Maternal high fat feeding and gestational dietary restriction: Effects on offspring body weight, food intake and hypothalamic gene expression over three generations in mice

Silvia Q. Giraudo [a,*], Mary Anne Della-Fera [b], Lindsey Proctor [a], Kathie Wickwire [a], Suresh Ambati [b], Clifton A. Baile [b]

[a] Department of Foods & Nutrition, University of Georgia, Athens, GA 30602-3622, United States
[b] Department of Animal & Dairy Science, University of Georgia, Athens, GA 30602, United States

ARTICLE INFO

ABSTRACT

Excessive gestational weight gain and maternal obesity have both been associated with increased incidence of obesity and metabolic disorder in offspring in both humans and animal models. The objectives of this study were to determine (1) whether mild gestational food restriction during the third trimester (GFR) would alter food intake and growth parameters of offspring, (2) whether effects of GFR depended on diet (high fat [HF] vs chow), (3) whether effects of excessive gestational weight gain (WG) would become magnified across generations, and (4) whether diet and GFR would alter hypothalamic gene expression in adult offspring. Three generations of female C57BL/6 mice were fed chow or HF diet, mated at 11 weeks of age and assigned to ad libitum feeding or 25% GFR. Offspring were fed the same diet as their mothers. Results showed (1) maternal gestational WG was positively correlated with offspring WG. (2) HF offspring weighed less ($p<0.01$) at weaning (WWT) but gained more during the 8 weeks after weaning than chow-fed offspring ($p<0.05$), resulting in higher final body weights (BW) ($p<0.01$). (3) HF males from GFR mothers had higher WWT ($p<0.05$), but subsequent WG and final BW were less ($p<0.05$) compared to males from ad lib mothers. (4) In the HF group, GFR also resulted in decreased FI ($p<0.05$) and FE ($p<0.07$) in offspring, compared to offspring from ad lib mothers. (5) In generation 3, hypothalamic expression of tyrosine hydroxylase was lower in HF males from GFR mothers compared to HF males from ad lib mothers ($p<0.05$). In conclusion, gender and maternal GFR had independent effects on growth and FI, and hypothalamic gene expression was dependent on both gender and maternal GFR in HF offspring. Even mild food restriction of obese mothers during pregnancy may have beneficial effects in reducing the risk or degree of obesity in offspring.

© 2010 Elsevier Inc. All rights reserved.

1. Introduction

Nearly two decades ago, Barker and colleagues presented the hypothesis that abdominal adiposity, coronary heart disease and other abnormalities associated with the metabolic syndrome may occur as a result of undernutrition during middle to late gestation (Barker, 1998; Barker and Fall, 1993; Hales et al., 1991; Law et al., 1992). Epigenetic effects are increasingly being recognized as playing important roles in influencing growth and body composition of offspring (Gillman et al., 2007; Levin, 2008; Symonds et al., 2009).

Both maternal food restriction and overnutrition during pregnancy have been shown to predispose offspring to develop obesity and metabolic syndrome, in some cases regardless of whether the offspring themselves are exposed to an obesogenic diet (Bayol et al., 2008; Bellinger et al., 2006; Chen et al., 2008; Ravelli et al., 1999). In addition, some studies have shown that the propensity of offspring to develop increased adiposity and insulin resistance due to both undernutrition and overnutrition during gestation can be transmitted beyond the first generation (Jimenez-Chillaron et al., 2009; Painter et al., 2008; Waterland et al., 2008).

Although maternal undernutrition is still an issue in poor and developing countries, in Western societies, the more pressing problem is the increasing prevalence of overweight and obesity in women of childbearing age. A recent study found that over 60% of women of childbearing age were overweight or obese (Sarwer et al., 2006), and non-Hispanic black and Hispanic white women were 2.25 times more likely to be overweight or obese compared to non-

Hispanic white women (Vahratian, 2009). Even in non-obese women, excessive gestational weight gain increases the risk of obesity in offspring (Olson et al., 2008; Stuebe et al., 2009), and over 40% of women were found to exceed the Institute of Medicine Guidelines for optimal weight gain during pregnancy (Sarwer et al., 2006). Because of these alarming statistics, a number of investigators have begun calling for new guidelines for gestational weight gain for all women, and development of specific recommendations for women who are overweight or obese (Davis and Olson, 2009; Olson et al., 2008; Ramachenderan et al., 2008; Sarwer et al., 2006; Stuebe et al., 2009).

Numerous studies have been carried out in animal models of gestational obesity to define the behavioral, biochemical and metabolic consequences of maternal obesity in offspring; however, very few have been designed to ameliorate these effects through specific maternal treatments. In one study dietary genistein provided to pregnant obese heterozygous viable yellow agouti (A(vy/a) mice reduced the susceptibility to adult onset obesity in the offspring (Dolinoy et al., 2006). Another study using the same mouse model showed that dietary supplementation of pregnant obese dams with extra folic acid, vitamin B12, betaine and choline shifted the population distribution towards lower body weights over several generations (Waterland et al., 2008).

The main objective of the current study was to determine whether mild dietary restriction of high fat fed pregnant C57BL/6 mice would alter the development of obesity in offspring fed a high fat diet. A second objective was to determine whether there was an interaction between diet and gestational treatment in development of obesity in the offspring. Three generations of female mice were fed a high fat diet or chow, and half of each group was subjected to a 25% reduction of food during the third trimester of gestation. Male and female offspring were weaned at 3 weeks of age, and fed the same diet as their mothers. Food intake and body weight were measured for the subsequent 8 weeks. We found specific diet and gestational treatment effects on body weight and weight gain of offspring that suggest that mild dietary restriction of mothers on an obesogenic diet may partially offset the increased risk of obesity development in offspring, but dietary restriction of mothers on a chow diet provided no benefit nor did it increase the risk of offspring developing obesity.

2. Materials and methods

All experimental procedures in this study were approved by the Animal Care and Use Committee at The University of Georgia and were carried out in accordance with the Guide for the Care and Use of Laboratory Animals.

2.1. Animals

C57BL/6 mice were used throughout the study. G0 females ($N = 14$) were provided by the Minneapolis Veterans Administration Medical Center, housed in individual cages and provided with tap water and standard chow (Purina Mouse Diet #5015: 17% protein, 11% fat, 3% fiber, 6.5% ash, 2.5% minerals; 3.73 kcal/g, 88.1% Total Digestible Nutrients) ad libitum. There were two dietary groups. In one group all mice, through the third generation, received chow (C). In the second group all mice were fed a high fat diet (HF) (20% protein, 35% carbohydrate, 45% fat; 4.73 kcal/g (Research Diets, 2006)).

At 11 weeks of age the G0 breeding females were each moved to a cage with a C57BL/6 male (all breeding males were chow-fed); group 1 continued to receive chow, while group 2 were switched to HF diet. Body weights were recorded daily. Weight gain of approximately 10 g was used to indicate the beginning of the third trimester of pregnancy, and males were removed. At this point the females in each dietary group were randomly assigned to one of two gestational treatments: ad libitum (ad lib) or 25% food restriction (R, based on average daily food intake from the previous week) until pups were born. F1 pups

were born within 7 days. Litter size and weight were recorded and pups remained with dams until they were weaned at 3 weeks of age. All dams were fed ad libitum during lactation.

At weaning F1 mice were weighed, moved to individual cages, and remained in the same dietary treatment group to which their dams had been assigned. Food intake was recorded daily and mice were weighed weekly for the next eight weeks. At 11 weeks of age, final body weights were recorded, a subgroup of females ($N = 14$) was randomly selected for breeding, and the rest of the F1 mice were killed by decapitation. In the HF group only, retroperitoneal fat pads (RPWAT) were collected from both male and female offspring, and epididymal fat pads (EWAT) were collected from the males. Brains were removed, frozen immediately in liquid nitrogen and stored at −80 C.

