Maternal Obesity Has Little Effect on the Immediate Offspring but Impacts on the Next Generation

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Maternal obesity during pregnancy has been linked to an increased risk of obesity and cardiometabolic disease in the offspring, a phenomenon attributed to developmental programming. Programming effects may be transmissible across generations through both maternal and paternal inheritance, although the mechanisms remain unclear. Using a mouse model, we explored the effects of moderate maternal diet-induced obesity (DIO) on weight gain and glucose-insulin homeostasis in first-generation (F1) and second-generation offspring. DIO was associated with insulin resistance, hyperglycemia and dyslipidemia before pregnancy. Birth weight was reduced in female offspring of DIO mothers (by 6%, $P = .039$), and DIO offspring were heavier than controls at weaning (males by 47%, females by 27%), however there were no differences in glucose tolerance, plasma lipids, or hepatic gene expression at 6 months. Despite the relative lack of effects in the F1, we found clear fetal growth restriction and persistent metabolic changes in otherwise unmanipulated second-generation offspring with effects on birth weight, insulin levels, and hepatic gene expression that were transmitted through both maternal and paternal lines. This suggests that the consequences of the current dietary obesity epidemic may also have an impact on the descendants of obese individuals, even when the phenotype of the F1 appears largely unaffected. **(*Endocrinology* 154: 2514–2524, 2013)**

The prevalence of obesity and its associated metabolic disorders such as type 2 diabetes is increasing worldwide (1). This affects women of childbearing age; indeed, a recent survey in the United States reported that 32% of women aged 20 to 4 years are obese (World Health Organization 2009). Adverse maternal environments during pregnancy, for example, undernutrition or exposure to stress or glucocorticoids or to inflammatory diseases, which all associate with restricted fetal growth, have repeatedly been linked to increases in the offspring's risks of cardiometabolic and neuropsychiatric disorders, a phenomenon attributed to developmental programming (2). Maternal obesity has well-recognized short-term complications for both mother and child (3), although it is commonly associated with fetal overgrowth rather than growth restriction (4). Some data have associated maternal obesity with later obesity and cardiometabolic risk (glucose/insulin homeostasis, hypertension, and vascular dysfunction) in offspring (5), raising the possibility that maternal obesity induces developmental programming of the offspring.

In human studies, it is often difficult to determine to what extent adverse effects on the health of offspring are due to shared genes, exposure to an adverse environment in utero (including maternal diabetes), and/or the effects of postnatal lifestyle factors (ie, maternal overfeeding) (6). Animal models have provided some evidence that maternal obesity and/or the use of a high-fat cafeteria diet during pregnancy changes offspring body composition, glucose-insulin homeostasis, and blood pressure (reviewed in Refs.

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Abbreviations: ACC, acetyl coenzyme-A carboxylase; CON, control diet; DIO, diet-induced obesity; E10.5, embryonic day 10.5; F1, first-generation; F2, second-generation; GR, glucocorticoid receptor; LPL, lipoprotein lipase; mCON, maternal CON; PEPCK, phosphoenolpyruvate carboxykinase; pCON, paternal CON; PPAR, peroxisome proliferator-activated receptor; PGC1α, PPAR-γ coactivator 1α.

5, 7, and 8). However, the extent of reported effects differs substantially between studies (9). The mechanisms by which maternal obesity might associate with programmed changes in the offspring include alteration in the development of key organs, eg, liver, muscle, adipose tissue, and pancreas (5, 10–12), altered appetite circuitry (12), and changes in leptin levels (13).

