	37.5 ± 2.6	84.2 ± 6.5[e]	151.4 ± 16.9[d]	247.4 ± 34.4[d,e]
Mesenteric WAT (mg)[a,b]	167.9 ± 6.3	252.9 ± 14.8[c]	361.2 ± 26.9[d]	453.5 ± 32.2[e,f]
Tibialis (mg)[a,b]	39.94 ± 0.94	52.42 ± 3.83[c]	49.49 ± 1.78[f]	71.88 ± 4.15[d,c]

Results are expressed as mean ± SEM. Data were analyzed by two-way ANOVA, followed by *post hoc* LSD tests. BW, Body weight.
[a] Significant overall maternal effect, $P < 0.05$.
[b] Significant overall litter effect, $P < 0.05$.
[c] $P < 0.01$, compared with pups from mothers fed same diet raised in normal-size litter.
[d] $P < 0.01$, compared with pups from chow fed mothers raised in same-size litter.
[e] $P < 0.05$, compared with pups from mothers fed same diet raised in normal-size litter.
[f] $P < 0.05$, compared with pups from chow fed mothers raised in same-size litter.

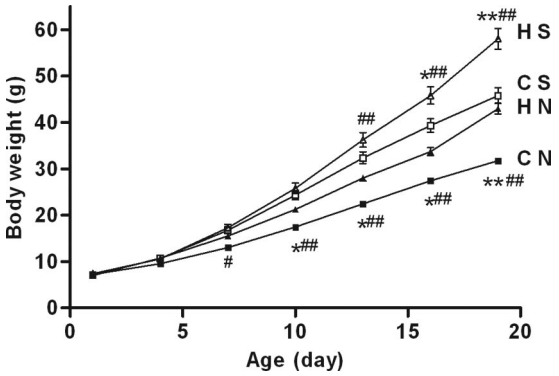

FIG. 1. Body weight changes during the suckling period. Results are expressed as mean ± SEM. Data were analyzed by ANOVA with repeated measures followed by a *post hoc* LSD test. *, $P < 0.05$; **, $P < 0.01$, significant maternal diet effect; #, $P < 0.05$; ##, $P < 0.01$, significant litter effect. CN, *black square* (n = 12); CS, *open square* (n = 13); HN, *black triangle* (n = 12); HS, *open triangle* (n = 12).

nutrition affected weight over time (Fig. 1). Pups raised in small litters had greater weight gain than those from normal-sized litters, independent of maternal diet ($P < 0.05$ between normal and small litters within the same maternal dietary group, Fig. 1). At d 19, the body weights of CS and HN were very similar, with clear separation for HS and the other three groups (Fig. 1). At the end point, both pre- and postnatal overnutrition had caused a similar 43% increase in body weight over the CN group. Pups from obese mothers (HS) were 29% heavier if they were raised in small litters ($P < 0.05$ HS *vs.* HN, Table 2).

In addition to an effect on body weight, pre- and postnatal overnutrition significantly increased N-A and tibia length as well as weight of organs, fat pads, and skeletal muscles ($P < 0.05$ maternal diet and litter size effects, Table 2). When standardized by body weight, liver and pancreas remained heavier in pups from obese mothers with no litter size effect ($P < 0.05$). RpWAT and testicular WAT were still significantly increased by both pre- and postnatal overnutrition, whereas the standardized mesenteric WAT was elevated only by maternal overnutrition ($P < 0.05$). The adipose-derived hormone, leptin, was also increased significantly by pre- and postnatal overnutrition (Table 3), and the increase was higher in pups from obese mothers (HN and HS).

Central appetite regulators and fuel processors. Hypothalamic NPY mRNA expression (Fig. 2A) was reduced, and POMC mRNA expression was increased (Fig. 2B) by both pre- and postnatal overnutrition. Changes in NPY and POMC mRNA expression were greater in the HS group than CS and HN. When the four groups were combined, plasma leptin was negatively correlated with NPY expression and positively correlated with POMC expression (r = −0.40 for NPY, $P = 0.02$; r = 0.68 for POMC, $P < 0.01$; n = 24). Y1 receptor expression was increased by maternal obesity (Fig. 2C), whereas MC4R mRNA was not significantly different between groups (Fig. 2D). Hypothalamic Ob-Rb mRNA tended to be reduced in the small litter groups (CS, HS), without reaching statistical significance (Fig. 3A), whereas its downstream signal, STAT3 was significantly increased in rats raised in small litters (CS, HS, Fig. 3B) and was positively correlated with plasma leptin concentration (r = 0.32, $P = 0.04$, n = 24). SOCS3 expression was not different between groups (data not shown). Hypothalamic mTOR and GLUT4 expression (Fig. 4, A and B) was significantly lower in pups from obese mothers (HN and HS). When the four groups were combined, mTOR expression was negatively correlated to plasma leptin levels (r = −0.37, n = 24, $P = 0.02$).