F1 breeding females were housed with chow-fed males who were not part of the study and subsequently randomly assigned to third trimester ad lib or restricted groups, as described above for the F0 females. Litter size and weight were recorded at parturition and dams were all placed on ad libitum food (either chow or HF) during lactation. F2 progeny were weaned at 3 weeks and moved to individual cages. Food intakes and body weights were measured for the next 8 weeks, as described for F1. At 11 weeks of age, mice were weighed and a subgroup of females was again randomly selected for breeding. The rest of the F2 mice were killed by decapitation and fat pads and brains were collected from the mice in the HF group.

The process was repeated once more to produce F3 progeny. Those mice were killed at 11 weeks of age, and adipose tissue and brains were collected from mice in the HF group.

2.2. Extraction of total RNA, reverse transcription (RT) and real-time PCR

For the gene expression analysis the brains were removed from the freezer and the hypothalamic tissue was rapidly excised using surface morphology of the brain to provide consistent landmarks. The medial basal hypothalamus borders were the optic chiasm, the lateral hypothalamic fissures and the mammillary bodies and extended dorsally to the level of the anterior commissure (Paxinos and Watson, 1986). The tissue was immediately placed into Trizol reagent (Invitrogen, Carlsbad, CA) for RNA extraction.

Total RNA was extracted from hypothalamic tissue samples using Trizol Reagent in accordance with the manufacturer's protocol. The integrity of the total RNA obtained from all the samples was verified using the RNA 6000 Nano Assay and the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). The RT reaction was performed on 100 ng of total RNA per sample in a 20 µl reaction mixture using the cDNA Archive Kit with MultiScribe™ Reverse Transcriptase (ABI; Applied Biosystems, Inc., Foster City, CA; Part #4322171) according to the manufacturer's instructions. Reactions were incubated initially at 25 °C for 10 min and subsequently at 37 °C for 120 min. Quantitative PCR (Taqman™) assays chosen for the transcripts to be evaluated were from Assays-On-Demand™ (ABI), a pre-validated library of qPCR assays, and were incorporated into 384-well MicroFluidic™ cards. All of the oligonucleotide primer and fluorogenic probe sets for Taqman™ real-time PCR were from ABI (Table 1). Two µl of the complementary DNA (cDNA) along with 50 µl of $2 \times$ PCR master mixes were loaded into respective channels on the microfluidic card followed by a brief centrifugation (330 g for 1 min, then repeated). The card was then sealed and real-time PCR and relative quantification were carried out using the ABI PRISM 7900 Sequence Detection System. The cycle conditions were: 94.5 °C for 15 min, followed by 40 cycles of 97 °C for 30 s, 59.7 °C for 1 min. Data were analyzed using Sequence Detection Systems software (ABI) and the Relative Quantification (RQ) method. Expression levels of mRNAs were normalized with 18 S as an endogenous control to correct the differences in the amount of total RNA added to each reaction.

Table 1
Gene symbols, names and ABI primer ID numbers.

Gene symbol	Gene name	ABI primer ID no.
ACTb	β-actin (housekeeping gene)	Actb-Mm00607939_s1
AGRP	Agouti related peptide	Agrp-Mm00475829_g1
AVP	Arginine vasopressin	Avp-Mm00437761_g1
B2M	β2-microglobulin	B2m-Mm00437762_m1
CART	Cocaine and amphetamine regulated transcript	Cart-Mm00489086_m1
CREB1	cAMP responsive element binding protein 1	Creb1-Mm00501607_m1
FOS	FBJ osteosarcoma oncogene	Fos-Mm00487425_m1
GABRD	Gamma-aminobutyric acid A receptor, delta	Gabrd-Mm00433476_m1
GHRH	Growth hormone releasing hormone	Ghrh-Mm00439098_m1
JAK2	Janus kinase 2	Jak2-Mm00434561_m1
MAPK1	Mitogen activated protein kinase 1	Mapk1-Mm00442479_m1
NPY	Neuropeptide Y	Npy-Mm00445771_m1
OXT	Oxytocin	Oxt-Mm00726655_s1
POMC1	Pro-opiomelanocortin-alpha	Pomc1-Mm00435874_m1
PPID	Peptidylprolyl isomerase D (cyclophilin D)	Ppid-Mm00835365_g1
PTGES	Prostaglandin E synthase	Ptges-Mm00452105_m1
SCT	Secretin	Sct-Mm00441235_g1
SOCS3	Suppressor of cytokine signaling 3	Socs3-Mm00545913_s1
STAT3	Signal transducer and activator of transcription 3	Stat3-Mm00456961_m1
TH	Tyrosine hydroxylase	Th-Mm00447546_m1
TNF	Tumor necrosis factor	Tnf-Mm00443258_m1
UCN3	Urocortin 3	Ucn3-Mm00453206_s1
VIP	Vasoactive intestinal polypeptide	Vip-Mm00660234_m1

2.3. Statistical analysis

Data were analyzed by ANOVA using the GLM procedure (Statistica, ver 7.1; Statsoft, Inc., Tulsa, OK). Generally, main factors included generation, gender, dietary treatment (chow or HF diet), and maternal gestational treatment (ad lib or food restricted during the third trimester). Food intake data from chow-fed F1 and F2 mice were not available for analysis. For all other mice, weekly and cumulative food intakes (FI) and feed efficiencies (FE) were calculated. For generation effects and analyses showing significant interactions, Tukey's test and Fisher's LSD test were used to determine significance of differences among individual means. Data presented are means ± SEM.

Regression analyses were also carried out to identify whether there were significant correlations between maternal body weight or weight gain and offspring post-weaning weight gain.

3. Results

3.1. Maternal weight gain, body weight, litter size and weight

Females fed the HF diet during pregnancy gained significantly more weight ($p < 0.0001$; Fig. 1A) and weighed more at the end of
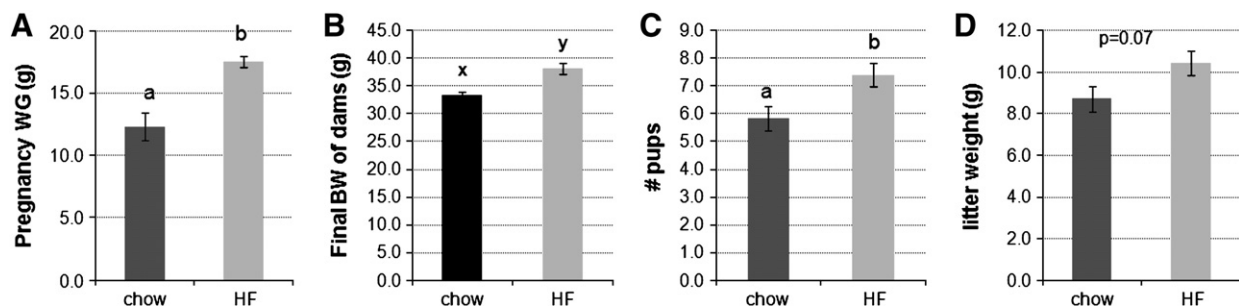
Table 2
Main effects of generation, gender, diet and treatment on weaning weight (WWT), final body weight (BW), and total weight gain (WG). Mean (g) ± SEM.