Evidence both from human and from animal studies suggests that programmed changes may not be limited to the directly exposed first-generation (F1) but may be transmitted to subsequent generations without re-exposure (14–18). In humans, data from Overkalix in northern Sweden suggest that grandparental overnutrition during the prepubertal period associates with increased cardiovascular disease risk in grandchildren (19), although this appears to reflect more a grandpaternal than a grandmaternal effect. Moreover, grandchild obesity is linked to grandparental obesity independent of parental weight (20). The transmission of programmed effects to subsequent generations has been reported in a number of animal models including prenatal glucocorticoid overexposure (14, 21), maternal undernutrition (18, 22, 23), neonatal overnutrition (24), and maternal overnutrition (25). These effects may be transmissible through both maternal and paternal lines (14, 18, 23, 24, 26). Although initial studies suggested that programmed effects occurring as a result of an insult in one generation may result in similar phenotypes in successive generations (14, 23), emerging evidence suggests that the effects in subsequent generations may differ from those seen in the F1 who were exposed directly to an insult in utero (21, 26). Indeed, we have demonstrated parent-of-origin–specific effects on fetal and placental growth and gene expression in a second-generation (F2) of animals after in utero glucocorticoid overexposure (14, 21). In this study, we used a model of moderate maternal diet-induced obesity (DIO) to explore effects on postnatal weight gain and glucose-insulin homeostasis in the first- and F2 offspring.

Materials and Methods

Animal model

For all studies, animals were maintained under conditions of controlled lighting (lights on 7:00 AM to 7:00 PM) and temperature (22°C) and allowed free access to food. All studies were conducted under licensed approval by the United Kingdom Home Office, under the Animals (Scientific Procedures) Act, 1986, and with local ethical committee approval.

Model of maternal obesity

Female C57BL/6 mice were bred in house and, at 5 weeks of age, placed on a high-fat/high-sugar cafeteria diet (DIO: 58% kcal fat, 25.5% kcal carbohydrate as sucrose; Diet D12331) or matched control diet (CON: 10.5% kcal fat and 73.1% kcal carbohydrate as corn starch; Diet D12328) (both from Research Diets, New Brunswick, New Jersey). Details of dietary constituents are given in Supplemental Table 1 (published on The Endocrine Society's Journals Online web site at http://endo.endojournals.org). At 17 weeks of age, 1 group of females underwent ip glucose tolerance testing and were killed; tissues were dissected, weighed, and snap frozen. A second group was time-mated with C57BL/6 males that had been maintained on standard laboratory chow (RMI 801002; Special Diets Services, Witham, Essex, United Kingdom). Groups of pregnant females either had ip glucose tolerance testing performed at gestational day 18.5 and were then killed and tissues collected or were allowed to litter undisturbed. Females remained on their experimental diets through pregnancy and lactation. Dietary intake was estimated by weighing food.

F1 offspring

At postnatal day 1, litters were weighed and culled to 5; only litters from females that had remained undisturbed during pregnancy were studied. Animals remained with their mothers until weaning at 3 weeks when they were placed onto standard chow diet (RMI 801002; Special Diets Services). Groups of offspring (1 male and 1 female pup per litter) were individually housed at 6 months (males, F1 DIO n = 10 and F1 CON n = 6; females, F1 DIO n = 5 and F1 CON n = 6) and left to acclimatize for at least 5 days before undergoing glucose tolerance testing. Animals were killed ~7 to 10 days later, and tissues were dissected, weighed, and snap-frozen on dry ice.

F2 offspring

F2 offspring were generated by timed mating of F1 males and females at 12 weeks of age, all on standard chow diet as above. Animals were mated in all combinations to give 4 groups of F2 offspring: 1) offspring from F1 CON mother and father (maternal CON [mCON]/paternal CON [pCON], n = 11 litters), 2) offspring from F1 CON mother and F1 DIO father (mCON/pDIO, n = 8 litters), 3) offspring from F1 DIO mother × F1 CON father (mDIO/pCON, n = 9 litters), and 4) offspring from F1 DIO mother and father (mDIO/pDIO, n = 9 litters). F2 offspring were weighed at birth and remained with their mother for 3 weeks after which they were weaned onto standard chow and weighed monthly. After glucose tolerance testing at 6 months, offspring were killed and tissues dissected and weighed.