Peripheral glucose metabolism. During IPGTT, baseline (0 min) glucose levels were not significantly different between groups. The HS group had the highest glucose levels at 15 and 30 min (Fig. 5A), and highest area under curve (Fig. 5B); both maternal obesity and litter size reduction led to increased glucose peak at 15 min after injection ($P < 0.05$, maternal and litter effects, Fig. 5A). Plasma insulin levels were doubled by prenatal overnutrition at the time the animals were killed (HN and HS groups, Table 3).

Both pre- and postnatal overnutrition significantly reduced GLUT4 protein levels in skeletal muscle ($P < 0.05$, CS, HN, and HS *vs.* CN, Fig. 5C). GLUT4 protein expression was also negatively correlated with plasma triglycerides (r = −0.4, $P < 0.05$, n = 24). TNFα mRNA in WAT was markedly increased only by postnatal overnutrition (CS, HS, Fig. 6C).

Lipid regulators. Maternal overnutrition significantly increased plasma triglyceride levels in offspring ($P < 0.05$, Table 3). In WAT, mRNA expression of lipid metabolic marker CPT-1 and lipase ATGL was significantly increased by postnatal overnutrition (CS, HS, Fig. 6) to similar levels, regardless of maternal diet.

Discussion

Both intrauterine and early postnatal environments have a significant influence on hypothalamic appetite regulators, glucose homeostasis, and adiposity in later life. This study investigated the impact of established maternal obesity on hypothalamic appetite regulators and fuel sensors, adiposity, and glucose and lipid metabolism in offspring at normal

TABLE 3. Plasma insulin, leptin, and triglyceride concentrations at 20 d of age

	CN (n = 12)	CS (n = 13)	HN (n = 12)	HS (n = 12)
Insulin (ng/ml)[a]	0.22 ± 0.04	0.27 ± 0.04	0.51 ± 0.06[b]	0.51 ± 0.08[b]
Leptin (ng/ml)[a,c]	0.99 ± 0.06	2.54 ± 0.26[d]	5.95 ± 0.64[e]	8.71 ± 0.54[e]
Triglyceride (mM)[a]	0.74 ± 0.07	0.96 ± 0.11	1.54 ± 0.18[e]	1.47 ± 0.16

Results are expressed as mean ± SEM. Data were analyzed by two-way ANOVA, followed by *post hoc* LSD tests.
[a] Significant overall maternal effect, $P < 0.05$.
[b] $P < 0.05$, compared with pups from chow-fed mothers raised in same-size litter.
[c] Significant overall litter effect, $P < 0.05$.
[d] $P < 0.01$, compared with pups from mothers fed same diet raised in normal size litter.
[e] $P < 0.01$, compared with pups from chow-fed mothers raised in same-size litter.