	WWT	Final BW	WG	N
Generation				
1	10.9 ± 0.2^a	24.0 ± 0.4	13.0 ± 0.4^b	86
2	11.2 ± 0.2^a	23.5 ± 0.5	12.4 ± 0.5^a	77
3	11.7 ± 0.2^b	24.0 ± 0.4	12.3 ± 0.4^a	74
Gender				
M	11.4 ± 0.2	27.0 ± 0.2^x	15.6 ± 0.3^x	117
F	11.1 ± 0.2	20.7 ± 0.2^y	9.6 ± 0.2^y	120
Diet				
HF	10.7 ± 0.1^x	24.1 ± 0.3^y	13.4 ± 0.3^x	144
Chow	12.0 ± 0.2^y	23.4 ± 0.4^x	11.3 ± 0.4^y	93
Gestational treatment				
Ad lib	11.2 ± 0.2	24.2 ± 0.3^b	13.0 ± 0.4^b	130
Restricted	11.3 ± 0.2	23.4 ± 0.4^a	12.1 ± 0.3^a	107

Means without a common superscript are different: [a,b] $p < 0.05$; [x,y] $p < 0.01$.

their pregnancy than females fed chow ($p < 0.001$; Fig. 1B). Females fed the HF diet also had significantly more pups/litter ($p < 0.05$; Fig. 1C). There were no generation or gestational treatment effects on female initial or final body weights, weight gain during pregnancy, litter size, or litter weight, and there were no significant interactions (data not shown).

3.2. Offspring weaning weight, final body weight and weight gain

3.2.1. Main effects (generation, gender, diet, and gestational treatment)

There was a significant main effect of generation on weaning weight ($p = 0.002$; WWT), so that by generation 3, mice were significantly heavier at weaning than in generation 1 (Table 2). However, WG (weight gain) declined in generation 2 compared to generation 1, and final body weight (BW) did not differ significantly over the three generations.

There were significant gender effects on final BW ($p < 0.0001$) and WG ($p < 0.0001$), but not WWT. Males gained more weight and had higher final body weights than females (Table 2).

Compared to chow diet, the high fat diet was associated with significantly higher WG ($p < 0.0001$) and final BW ($p < 0.001$), but significantly lower weaning weight ($p < 0.0001$). Mice whose mothers were food restricted during the final trimester weighed the same at weaning as mice whose mothers were fed ad libitum. However, mice from restricted mothers gained less weight ($p < 0.05$) and their final BW was significantly less at 8 weeks of age than mice from mothers fed ad lib ($p < 0.05$; Table 2).

3.2.2. Gender × diet

As noted above for overall gender effects, there were no differences in WWT between males and females, but there was a strong diet effect. Both males and females from mothers fed chow
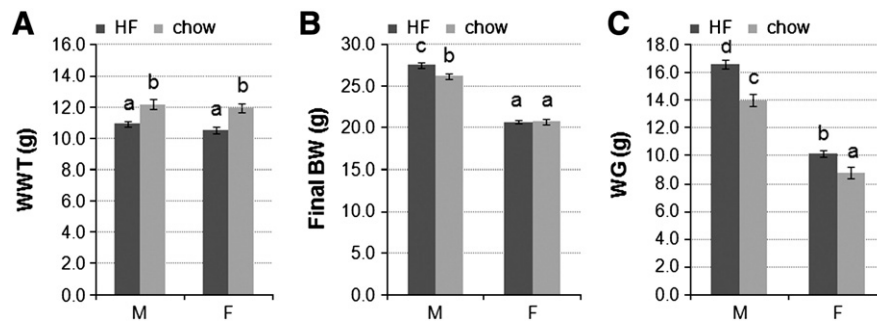

Fig. 1. Maternal weight gain (A), final body weight (B), litter size (C) and litter weight (D). Three generations of female C57BL/6 mice were mated at 11 weeks of age and provided with chow or HF diet. Half of each group was randomly assigned to ad libitum feeding or 25% food restriction during the third trimester. Data are means ± SEM. Means that do not share a common letter are different: a,b $p < 0.05$; x,y $p < 0.01$.

Fig. 2. Gender × diet effects on weaning weight (WWT, A), final body weight (final BW, B), and total weight gain (WG, C) of three generations of male (M) and female (F) offspring. Offspring of dams fed ad lib or food restricted by 25% during the third trimester were weaned at 3 weeks of age and fed either chow or high fat (HF) diet for the next 8 weeks. Data are means ± SEM. Means that do not share a common letter are different: a,b,c,d $p < 0.05$.

were heavier at weaning than males and females from mothers fed the HF diet ($p < 0.05$; Fig. 2A). After 8 weeks, the males fed the HF diet were significantly heavier than males fed chow ($p < 0.05$), but there was no difference in final BW between females fed chow and females fed the HF diet (Fig. 2B). However, both males and females fed the HF diet gained more weight than chow-fed males and females ($p < 0.05$; Fig. 2C).

3.2.3. Diet × treatment

There was a significant diet × treatment interaction for WWT ($p < 0.001$) and WG ($p < 0.05$; Fig. 3A and C). Mice on the HF diet whose mothers were food restricted during the third trimester were heavier at weaning than mice whose mothers had food ad libitum during gestation; in contrast there was no difference between maternal gestational treatments for mice fed chow (Fig. 3A). In addition only mice on the HF diet gained less weight when their mothers were food

restricted during the third trimester (Fig. 3C). There was no significant diet × treatment effect on final BW.

3.2.4. Generation × diet

There were significant generation × diet effects on WWT ($p < 0.0001$), final BW ($p < 0.05$) and WG ($p < 0.0001$; Fig. 4). The main finding of interest was that the chow-fed offspring in generations 2 and 3 weighed more at weaning (Fig. 4A), but gained less weight after weaning (Fig. 4C) so that final BW actually declined slightly over the generations (Fig. 4C).

3.3. Food intake and feed efficiency

Two separate analyses were run because of missing food intake data for chow-fed generations 1 and 2. In the first analysis data from all three generations of the HF mice were analyzed in a 3-way factorial design (generation, gender, treatment). In the second analysis, data
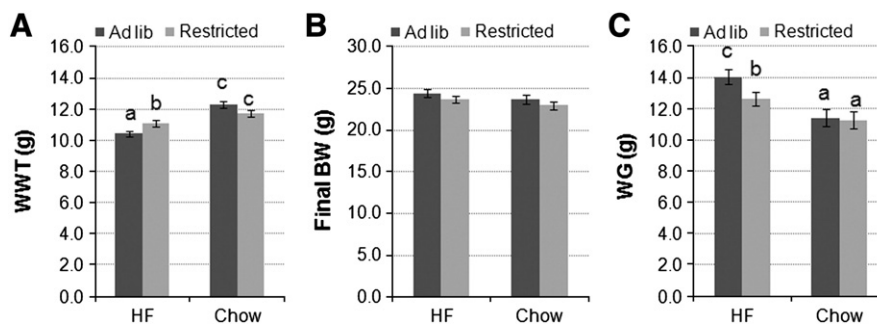

Fig. 3. Diet × treatment effects on weaning weight (WWT, A), final body weight (final BW, B), and total weight gain (WG, C) of three generations of offspring. Offspring of dams fed ad lib or food restricted by 25% during the third trimester were weaned at 3 weeks of age and fed either chow or high fat (HF) diet for the next 8 weeks. Data are means ± SEM. Means that do not share a common letter are different: a,b,c $p < 0.05$.
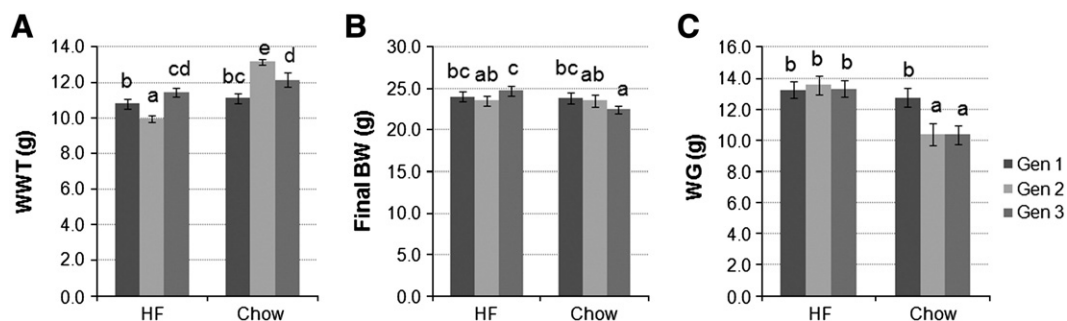

Fig. 4. Generation × diet effects on weaning weight (WWT, A), final body weight (final BW, B), and total weight gain (WG, C) of three generations of offspring. Offspring of dams fed ad lib or food restricted by 25% during the third trimester were weaned at 3 weeks of age and fed either chow or high fat (HF) diet for the next 8 weeks. Data are means ± SEM. Means that do not share a common letter are different: a,b,c,d,e $p < 0.05$.