Plasma measurements

Intraperitoneal glucose tolerance tests were performed after a 6-hour fast. A fasting tail nick blood sample was taken immediately before glucose injection and within 1 minute of cage disturbance, after which mice received an ip injection of glucose (2 g/kg body weight). Tail blood was taken into EDTA-coated tubes at 15, 30, 60, and 90 minutes after glucose injection. Blood samples were placed on ice and centrifuged at 2.3*g* for 10 minutes at 4°C, and plasma was stored at −20°C. Plasma glucose levels were determined by the hexokinase/glucose-6-phosphate dehydrogenase method (Thermo Fisher Scientific, Loughborough, United Kingdom) and plasma insulin by ELISA (Crystal Chem Inc, Downers Grove, Illinois). Plasma triglyceride (fasting) and cholesterol levels were measured using an enzymatic assay fol-

Table 1. Food Intake, Body and Organ Weights, and Plasma Lipids in Nonpregnant and Pregnant Females[a]

	Control Diet (CON)	Cafeteria Diet (DIO)	*P* value
Nonpregnant			
Food intake/d, g	2.53 ± 0.12	2.77 ± 0.08	.17
Energy intake/d, kcal	10.31 ± 0.51	15.45 ± 0.46	.002
Weight at cull, g	23.32 ± 0.47	35.18 ± 1.13	<.0001
Liver weight, g	0.94 ± 0.05	1.15 ± 0.11	.11
Mesenteric fat weight, g	0.16 ± 0.02	0.62 ± 0.07	<.01
Subcutaneous fat weight, g	0.16 ± 0.01	0.59 ± 0.11	<.001
Retroperitoneal fat weight, g	0.036 ± 0.003	0.18 ± 0.03	<.001
Plasma cholesterol, mmol/L	2.18 ± 0.16	3.67 ± 0.21	<.001
Plasma triglycerides, mmol/L	0.54 ± 0.03	0.68 ± 0.04	.017
Pregnant			
Body weight at mating, g	22.17 ± 0.37	30.16 ± 0.88	<.0001
Body weight E10, g	25.21 ± 0.44	32.31 ± 0.99	.006
Body weight E18, g	33.89 ± 0.39	38.34 ± 1.09	<.001
Weight gain in pregnancy E1--E18, g	11.72 ± 0.56	8.18 ± 1.12	.006
Food intake E1--E18, g	51.99 ± 1.536	39.17 ± 2.692	.001
Energy intake E1--E18, kcal	207.6 ± 3.6	213.8 ± 12.2	.65
Energy intake/d E1--E18, kcal	11.75 ± 0.35	12.16 ± 0.81	.65
Protein intake E1--E18, g	8.57 ± 0.14	8.84 ± 0.50	.60
Maternal body weight at E18 (uterus removed), g	26.1 ± 1.4	27.8 ± 0.9	.33
Liver weight at E18, g	1.58 ± 0.06	2.15 ± 0.20	.04
Mesenteric fat weight at E18, g	0.19 ± 0.02	0.19 ± 0.01	.12
Retroperitoneal fat weight at E18, g	0.06 ± 0.01	0.11 ± 0.02	.30
Subcutaneous fat weight at E18, g	0.23 ± 0.03	0.36 ± 0.07	.33
Plasma cholesterol at E18, mmol/L	1.41 ± 0.09	2.53 ± 0.17	<.001
Plasma triglycerides at E18, mmol/L	0.73 ± 0.02	0.77 ± 0.07	.61

For nonpregnant females, n = 6 per group and 7 per group for pregnant females

lowing the manufacturer's instructions (Infinity kits; Thermo Fisher Scientific). Plasma corticosterone concentrations were measured as previously described (27).

Quantification of mRNA by real-time PCR

Total RNA was extracted from snap-frozen liver using an RNeasy mini kit (QIAGEN, Crawley, United Kingdom) following the manufacturer's instructions. Total RNA (500 ng) was reverse transcribed using a Promega reverse transcription kit (Promega United Kingdom Ltd, Southampton, United Kingdom). Real-time PCR was performed using either the UPL system from Roche Diagnostics Ltd (Burgess Hill, United Kingdom) or predesigned assays from Applied Biosystems (Warrington, United Kingdom), using the Roche LightCycler 480 as previously described (28). Primer sequences and assay details are listed in Supplemental Table 2. Results were normalized to the expression of the housekeeping gene cyclophilin (Applied Biosystems gene expression assay Mm02342430_gl).