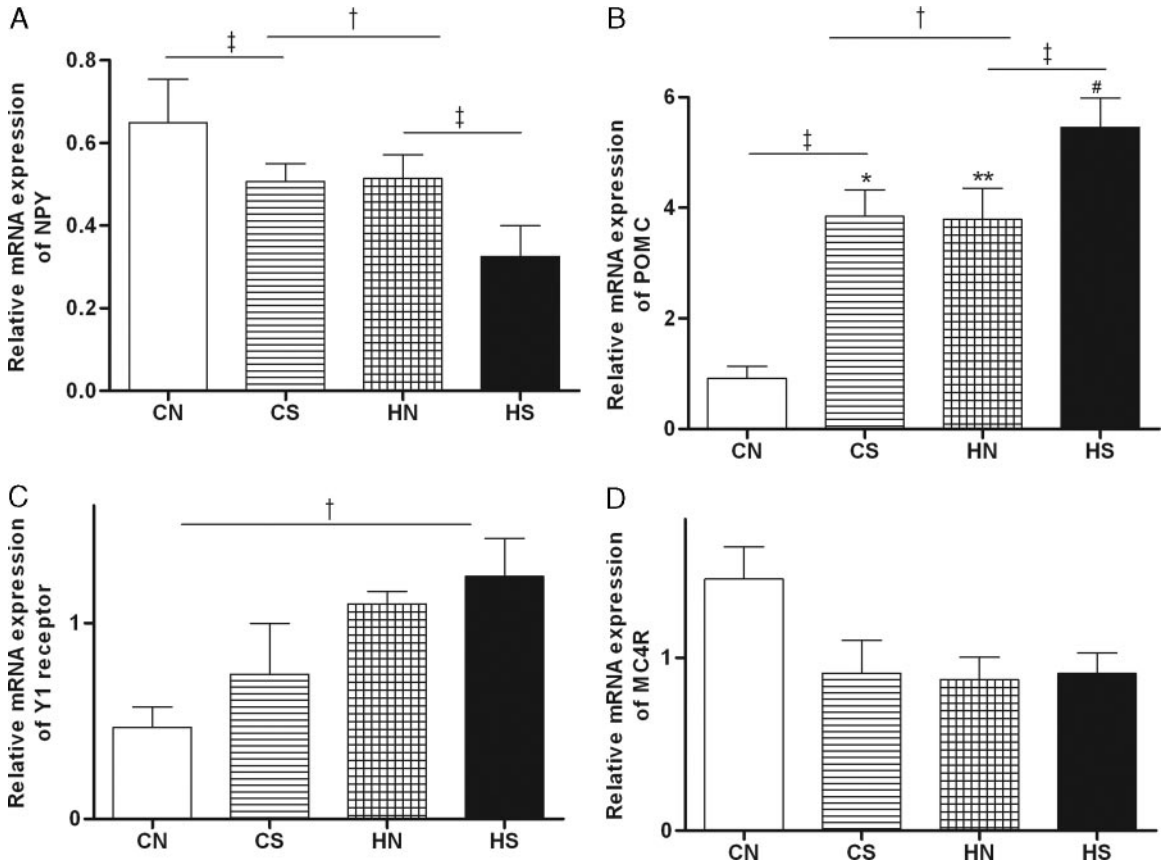

FIG. 2. mRNA expression of NPY (A), POMC (B), Y1 receptor (C), and MC4R (D) in the hypothalamus at 20 d (n = 8–9/group). Results are expressed as mean ± SEM. Data were analyzed by two-way ANOVA followed by a *post hoc* LSD tests. †, Significant overall maternal diet effect, $P < 0.05$; ‡, significant overall litter effect, $P < 0.05$; *, $P < 0.05$; **, $P < 0.01$, significantly different from CN; #, $P < 0.05$, significantly different from CS and HN.

weaning age in rats. Moreover the impacts of maternal obesity and overnutrition during postnatal suckling periods were compared. Maternal obesity caused overnutrition *in utero* and appeared to exert a stronger adverse influence on offspring than postnatal overnutrition alone in programming brain appetite and fuel-sensing regulators, which led to early-onset obesity, increased adiposity, hyperlipidemia, and glucose intolerance. Importantly, the effects of maternal obesity were amplified by additional postnatal overnutrition.

Maternal intake of a HFD and the consequent obesity during pregnancy and lactation promoted adiposity in offspring. The richer milk composition of obese mothers most likely to be delivered to pups enhanced nutrition as previously reported (19). Furthermore, high circulating maternal leptin can also be transported across the blood-placenta barrier into the fetal circulation to affect endocrine systems because leptin is important for fetal development (20). As a result, at weaning, offspring from obese rats had larger body