Table 3
Main effects of gender, gestational treatment and generation on cumulative food intake (FI, kcal) and feed efficiency (FE, g weight gain/kcal consumed) of high fat fed offspring.

	Cumulative FI (kcal) mean ± SEM	FE (g wt gain/kcal) mean ± SEM
Gender		
M	782.3 ± 6.4[x]	0.1005 ± 0.0015[x]
F	734.8 ± 5.7[y]	0.0654 ± 0.0014[y]
Gestational treatment		
Ad lib	768.1 ± 6.6[a]	0.0856 ± 0.0025
Restricted	747.2 ± 6.5[b]	0.0797 ± 0.0026
Generation		
1	751.4 ± 7.3[a]	0.0831 ± 0.0031
2	751.6 ± 6.7[a]	0.0846 ± 0.0034
2	772.6 ± 10.0[b]	0.0810 ± 0.0029

Means that do not share a common letter are different: [a,b] $p < 0.05$; [x,y] $p < 0.01$.

from both chow-fed and HF-fed generation 3 mice were analyzed in a 3-way factorial design (gender, diet, treatment). Food intakes (FI) were converted to kcal and cumulative FI was calculated for each week and for the total 8 week period. Feed efficiency (FE, g weight gained/kcal consumed) was also calculated for each week and for the total 8 week period. Results summarized below refer only to the 8 week cumulative FI and FE because there were no consistent weekly trends related to any of the main factors, except for gender.

3.3.1. HF mice − 3 generations

For the HF mice, there were significant main effects of gender on cumulative FI and FE ($p < 0.0001$): males ate more than females and were more efficient (Table 3). There was a significant effect of gestational treatment on cumulative FI ($p = 0.02$): offspring from mothers fed ad lib during the third trimester ate more compared to offspring whose mothers were food restricted during the third trimester. There were no significant interactions for FI or FE.

3.3.2. Generation 3 HF-fed mice vs chow-fed mice

For generation 3 mice there were main effects of gender on FE ($p < 0.0001$), but not FI, with males more efficient than females (Table 4). There were significant diet effects on both FI ($p < 0.0001$) and FE ($p < 0.0001$). Interestingly, mice fed chow ate more than mice fed the HF diet, but the chow-fed mice were less efficient. There also was a significant diet × gender effect for FI ($p = 0.01$), showing that males on the HF diet ate more than females on the HF diet, but intakes of chow-fed males and females were similar (Table 5). There were no significant gestational treatment effects on FI or FE for generation 3 mice.

3.4. Fat pad weights (HF mice only)

There were no significant gender, generation or treatment effects on RPWAT weight. There was a significant gender effect on RPWAT as

Table 4
Main effects of gender and diet on cumulative food intake (FI, kcal) and feed efficiency (FE, g weight gain/kcal consumed) of generation 3 offspring.

	Cumulative FI (kcal) mean ± SEM	FE (g wt gain/kcal) mean ± SEM
Gender		
M	830.2 ± 14.2	0.0331 ± 0.0007[x]
F	824.1 ± 19.8	0.0263 ± 0.0007[y]
Diet		
HF	772.6 ± 10.0[x]	0.0320 ± 0.0006[x]
Chow	927.2 ± 18.0[y]	0.0246 ± 0.0008[y]

Means that do not share a common letter are different: [x,y] $p < 0.01$.

Table 5
Cumulative food intake (FI, kcal) and feed efficiency (FE, g weight gain/kcal consumed) of generation 3 chow and high fat (HF) fed male (M) and female (F) offspring.

Gender	Diet	Cumulative FI (kcal) mean ± SEM	FE (g wt gain/kcal) mean ± SEM
Male	HF diet	800.4 ± 13.8[a]	0.0353 ± 0.0005
	Chow	901.7 ± 22.8[b]	0.0278 ± 0.0009
Female	HF diet	744.7 ± 12.2[c]	0.0287 ± 0.0005
	Chow	943.2 ± 25.2[c]	0.0225 ± 0.0009

Means that do not share a common letter are different: [a,b,c] $p < 0.05$.

percent of body weight ($p < 0.001$): in females the RPWAT was a higher % of their BW than in males (Fig. 5A). There was a significant generation effect on EWAT weight ($p < 0.05$) and EWAT as a % of final BW ($p < 0.05$; Fig. 7B): generation 1 mice had a lower % fat as EWAT than generation 2 or 3 mice. There were no other main effects or interactions for EWAT.

3.5. Regression analyses

Regression analyses were carried out for maternal gestational weight gain vs offspring post-weaning weight gain. Across all generations, diets and gestational treatments, there was a significant correlation between maternal WG and post-weaning WG of male offspring ($R = 0.32$, $p < 0.03$) (Fig. 6A and B).

Separate regression analyses were then run for each generation. In generation 1 (F0 mothers), there was no significant correlation between maternal gestational WG and post-weaning WG of males or females (Fig. 7A and B). In generation 2 there was still no significant correlation, but slopes for the calculated lines for both males and females were positive (Fig. 7C and D). In generation 3, the correlations were significant for both males ($p = 0.006$) and females ($p = 0.03$) (Fig. 7E and F).

3.6. Hypothalamic gene expression

There was a significant gender effect on expression of CART, CREB, FOS, PPID, SOCS3, TNF, and UCN3 (Table 6). Expression of CART, FOS and SOCS3 was higher in males than females, whereas expression of CREB, PPID, TNF and UCN3 was higher in females.