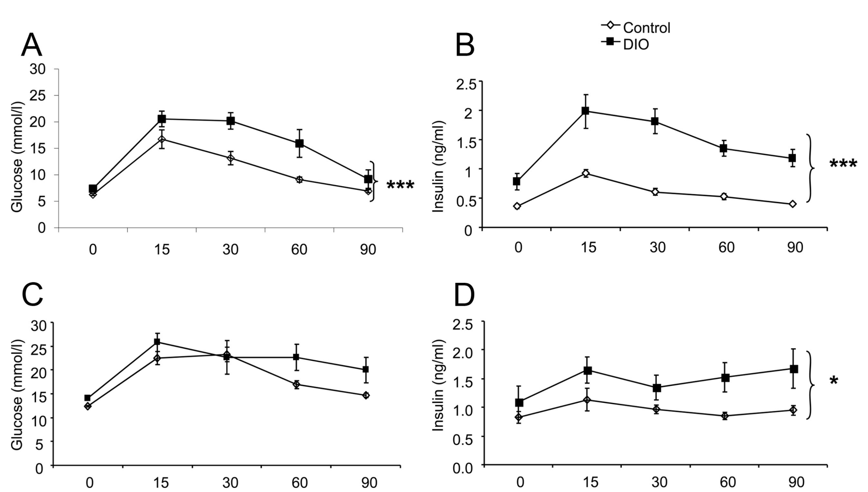

Figure 1. A and B, Glucose tolerance tests in nonpregnant and pregnant females at E18.5. DIO females were both hyperglycemic (A) and hyperinsulinemic (B) before pregnancy. C and D, DIO females were no longer hyperglycemic (C) but remained hyperinsulinemic (D) at E18.5. Statistical analysis was by unpaired *t* tests for areas under the curve; data are mean ± SEM for n = 6 per group for nonpregnant and 7 per group for pregnant females. *, *P* < .05; ***, *P* < .005.

Measurement of hepatic triglyceride content

Liver tissue (~100 mg) was digested at 55°C overnight in 10% (wt/vol) potassium hydroxide in ethanol; undigested tissue was removed by centrifugation at 10 000*g* for 5 minutes. Supernatant was removed and mixed with 1M magnesium chloride (1:1), and samples were then vortexed, incubated on ice for 10 minutes, and centrifuged at 10 000*g* for 5 minutes. The supernatant was used to measure triglyceride using an enzymatic assay following the manufacturer's instructions (Infinity kits; Thermo Fisher Scientific).

Statistics

Data are expressed as mean ± SEM. Groups were compared by independent *t* tests, 1-way ANOVA with post hoc least significant difference testing, area under the curve, 2-way ANOVA or repeated-measures ANOVA as appropriate.

Results

Maternal weight gain and metabolic status prepregnancy

Nonpregnant females on the cafeteria diet (DIO) consumed more calories than CON (Table 1) and were significantly heavier from 7 weeks of age. By 17 weeks, DIO females were 22.9% heavier than CON with increased mesenteric, sc, and retroperitoneal fat pad weights (Table 1). DIO females were hyperglycemic (Figure 1A) and hyperinsulinemic (Figure 1B) on glucose tolerance testing and had increased circulating levels of cholesterol and triglycerides (Table 1).

Maternal dietary intake, weight gain, and metabolic status during pregnancy

During pregnancy, DIO females consumed the same number of calories per day as CON females. Although DIO females were significantly heavier at embryonic day 10.5 (E10.5) and E18.5 (Table 1), by E18.5, once the uteri containing pups had been removed, there was no longer a difference in maternal weights between groups. Additionally, there were no longer any significant differences in the weights of mesenteric, sc, or retroperitoneal fat pads between the groups at E18.5 (Table 1). By E18.5, glucose and insulin concentrations were higher in both groups than in the nonpregnant state; however, glucose levels in pregnant DIO females were no longer different from CON females (Figure 1C), although insulin (Figure 1D) and cholesterol (Table 1) concentrations were still higher in DIO females compared with CON females.