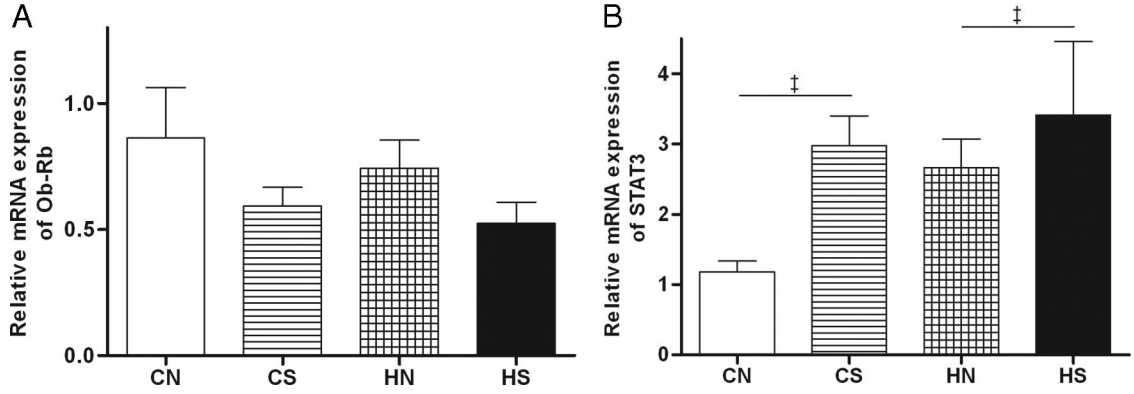

FIG. 3. mRNA expression of Ob-Rb (A) and STAT3 (B) in the hypothalamus at 20 d (n = 8–9/group). Results are expressed as mean ± SEM. Data were analyzed by two-way ANOVA. ‡, Significant overall litter effect, $P < 0.05$.

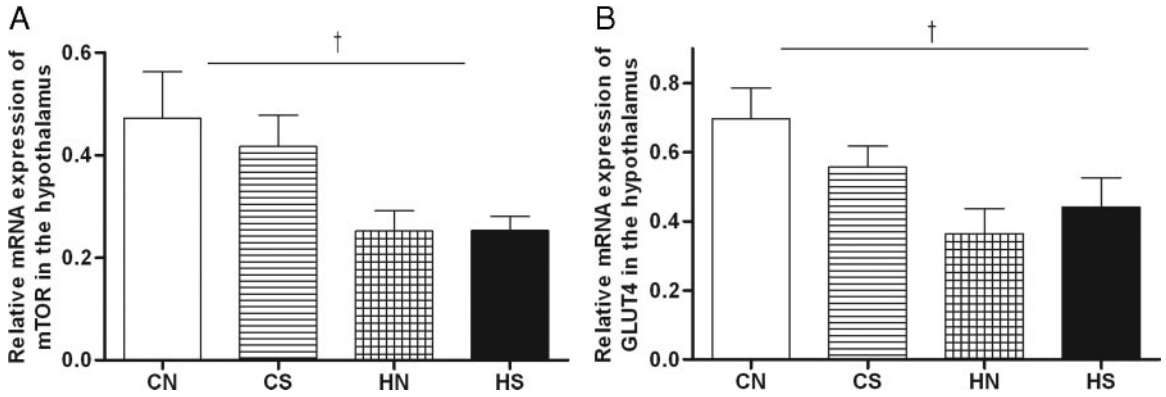

FIG. 4. mRNA expression of mTOR (A) and GLUT4 (B) in the hypothalamus at 20 d (n = 9/group). Results are expressed as mean ± SEM. Data were analyzed by two-way ANOVA. †, Significant overall maternal diet effect, $P < 0.05$.

weight and body length than those from lean mothers, and additional postnatal overnutrition imposed by reduced competition for milk exaggerated this effect. Although body weight of HN and CS are similar, the adiposity in HN pups was much more severe than CS along with significantly higher plasma insulin and triglyceride levels, highlighting maternal overnutrition as a more potent factor to increase metabolic disorders in offspring. Gestational diabetes is common in obese pregnant women. Although insulin and HOMA were higher in the HFD-fed mothers, fasting glucose levels were not significantly increased. Thus, in the current cohort, maternal insulin resistance is a potential factor to influence fetal development in addition to high triglyceride and leptin levels; however, it may not reach the level of gestational diabetes.

Plasma leptin concentration was increased by both prenatal and postnatal overnutrition, consistent with the increased adiposity in response to both interventions (21). The key signaling form, the long form of the leptin receptor, Ob-Rb, is found in the arcuate nucleus of POMC- and NPY-containing cells. Leptin binding to Ob-Rb results in the phosphorylation of STAT3, which in turn induces expression of SOCS3 as a negative feedback to inhibit STAT3 signaling (22). In the current study, hypothalamic STAT3 appeared to be