There were significant generation, generation × gender, generation × gestational treatment, and generation × gender × gestational treatment on expression of TH (Fig. 8). Expression of TH was higher in generation 3 offspring compared to generation 1 and 2 (Fig. 8A). This increase occurred primarily in generation 3 males from mothers fed ad lib throughout pregnancy (Fig. 8D). In females, there was no generation or maternal treatment effect on TH expression (Fig. 8B and D).
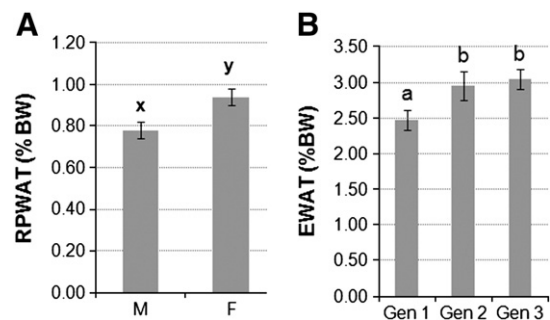
Fig. 5. Retroperitoneal fat pad (RPWAT, A) of high fat fed male (M) and female (F) offspring and epididymal fat pad (EWAT, B) of high fat fed male offspring. Offspring of dams fed ad lib or food restricted (R) by 25% during the third trimester were weaned at 3 weeks of age and fed a high fat diet for the next 8 weeks. Data are means ± SEM. Means that do not share a common letter are different: a,b $p < 0.05$; x,y $p < 0.01$.
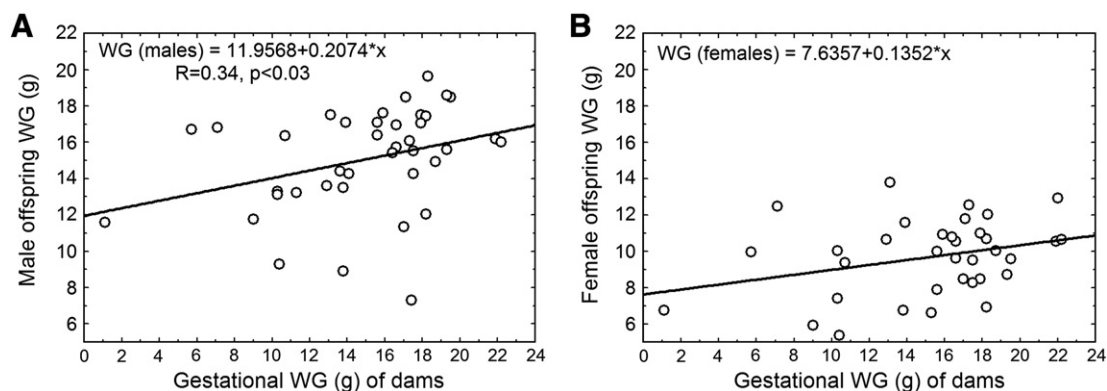
Fig. 6. Scatterplots showing correlation between gestational weight gain (WG) of dams and total WG of male (A) and female (B) offspring across three generations.

4. Discussion

The main objectives of this study were to determine (1) whether short-term food restriction during gestation would alter food intake and growth parameters of offspring, (2) whether effects of gestational food restriction were dependent on diet (obesogenic diet vs chow), (3) whether effects on offspring of excessive maternal weight gain during gestation would become magnified over several generations,
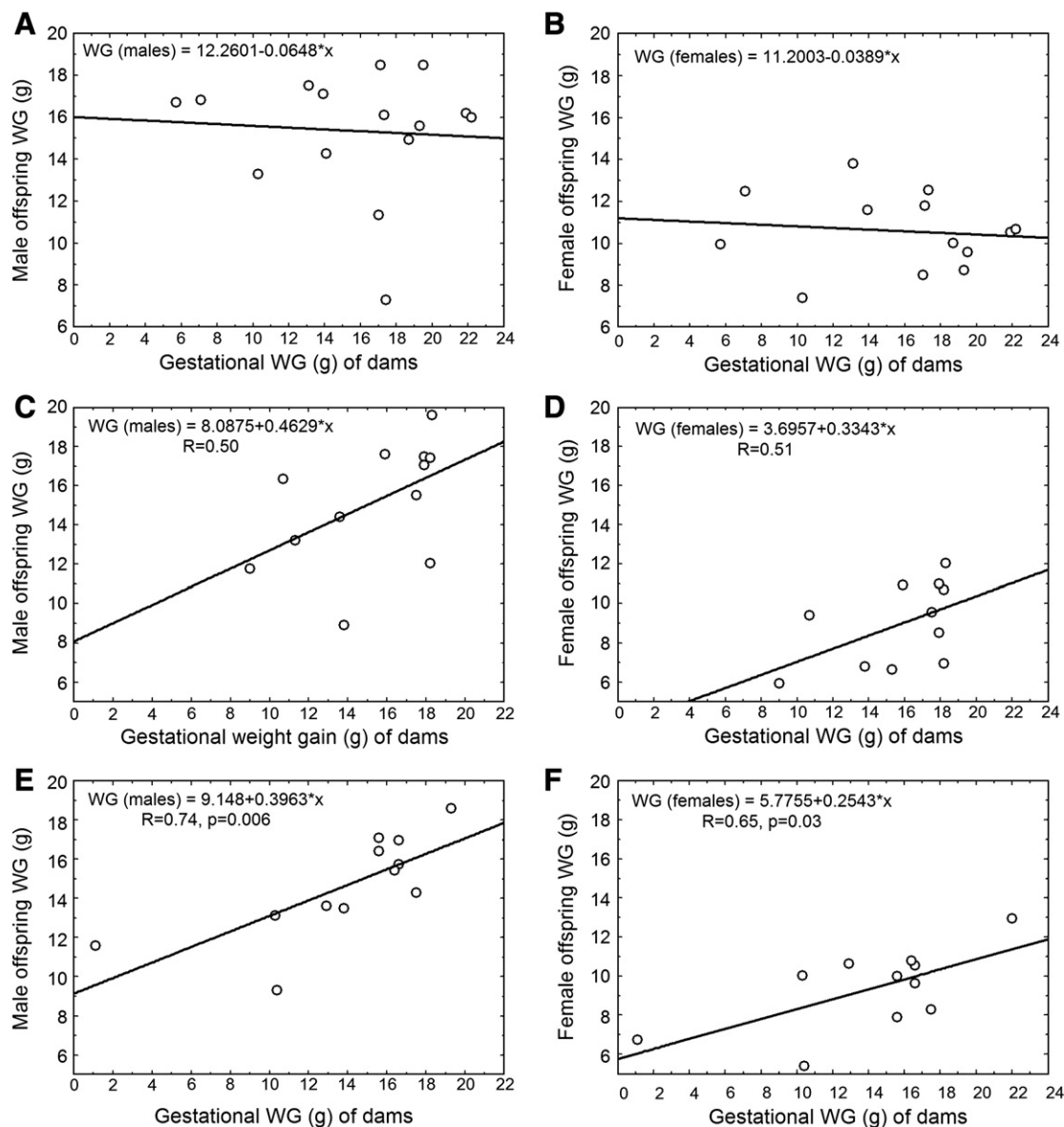

Fig. 7. Scatterplots showing correlation between gestational weight gain (WG) of dams and total WG of male (A, C, E) and female (B, D, F) offspring in generation 1 (A, B), generation 2 (C, D) and generation 3 (E, F).

Table 6
Hypothalamic gene expression (RQ) in female and male HF-fed mice (mean ± SEM).

Gene	Females	Males
CART	0.903 ± 0.069	1.166 ± 0.067*
CREB	1.224 ± 0.064	0.932 ± 0.056**
FOS	1.286 ± 0.089	1.549 ± 0.112*
PPID	1.102 ± 0.050	0.893 ± 0.050**
SOCS3	1.027 ± 0.081	1.283 ± 0.089*
TNF	1.286 ± 0.122	0.936 ± 0.070*
UCN3	1.287 ± 0.117	0.814 ± 0.079**

Means are different: *$p < 0.05$, **$p < 0.01$.

and (4) whether expression of genes known to be involved in regulation of body weight would be affected differentially by gestational food restriction and across generations.