Effect of maternal obesity on F1 offspring

There was no difference in gestation length (CON 21.2 ± 0.2 vs DIO 20.5 ± 0.3 days, $P = .08$), although DIO females had fewer offspring than CON females (CON 6.5 ± 0.5 pups per litter from 21 litters vs DIO 5.0 ± 0.4 pups per litter from 22 litters, $P = .033$). Maternal DIO did not affect birth weight of male offspring (F1 CON 1.32 ± 0.02 g [62 pups from 21 litters] vs F1 DIO 1.28 ± 0.03 g [38 pups from 22 litters], $P = .25$); however, there was a reduction in birth weight of females (F1 CON 1.28 ± 0.02 g [69 pups from 21 litters] vs F1 DIO 1.21 ± 0.03 g [53 pups from 22 litters], $P = .039$). By weaning, both male and female F1 DIO offspring were significantly heavier than F1 CON offspring (F1 CON males 8.90 ± 0.56 g [n = 31] vs F1 DIO males 13.10 ± 0.64 g [n = 19], $P = .001$; and F1 CON females 7.87 ± 0.15 g [n = 41] vs F1 DIO females 10.01 ± 0.17 g [n = 42], $P < .0001$); however, this weight difference did not persist, so that at 3 and 6 months of age, there were no differences in body weight in male F1 DIO offspring, and indeed, female F1 DIO offspring were lighter than F1 CON at 6 months (Figure 2). There were no differences in food intake between F1 CON and F1 DIO offspring in either sex (F1 CON males 3.65 ± 0.06 vs F1 DIO males 3.83 ± 0.07 g/d, $P = .08$; and F1 CON females 3.11 ± 0.04 vs F1 DIO females 3.09 ± 0.07 g/d, $P = .76$). No differences were found in organ weights, including fat pad weight, in either sex at 6 months (Supplemental Table 3).

Maternal DIO had no effects on plasma glucose or insulin concentrations during glucose tolerance testing (Figure 3) or on plasma triglyceride or cholesterol levels at 6 months in male or female F1 offspring (Supplemental Table 3). We analyzed expression of key genes implicated in programming effects in F1 offspring liver at 6 months of age. These included genes important in glucose and lipid metabolism (lipoprotein lipase [*LPL*], phosphoenolpyruvate carboxykinase [*PEPCK*] and peroxisome proliferator-activated receptor [PPAR]-α [*PPAR*α]) and genes involved in glucocorticoid signaling and metabolism (glucocorticoid receptor [*GR*], 5α-reductase [*5*α*r*], 5β-reductase [*5*β*r*], and 11β-hydroxysteroid dehydrogenase type 1 [*Hsd11b1*]). There were no effects of maternal DIO on hepatic gene expression in either male (Figure 4A) or female (Figure 4B) F1 offspring at 6 months of age.

Effects on F2 offspring

The F1 offspring of DIO and CON mothers, without any further manipulations, were mated in all combinations to generate a second generation of offspring. There were no differences in litter numbers or gestation length in any group. To determine any differences between F2 offspring groups, initial analysis was performed by 1-way

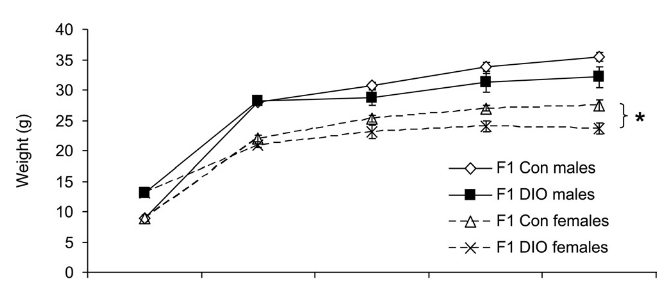

Figure 2. Postweaning body weight in F1 offspring of lean and obese females. Both male and female F1 offspring of DIO mothers were heavier than controls at weaning (3 weeks). However, by 6 months, there were no differences in weight in males, whereas female offspring of DIO mothers were lighter than offspring of CON mothers. Data are mean ± SEM and were analyzed by repeated-measures ANOVA. *, $P < .05$ (n = 5–10 per group).