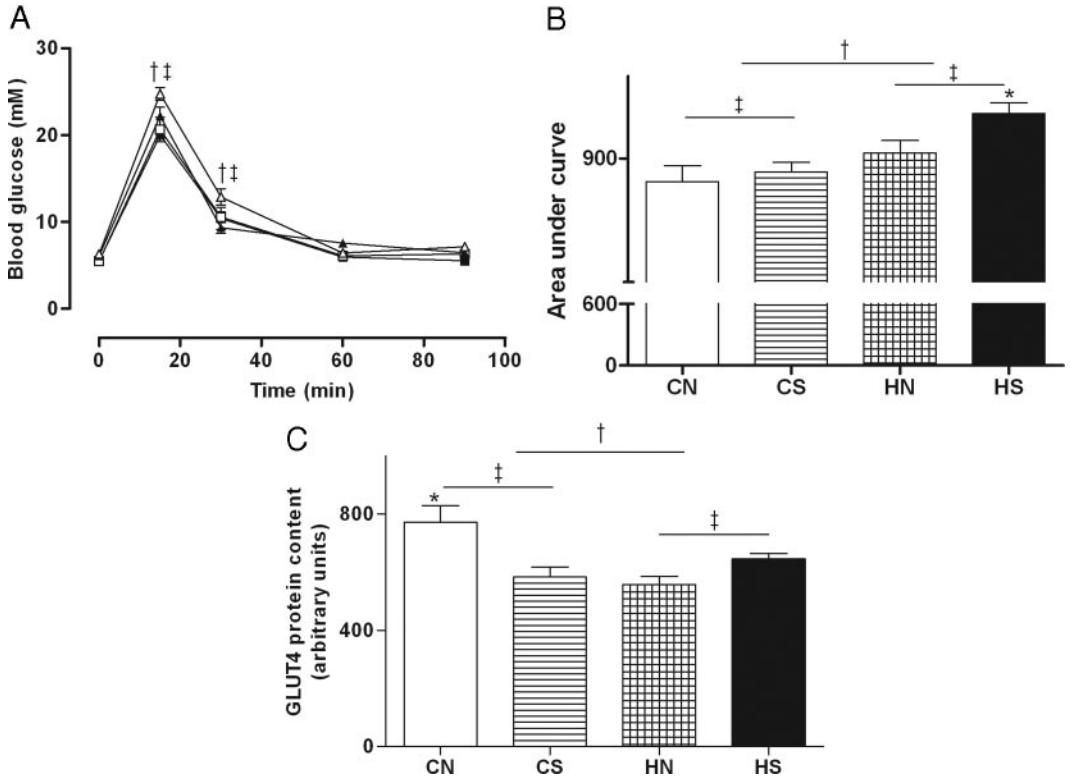

FIG. 5. A, IPGTT at 20 d (glucose 2 g/kg, n = 10–12/group). Results are expressed as mean ± SEM. Data were analyzed by ANOVA with repeated measures followed by a *post hoc* LSD test. Area under the curve is shown in B. C, GLUT4 protein expression in skeletal muscle at 20 d (n = 8–9/group). Results are expressed as mean ± SEM. Data in B and C were analyzed by two-way ANOVA followed by a *post hoc* LSD test. †, Significant overall maternal diet effects, $P < 0.05$; ‡, significant overall litter effects, $P < 0.05$; *, significantly different from the other three groups, $P < 0.05$. CN, *solid square*; CS, *open square*; HN, *solid triangle*; HS, *open triangle*.