Our main findings showed (1) across dietary treatments and gestational treatments, maternal weight gain was positively correlated with weight gain in the offspring, and this correlation became more prominent across generations. (2) Maternal diet differentially impacted offspring prior to weaning: mothers on the HF diet gained more weight during gestation and had larger litters, but at weaning the individual pups weighed less than those from mothers on chow. Offspring on the HF diet gained more weight after weaning than offspring fed chow, and BW at 11 weeks of age was higher than that of chow-fed mice. Interestingly, mice fed chow consumed more kcal but were less efficient than mice fed HF diet. (3) Food restriction during the third trimester did not affect maternal weight gain, but had differential effects on offspring growth that were dependent on the diet. Overall, mice whose mothers were food restricted during gestation gained less weight and weighed less at 11 weeks of age than mice from mothers fed ad lib; however, this effect occurred only in the HF-fed group. In the HF group, gestational food restriction also

resulted in decreased FI and FE in the offspring, compared to offspring whose mothers had ad lib access to food during gestation. (4) In generation 3 HF-fed males from food restricted mothers had significantly lower expression of tyrosine hydroxylase (TH), which is involved in dopamine synthesis, compared to HF-fed males from mothers fed ad lib during gestation.

Several recent papers have documented the association between excessive maternal weight gain and increased risk of obesity in the offspring. Mamun et al. showed that for every 0.1 kg/week gain over the average pregnancy weight gain, offspring BMI at age 21 was 0.3 kg/m^2 greater (Mamun et al., 2009). Wrotniak et al. found that the odds of overweight in offspring at age 7 years increased by 3% for every 1 kg of gestational weight gain. The odds of overweight was 48% greater for children of mothers who gained more than the Institute of Medicine weight gain recommendations than for children of mothers who met the weight gain guidelines (Wrotniak et al., 2008). A linear association has been demonstrated between pregnancy weight gain and risk of obesity in adolescent offspring (Oken et al., 2008) and offspring at age 3 (Olson et al., 2008). In addition, Olson et al. showed that the risk was greater if mothers were overweight or obese. Stuebe et al. found an increased risk of obesity in female offspring in both adolescence and adulthood when mothers had either below normal or above normal gestational weight gain, and the risk was increased if the mother was obese prior to pregnancy (Stuebe et al., 2009).

Heinsbroek and VanDijk developed a rat model of increased gestational weight gain by chronically infusing a melanocortin receptor blocker into the third cerebral ventricle of female rats throughout pregnancy and lactation (Heinsbroek and van Dijk, 2009). Both mothers and offspring were fed chow. In contrast to our study, they found that mothers with increased weight gain had litters that were smaller and lower in weight. However, offspring of mothers with increased gestational weight gain had increased body weight
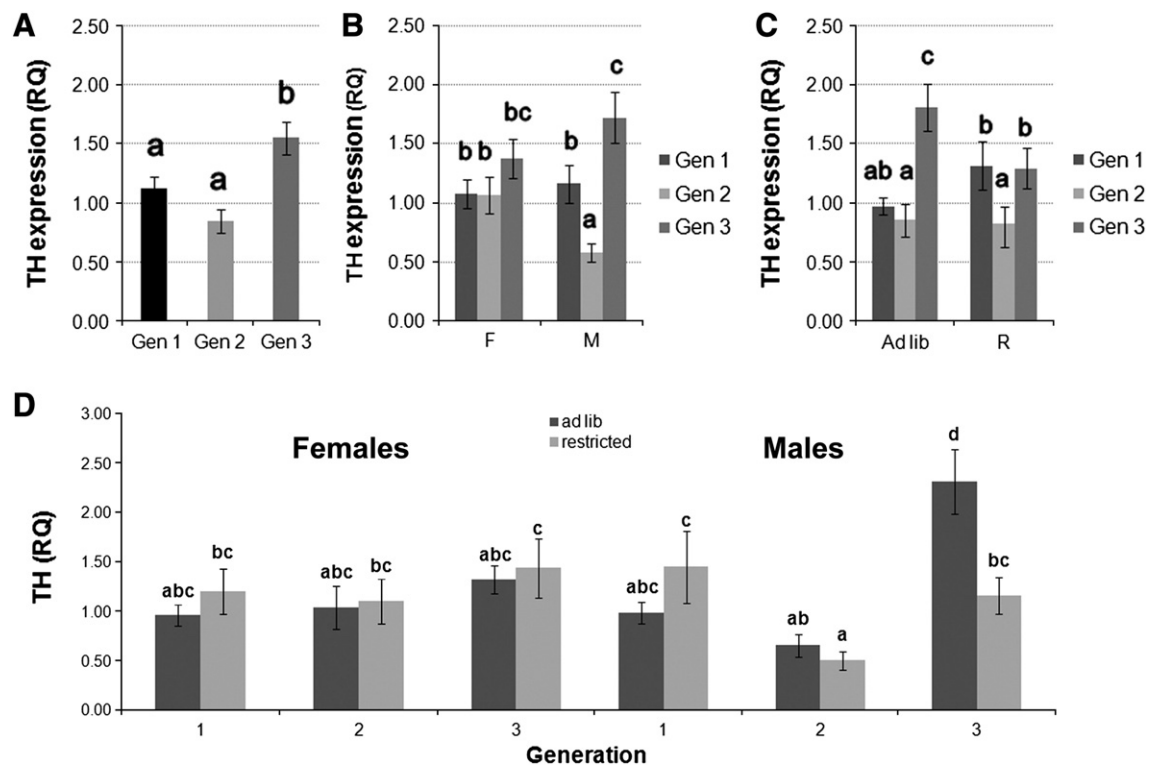

Fig. 8. Hypothalamic mRNA expression of tyrosine hydroxylase (TH) from 11 week old male (M) and female (F) offspring of dams fed ad lib or food restricted (R) by 25% during the third trimester. Offspring were weaned at 3 weeks of age and fed a high fat diet for the next 8 weeks. (A) Difference in TH expression across generations. (B) Difference in TH expression between males and females. (C) Difference in TH expression between offspring of ad lib fed and restricted dams. (D) Gender × generation × gestational treatment effects on TH expression. Data are means ± SEM. Means that do not share a common letter are different: a,b,c,d $p < 0.05$.

and adiposity through 9 months of age, and the effect was more prominent in males than females, as we also found.

Maternal protein and/or calorie restriction during gestation is well known to increase the risk of obesity in offspring in both humans and rodents (Bellinger et al., 2004; Ravelli et al., 1999). Our objective was not to test the effect of severe nutritional restriction, but rather to impose a mild restriction and limit exposure to only the third trimester, when effects on critical periods of organogenesis should be minimal. In fact, as noted above, food restriction of 25% during the third trimester had no effect on weight gain of the dams and did not alter litter size or weight in either the chow-fed or HF-fed groups. Rather than enhancing obesity in the offspring, this mild restriction appears to have had a beneficial effect by reducing WG and BW in the HF-fed offspring. Food restriction during different stages of gestation has not been studied extensively. However, a series of papers on gestational food restriction in sheep has shown that timing of the restriction can have differential effects on offspring growth and metabolic characteristics (Chan et al., 2009; Sebert et al., 2009a,b; Sharkey et al., 2009a,b; Yiallourides et al., 2009).

There is increasing evidence demonstrating the multi-generational transmission of increased obesity risk related to exposure in utero to predisposing factors. Indirect evidence has suggested that both low and high birthweight, both of which are associated with increased risk of development of obesity in first generation offspring, are traits that are transmitted to subsequent generations (Ahlsson et al., 2007; Collins et al., 2003; Selling et al., 2006). Although these studies do not rule out common genetic causes of the intergenerational transmission of these traits, a recent study showed increased risk of developing obesity and poor health later in life in F2 children of parents (F1) exposed in utero to the effects of maternal (F0) starvation during the Dutch famine (Painter et al., 2008). Jimenez-Chillaron et al. used a mouse model to demonstrate the transmission to the F2 generation of increased obesity and glucose intolerance resulting from gestational food restriction in the F0 generation (Jimenez-Chillaron et al., 2009). Similarly, a study in rats showed that low protein intake of F0 female rats during gestation led to changes in glucose and insulin sensitivity in the F2 progeny (Pinheiro et al., 2008). Dietary restriction of female F0 guinea pigs during either the first or second half of pregnancy was also shown to adversely affect the cardiovascular system and hypothalamo-pituitary-adrenal function of both F1 and F2 male offspring (Bertram et al., 2008). The mechanisms involved in the multi-generational transmission of metabolic disorder and obesity are not well understood yet. However, one likely contributing factor is the development of insulin resistance in each generation of offspring, which predisposes pregnant females to develop gestational diabetes, which then predisposes the next generation to develop metabolic disorder (Boney et al., 2005).