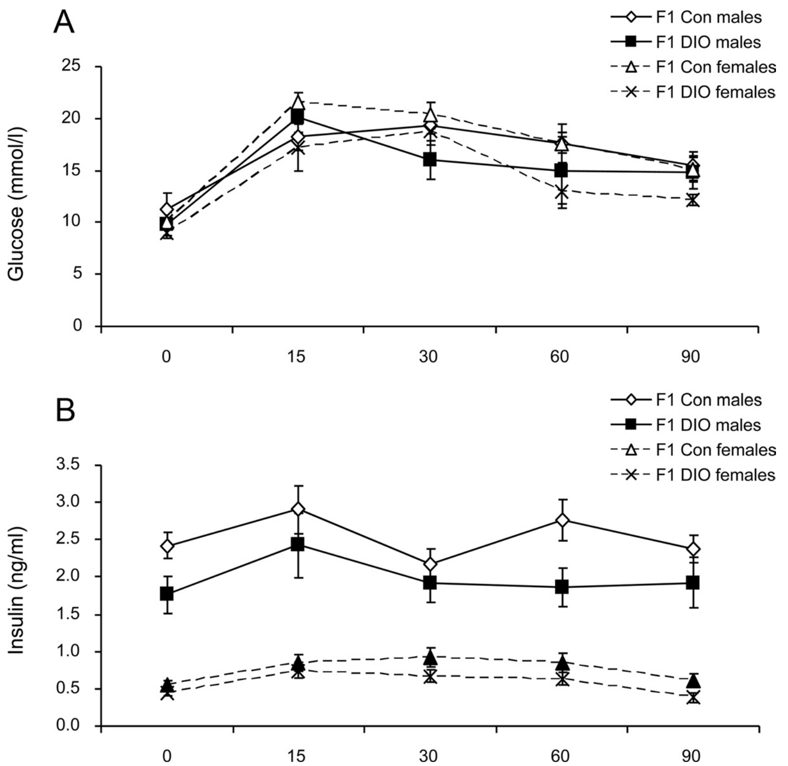

Figure 3. Glucose tolerance tests in F1 offspring males and females at 6 months of age. A, Plasma glucose. B, Plasma insulin. There was no statistically significant difference in plasma glucose or insulin concentrations between groups in male or female offspring. Data are mean ± SEM and were analyzed by unpaired *t* tests for areas under the curve (n = 5-10 per group).

ANOVA. Birth weight was reduced in all male DIO F2 offspring groups compared with mCON/pCON offspring (Table 2; $F = 3.61$, $P = .015$). Among female offspring, birth weight was lower in mDIO/pDIO F2 offspring compared with mCON/pCON (Table 2; $F = 3.39$, $P = .02$). At weaning, there had been catch-up growth in the lower-birth-weight DIO F2 groups such that there were no persisting group differences in weight in either male or female pups (Table 2). At 6 months of age, although there were no differences in body weight in F2 male offspring (Table 2), there was an increase in the weight of sc adipose tissue in mDIO/pDIO and mDIO/pCON male offspring compared with mCON/pCON (Table 2; $F = 3.43$, $P = .03$). Additionally, in the F2 males, plasma cholesterol was lower in mDIO/pCON and mCON/pDIO compared with mCON/pCON offspring (Table 2; $F = 3.89$, $P = .002$). In F2 female offspring at 6 months of age, there were no differences in body weight, organ weight, or lipid profile (Table 2).

Recent data suggest that the exposure status of either parent might contribute to the phenotype of F2 offspring, with studies showing that the effects of prenatal glucocorticoid overexposure and prenatal undernutrition can be transmissible to the F2 through either maternal or paternal lines (21, 29). Thus, to determine the contribution to the F2 phenotype that might be attributable to either maternal or paternal early-life exposure to obesity and, additionally, whether there was any interaction between these 2 variables, data were further analyzed by 2-way ANOVA. We found an effect of maternal early-life exposure to obesity to decrease birth weight in F2 males (Table 2; maternal effect $F = 4.30$, $P < .05$) and of paternal early-life exposure to obesity to decrease birth weight in F2 females (Table 2; paternal effect $F = 8.77$, $P < .01$). At 6 months of age, analysis of parental effects revealed an effect of maternal early-life exposure to obesity specifically in male offspring to increase the weight of mesenteric ($F = 4.93$, $P < .05$), sc ($F = 9.87$, $P < .01$), and retroperitoneal ($F = 4.93$, $P < .05$) adipose tissue (Table 2). There was also an effect of maternal early-life exposure to increase insulin levels during glucose tolerance testing in male offspring, with the highest insulin responses to a glucose load in offspring of mothers exposed to obesity in early life (F2 male mDIO/pDIO and F2 male mDIO/pCON; Figure 5B; $F = 9.36$, $P < .01$). In contrast, in F2 females, there was an interaction between maternal and paternal early-life exposure to obesity to reduce insulin levels during glucose tolerance testing, with the lowest insulin responses in F2 female mDIO/pCON and mCON/pDIO offspring (Figure 5D; $F = 5.47$, $P < .05$).