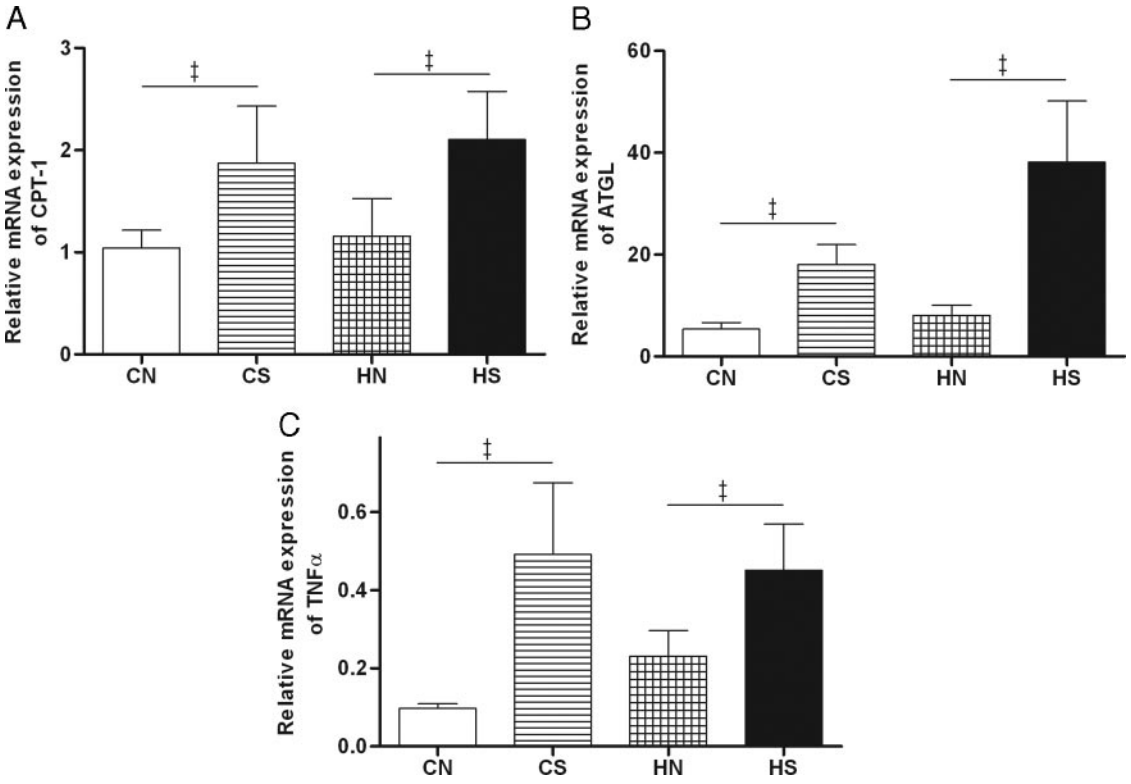

FIG. 6. mRNA expression of CPT-1 (A), ATGL (B), and TNFα (C) in RpWAT (n = 8–9/group) at 20 d. Results are expressed as mean ± SEM. Data were analyzed by two-way ANOVA. ‡, Significant overall litter effect, $P < 0.05$.

more affected by postnatal overnutrition. The increased STAT3 expression could be due to increased plasma leptin levels, as supported by the positive correlation between these two. In the current study, production of hypothalamic POMC and NPY seemed to be regulated by circulating leptin because down-regulated hypothalamic NPY and up-regulated POMC expression in overnourished pups were correlated with circulating leptin levels. This adaptive response in pups could mirror the changes in adult-onset obesity models, in which reduced NPY and increased POMC or α-MSH was observed in the face of increased circulating leptin levels (23–25). However, we cannot conclude here whether leptin resistance exists in these overnourished animals early in life. Further work is required to examine the feeding response to exogenous leptin injection.

The NPY Y1 receptor is one of the receptors mediating the orexigenic effect of NPY and is expressed in hypothalamic arcuate nucleus, paraventricular nuclei, lateral hypothalamic nucleus, and dorsomedial and ventromedial hypothalamic nuclei (26). Blockade of the Y1 receptor with antagonists can reduce NPY-induced and fasting-induced feeding (27–29). However, most studies reported unchanged regional Y1 receptor mRNA expression in response to HFD or programming (19, 30). In the current study, reciprocal changes in Y1 receptor and NPY mRNA expression were observed. These complementary responses could diminish the effects of adaptive changes of NPY to counteract overnutrition. Furthermore, as evidenced after restricted intrauterine nutrition (7), increased brain NPY expression could potently contribute to catch-up growth and obesity. In the current study, hypothalamic NPY and POMC mRNA was measured in nonfasted animals, and NPY levels can be increased in the hungry state (1). If up-regulated Y1 receptors are available, the animals would be expected to have greater appetite and overeat. Indeed in another cohort, animals experiencing pre- and/or postnatal overnutrition displayed 15–40% higher caloric intakes (CS, HN, and HS) soon after they were weaned onto a HFD, compared with animals with normal pre- and/or postnatal nutrition consuming the same diet (Chen, H., and M. J. Morris, unpublished data). This suggests that overnutrition during the preweaning period is a significant risk factor for developing hyperphagia and subsequent obesity in later life.