In our study there were small changes in WWT and WG over the generations; however, final BW overall was the same across generations. The most prominent change over generations occurred in the chow-fed group, which gained less weight in generations 2 and 3 and weighed slightly, but significantly, less in generations 2 and 3. We had predicted an intergenerational magnification of obesity in the HF group; however, we did not find changes in final BW in the HF group over generations. We did find an increase in EWAT (% BW) in the HF males that suggests an increase in adiposity over generations, but not in RPWAT in either males or females. Several factors may have contributed to the lack of change in BW over generations. First, we did not standardize the number of pups/litter at birth, and unexpectedly, the HF-fed mothers had significantly larger litter sizes. Thus, it is likely that there was increased competition for food prior to weaning, resulting in the decreased WWT of individual offspring from HF-fed mothers. There may have also been increased competition for nutrients in utero, as well. Although we did not weigh individual pups at birth, there was no significant difference in mean weight/pup (litter weight/number of pups) between HF and chow-fed offspring (data not shown). Thus it is unlikely there was relative malnutrition of HF offspring in utero compared to chow offspring.

Although WG was increased in the HF group, those mice first had to catch up to the chow-fed group. The length of our study also may have been too short to detect progressive increases in BW in the HF group compared to the chow group, given the time required for catch-up growth. A second factor that may have contributed to the overall lack of increased BW over generations is that half of all offspring were from mothers that had been food restricted during gestation, a treatment which resulted in reduced WG and BW in the HF group only. Finally, there was a significant gender × diet difference in final BW, with HF-fed males weighing significantly more than chow-fed males, but HF and chow-fed females weighed the same at 11 weeks of age. Gender differences in growth in response to different maternal dietary manipulations have been previously reported (Bellinger et al., 2004; Knight et al., 2007). Thus, the gestational treatment and gender effects may have diluted the overall generation effect in the HF group.

Changes in hypothalamic gene expression in offspring of mothers with either overnutrition or undernutrition during pregnancy have been reported. Gupta et al. found that term fetuses of HF female rats had increased expression of leptin long receptor (ObRb), insulin receptor beta subunit, NPY, agouti-related polypeptide, pro-opiate-melanocortin (POMC) and melanocortin receptor-4 (Gupta et al., 2008). In contrast Morris et al. found decreased mRNA expression of ObRb, NPY, POMC, and STAT3 in one day old pups from obese dams (Morris and Chen, 2009). Page et al. investigated the effects of maternal and post-weaning HF diet on hypothalamic mRNA expression in 120 day old offspring (Sprague Dawley rats). They found increased expression of ObRb, POMC, and NPY in offspring exposed to the HF diet during gestation and after weaning, whereas NPY1 receptor was down-regulated. Expression of STAT3 was also higher in offspring from HF-fed dams compared to controls.

In rat pups from Sprague Dawley dams that were restricted to 50% of control intake during the third trimester of gestation, Delahaye et al. showed that hypothalamic expression of POMC was decreased from 14 to 30 days of age, but NPY expression was not affected (Delahaye et al., 2008). In 24 week old HF-fed offspring of Wistar rats fed 30% of ad lib intake during gestation, Ikenasio-Thorpe et al. found increased hypothalamic mRNA expression of POMC, NPY and ObRb compared to chow-fed offspring of restricted mothers of HF-fed offspring of ad lib fed mothers (Ikenasio-Thorpe et al., 2007).

In our study, we compared HF-fed offspring of restricted dams to HF-fed offspring of ad lib fed dams and found lower hypothalamic TH expression in generation 3 males from restricted dams compared to males from ad lib fed dams, whereas there was no treatment related effect on TH expression in the female offspring. This is interesting in light of the role played by dopamine in the central mechanisms of reward and our data that showed that mice from restricted mothers ate less and gained less weight than mice from ad lib fed mothers. Lee et al. recently showed that expression of TH and other genes involved in availability of dopamine in the hypothalamus was increased in HF-fed mice (Lee et al., 2009). In contrast, in some animal models of obesity (including leptin-deficiency *ob/ob* mice, Zucker rats or seasonally obese animals), dopamine activity is reduced (Pijl, 2003). Wang et al. suggested that low brain DA activity in obese subjects predisposes them to excessive use of food (Wang et al., 2002). However, our data suggest that the effect of the HF diet on TH expression and dopamine activity can be modified by mild food restriction of the mothers during the third trimester of gestation, and perhaps lower motivation to eat was responsible in part for the reduced food intake and weight gain in that group of offspring.

In summary, our study shows that diet, gender and maternal gestational food restriction had independent effects on growth and food intake of offspring, and hypothalamic gene expression was dependent on both gender and maternal gestational food restriction in high fat fed offspring. Even mild food restriction of obese mothers

during pregnancy may have beneficial effects in reducing the risk or degree of obesity in offspring.

Acknowledgements

References

Ahlsson F, Gustafsson J, Tuvemo T, Lundgren M. Females born large for gestational age have a doubled risk of giving birth to large for gestational age infants. Acta Paediatr 2007;96:358–62.

Barker DJ. In utero programming of chronic disease. Clin Sci (Lond) 1998;95:115–28.

Barker DJ, Fall CH. Fetal and infant origins of cardiovascular disease. Arch Dis Child 1993;68:797–9.

Bayol SA, Simbi BH, Bertrand JA, Stickland NC. Offspring from mothers fed a 'junk food' diet in pregnancy and lactation exhibit exacerbated adiposity that is more pronounced in females. J Physiol 2008;586:3219–30.

Bellinger L, Lilley C, Langley-Evans SC. Prenatal exposure to a maternal low-protein diet programmes a preference for high-fat foods in the young adult rat. Br J Nutr 2004;92:513–20.

Bellinger L, Sculley DV, Langley-Evans SC. Exposure to undernutrition in fetal life determines fat distribution, locomotor activity and food intake in ageing rats. Int J Obes (Lond) 2006;30:729–38.

Bertram C, Khan O, Ohri S, Phillips DI, Matthews SG, Hanson MA. Transgenerational effects of prenatal nutrient restriction on cardiovascular and hypothalamic-pituitary-adrenal function. J Physiol 2008;586:2217–29.

Boney CM, Verma A, Tucker R, Vohr BR. Metabolic syndrome in childhood: association with birth weight, maternal obesity, and gestational diabetes mellitus. Pediatrics 2005;115:e290–6.

Chan LL, Sebert SP, Hyatt MA, Stephenson T, Budge H, Symonds ME, et al. Effect of maternal nutrient restriction from early to midgestation on cardiac function and metabolism after adolescent-onset obesity. Am J Physiol Regul Integr Comp Physiol 2009;296:R1455–63.

Chen H, Simar D, Lambert K, Mercier J, Morris MJ. Maternal and postnatal overnutrition differentially impact appetite regulators and fuel metabolism. Endocrinology 2008;149:5348–56.

Collins Jr JW, David RJ, Prachand NG, Pierce ML. Low birth weight across generations. Matern Child Health J 2003;7:229–37.

Davis E, Olson C. Obesity in pregnancy. Prim Care 2009;36:341–56.