We initially analyzed the hepatic expression of the same genes studied in the F1 generation; however, after a number of significant differences were identified, we extended the analysis to include a number of other genes implicated in hepatic lipid metabolism (acetyl coenzyme-A carboxylase [*ACC*], carnitine palmitoyltransferase 1a, PPAR-γ coactivator 1α [*PGC1α*], fatty acid synthase [*FAS*], and *PPARγ*). Initial analysis of gene expression to identify differences between groups using 1-way ANOVA revealed effects in both male and female F2 offspring at 6 months of age. In males, mRNA encoding *PPARα*, *ACC*, *PGC1α*, *FAS*, and *GR* was reduced in all DIO groups compared with mCON/pCON (*PPARα*: $F = 3.36$, $P = .03$; *ACC*: $F = 36.81$, $P < .001$; *PGC1α*: $F = 10.90$, $P < .001$; *FAS*: $F = 8.53$, $P < .001$; *GR*: $F = 5.81$, $P = .004$) (Figure 6A). In female offspring, there were significant differences in the expression of *PPARα* (reduced in mDIO/pCON: $F = 4.52$, $P = .03$), *LPL* (reduced in mDIO/pCON and

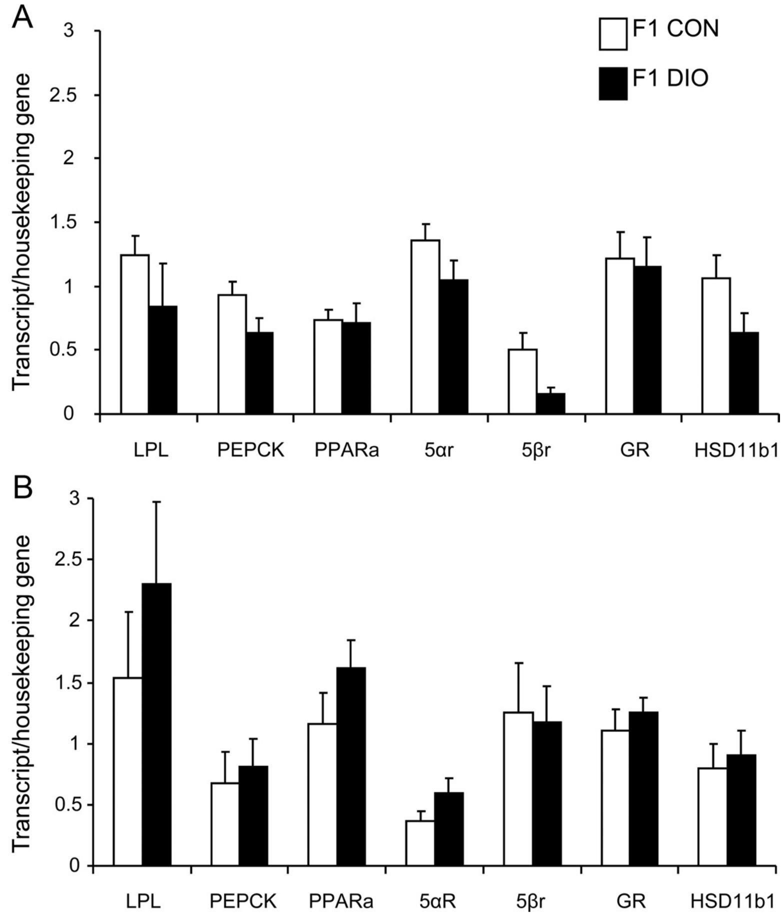

Figure 4. Gene expression analysis in liver from 6-month-old F1 offspring. There were no differences between offspring of DIO and CON mothers in hepatic mRNA expression in F1 males (A) or females (B). Data are mean ± SEM and were analyzed by unpaired Student's *t* testing (n = 7-8 per group).