Interestingly, we also observed a down-regulation of hypothalamic GLUT4 and mTOR expression, two important factors involved in brain glucose sensing, in response to maternal obesity. Leptin activates mTOR either directly or via AMP-activated protein kinase (31, 32). However, in this study, increased leptin levels in response to prenatal overnutrition was associated with low hypothalamic mTOR expression, suggesting either reduced fuel sensing ability or impaired response to leptin, the latter possibly representing the development of leptin resistance. Another contributor to the reduced expression of mTOR may be the reduced brain GLUT4 mRNA expression. Insulin sensitive GLUT4 is critical for facilitating cellular glucose uptake. GLUT4 is expressed in the rat ventromedial hypothalamus (33), which is important in glucose sensing and in the counter regulatory responses to hypoglycemia (34). The reduced GLUT4 could be due to systemic insulin resistance, which may lead to altered

glucose uptake limiting mTOR activation. Alternatively, sustained overnutrition could have desensitized central nervous system fuel sensing ability. Inhibition of mTOR has been shown to diminish the anorexigenic effect of leptin (2) and promote central leptin resistance in the long term, an adaptation commonly observed in obese individuals (35). Failure to sense increased nutrient storage can then lead to uncontrolled hyperphagia. In this study, this deficiency seems to be exclusively caused by maternal obesity.

All three groups of rats exposed to overnutrition in early life showed a delay in glucose uptake during the first 30 min of IPGTT. This can be directly linked to reduced muscle GLUT4 protein expression. Skeletal muscle plays a key role in systemic insulin sensitivity and accounts for about 80% of whole body glucose uptake (36). GLUT4 translocation is the rate-limiting step in insulin-stimulated glucose uptake. Increased circulating triglyceride levels in response to either pre- or postnatal overnutrition could lead to greater intramuscular lipid uptake and accumulation, thus resulting in down-regulation of GLUT4 (37, 38). This could directly impair glucose metabolism and lead to insulin resistance. TNFα also causes insulin resistance in obese subjects by interfering with insulin receptor signaling and GLUT4 translocation on the cell membrane (39). Thus, increased WAT TNFα expression, mainly in response to postnatal overnutrition, may also contribute to the down-regulation of GLUT4, suggesting early postnatal overnutrition is a potent predisposing factor for insulin resistance, suggesting pre- and postnatal overnutrition act through different pathways to affect GLUT4.

Circulating lipid levels are regulated by both central and peripheral mechanisms. Recently it has been suggested that glucose sensing and uptake by the brain plays a critical role in regulating hepatic secretion and release of lipoproteins into the blood (40). This is of high importance because free fatty acids are major contributors to peripheral insulin resistance. Thus, reduced brain glucose sensing mediators (mTOR) may contribute to the high triglyceride levels in response to prenatal overnutrition in this study. Furthermore, our data also suggest that fatty acid metabolism is more readily regulated by postnatal overnutrition, as evidenced by the increase in WAT CPT-1, the rate-limiting factor for fatty acid oxidation and ketogenesis (41), and WAT ATGL, necessary for triglyceride hydrolysis (42) and a contributor to basal lipolysis (43). This up-regulation of lipid metabolic markers could be an adaptation to counteract the rapid accumulation of adiposity in pups overfed early in life. However, our data also suggest that rats subjected to maternal obesity (HN group) lost such adaptation, although they had a higher level of fat deposition. Maternal overnutrition may up-regulate the threshold of lipid homeostasis in offspring, which increases their risks of excessive fat deposition and hyperlipidemia.

Conclusion

Both maternal obesity and postnatal overnutrition are important risk factors for the early onset of obesity in offspring. In the current study, maternal obesity exerted a stronger detrimental impact than overnutrition in the early postnatal period. However, pre- and postnatal nutritional excess interacted with each other to exaggerate the adverse programming of central appetite regulators as well as glucose and lipid metabolism in offspring. Changes in brain appetite regulatory systems induced by overnutrition may lead to hyperphagia as animals develop. Despite the adaptations in lipid metabolism in response to early-life overnutrition, excessive fat accumulation and hyperlipidemia occurred. The differential effects of pre- or postnatal overfeeding provide important new information regarding the metabolic risk during development. Further work is required to investigate the metabolic consequences into adulthood and whether postweaning nutritional manipulation can override the impact of early life overnutrition.

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Address all correspondence and requests for reprints to: Professor Margaret J. Morris, Department of Pharmacology, School of Medical Sciences, University of New South Wales, Sydney, New South Wales 2052, Australia. E-mail: m.morris@unsw.edu.au.

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