Delahaye F, Breton C, Risold PY, Enache M, Dutriez-Casteloot I, Laborie C, et al. Maternal perinatal undernutrition drastically reduces postnatal leptin surge and affects the development of arcuate nucleus proopiomelanocortin neurons in neonatal male rat pups. Endocrinology 2008;149:470–5.

Dolinoy DC, Weidman JR, Waterland RA, Jirtle RL. Maternal genistein alters coat color and protects Avy mouse offspring from obesity by modifying the fetal epigenome. Environ Health Perspect 2006;114:567–72.

Gillman MW, Barker D, Bier D, Cagampang F, Challis J, Fall C, et al. Meeting report on the 3rd International Congress on Developmental Origins of Health and Disease (DOHaD). Pediatr Res 2007;61:625–9.

Gupta A, Srinivasan M, Thamadilok S, Patel M. Hypothalamic alterations in fetuses of high fat diet-fed obese female rats. J Endocrinol 2009;200:293–300.

Hales CN, Barker DJ, Clark PM, Cox LJ, Fall C, Osmond C, et al. Fetal and infant growth and impaired glucose tolerance at age 64. BMJ 1991;303:1019–22.

Heinsbroek AC, van Dijk G. Gestational weight gain by reduced brain melanocortin activity affects offspring energy balance in rats. Int J Obes (Lond) 2009;33:104–14.

Ikenasio-Thorpe BA, Breier BH, Vickers MH, Fraser M. Prenatal influences on susceptibility to diet-induced obesity are mediated by altered neuroendocrine gene expression. J Endocrinol 2007;193:31–7.

Jimenez-Chillaron JC, Isganaitis E, Charalambous M, Gesta S, Pentinat-Pelegrin T, Faucette RR, et al. Intergenerational transmission of glucose intolerance and obesity by in utero undernutrition in mice. Diabetes 2009;58:460–8.

Knight BS, Pennell CE, Adamson SL, Lye SJ. The impact of murine strain and sex on postnatal development after maternal dietary restriction during pregnancy. J Physiol 2007;581:873–81.

Law CM, Barker DJ, Osmond C, Fall CH, Simmonds SJ. Early growth and abdominal fatness in adult life. J Epidemiol Commun Health 1992;46:184–6.

Lee AK, Mojtahed-Jaberi M, Kyriakou T, Aldecoa-Otalora Astarloa E, Arno M, Marshall NJ, et al. Effect of high-fat feeding on expression of genes controlling availability of dopamine in mouse hypothalamus. Nutrition 2010;26:411–22.

Levin BE. Epigenetic influences on food intake and physical activity level: review of animal studies. Obesity (Silver Spring) 2008;16(Suppl 3):S51–4.

Mamun AA, O'Callaghan M, Callaway L, Williams G, Najman J, Lawlor DA. Associations of gestational weight gain with offspring body mass index and blood pressure at 21 years of age: evidence from a birth cohort study. Circulation 2009;119:1720–7.

Morris MJ, Chen H. Established maternal obesity in the rat reprograms hypothalamic appetite regulators and leptin signaling at birth. Int J Obes (Lond) 2009;33:115–22.

Oken E, Rifas-Shiman SL, Field AE, Frazier AL, Gillman MW. Maternal gestational weight gain and offspring weight in adolescence. Obstet Gynecol 2008;112:999-1006.

Olson CM, Strawderman MS, Dennison BA. Maternal weight gain during pregnancy and child weight at age 3 years. Matern Child Health J 2009;13:839–46.

Painter RC, Osmond C, Gluckman P, Hanson M, Phillips DI, Roseboom TJ. Transgenerational effects of prenatal exposure to the Dutch famine on neonatal adiposity and health in later life. Bjog 2008;115:1243–9.

Paxinos G, Watson C. The rat brain in stereotaxic coordinates. Sydney: Academic Press; 1986.

Pijl H. Reduced dopaminergic tone in hypothalamic neural circuits: expression of a "thrifty" genotype underlying the metabolic syndrome? Eur J Pharmacol 2003;480: 125–31.

Pinheiro AR, Salvucci ID, Aguila MB, Mandarim-de-Lacerda CA. Protein restriction during gestation and/or lactation causes adverse transgenerational effects on biometry and glucose metabolism in F1 and F2 progenies of rats. Clin Sci (Lond) 2008;114:381–92.

Ramachenderan J, Bradford J, McLean M. Maternal obesity and pregnancy complications: a review. Aust N Z J Obstet Gynaecol 2008;48:228–35.

Ravelli AC, van Der Meulen JH, Osmond C, Barker DJ, Bleker OP. Obesity at the age of 50 y in men and women exposed to famine prenatally. Am J Clin Nutr 1999;70: 811–6.

Research Diets I. Product Data: D12451. New Brunswick, NJ: Research Diets, Inc.; 2006.

Sarwer DB, Allison KC, Gibbons LM, Markowitz JT, Nelson DB. Pregnancy and obesity: a review and agenda for future research. J Womens Health (2002) 2006;15:720–33.

Sebert S, Hyatt M, Chan L, Yiallourides M, Fainberg P, Patel N, et al. Influence of prenatal nutrition and obesity on tissue specific fat mass and obesity-associated (FTO) gene expression. Reproduction 2009a;139:265–74.

Sebert SP, Hyatt MA, Chan LL, Patel N, Bell RC, Keisler D, et al. Maternal nutrient restriction between early and midgestation and its impact upon appetite regulation after juvenile obesity. Endocrinology 2009b;150:634–41.

Selling KE, Carstensen J, Finnstrom O, Sydsjo G. Intergenerational effects of preterm birth and reduced intrauterine growth: a population-based study of Swedish mother-offspring pairs. Bjog 2006;113:430–40.

Sharkey D, Gardner DS, Fainberg HP, Sebert S, Bos P, Wilson V, et al. Maternal nutrient restriction during pregnancy differentially alters the unfolded protein response in adipose and renal tissue of obese juvenile offspring. Faseb J 2009a;23:1314–24.

Sharkey D, Symonds ME, Budge H. Adipose tissue inflammation: developmental ontogeny and consequences of gestational nutrient restriction in offspring. Endocrinology 2009b;150:3913–20.

Stuebe AM, Forman MR, Michels KB. Maternal-recalled gestational weight gain, pre-pregnancy body mass index, and obesity in the daughter. Int J Obes (Lond) 2009;33:743–52.

Symonds ME, Sebert SP, Hyatt MA, Budge H. Nutritional programming of the metabolic syndrome. Nat Rev Endocrinol 2009;5:604–10.

Vahratian A. Prevalence of overweight and obesity among women of childbearing age: results from the 2002 National Survey of Family Growth. Matern Child Health J 2009;13:268–73.

Wang GJ, Volkow ND, Fowler JS. The role of dopamine in motivation for food in humans: implications for obesity. Expert Opin Ther Targets 2002;6:601–9.

Waterland RA, Travisano M, Tahiliani KG, Rached MT, Mirza S. Methyl donor supplementation prevents transgenerational amplification of obesity. Int J Obes (Lond) 2008;32:1373–9.

Wrotniak BH, Shults J, Butts S, Stettler N. Gestational weight gain and risk of overweight in the offspring at age 7 y in a multicenter, multiethnic cohort study. Am J Clin Nutr 2008;87:1818–24.

Yiallourides M, Sebert SP, Wilson V, Sharkey D, Rhind SM, Symonds ME, et al. The differential effects of the timing of maternal nutrient restriction in the ovine placenta on glucocorticoid sensitivity, uncoupling protein 2, peroxisome prolif-erator-activated receptor-gamma and cell proliferation. Reproduction 2009;138: 601–8.