mCON/pDIO: $F = 4.52$, $P = .01$), *PGC1α* (reduced in mDIO/pCON and mCON/pDIO: $F = 3.48$, $P = .03$), *PEPCK* (reduced in all groups compared with mCON/pCON: $F = 5.94$, $P = .03$), and *GR* (reduced in mCON/pDIO: $F = 5.29$, $P = .005$) (Figure 6B).

To analyze any effect of maternal or paternal early-life exposure to obesity on gene expression, data were reanalyzed using 2-way ANOVA. In male offspring, there was an effect of maternal early-life exposure to obesity to reduce the expression of *PPARα* ($F = 4.49$, $P = .04$), *FAS* ($F = 5.78$, $P = .024$), *ACC* ($F = 35.36$, $P < .001$), and *PGC1α* ($F = 9.02$, $P = .006$) and an effect of paternal early-life exposure to obesity to reduce the expression of *FAS* ($F = 10.57$, $P = .03$), *ACC* ($F = 42.08$, $P < .001$), *PGC1α* ($F = 11.4$, $P = .002$), and *GR* ($F = 12.18$ $P = .002$). In female F2 offspring, there was an interaction between maternal and paternal early-life exposure to obesity to reduce the expression of *LPL* ($F = 9.23$ $P = .005$), *PEPCK* ($F = 11.98$ $P = .002$), *PPARα* ($F = 10.18$ $P = .003$), *PGC1α* ($F = 10.26$, $P = .004$), *GR* ($F = 12.6$ $P = .001$), and *5βr* ($F = 4.67$ $P = .04$). In view of the changes in the expression of genes involved in hepatic lipid metabolism in both sexes, we also quantified hepatic triglyceride content in the F2 offspring; however, there were no differences between groups in either sex (Table 2).

Discussion

Here we show that moderate maternal dietary obesity, sufficient to cause modest insulin resistance, hyperglycemia, and dyslipidemia, and which causes relatively few effects in her immediate offspring, nonetheless produces clear fetal growth restriction and persisting metabolic effects in the otherwise unexposed F2 via both maternal and paternal inheritance. These data echo the striking, if limited, human epidemiological findings from the Overkalix study (19) and suggest that the consequences of the current dietary obesity epidemic may have an impact well beyond the direct effects in obese individuals.

Previous studies have reported inconsistent findings with respect to effects on glucose and insulin homeostasis in offspring of mothers consuming a high-fat diet throughout pregnancy and lactation (9), with a number of studies reporting only transient effects on offspring glucose and insulin levels (30, 31). The inconsistencies between studies have been highlighted in a recent systematic review (9). Experiments have differed in terms of species (rat and mouse) and strain of animal used and the length of time females were exposed to the diet before mating. This is likely to be important in determining the extent of maternal metabolic dysfunction and thus offspring outcome (32). Importantly, the types of diet used and the dietary constituents have differed between studies; some have used diets only high in fat content (with the type of fat differing between studies), or high-fat combined with high-sugar cafeteria diets (9). Notably, many studies have used standard chow as the control diet, which may additionally differ from the experimental diet in both macro- and micronutrient content. We used a cafeteria diet that was high in both sucrose and fat, because the content of both macronutrients is higher in the diets of obese women.

Table 2. Body and Fat Pad Weights and Plasma Lipids in F2 Offspring

	F2 Generation			
	mCON/pCON (n = 11 litters)	**mDIO/pDIO (n = 9 litters)**	**mDIO/pCON (n = 9 litters)**	**mCON/pDIO (n = 8 litters)**
Mother	CON	DIO	DIO	CON
Father	CON	DIO	CON	DIO
Males				
Birth weight, g (number of pups)	1.34 ± 0.02 (37)	1.26 ± 0.02[b] (34)	1.26 ± 0.02[b] (31)	1.27 ± 0.02[b] (26)
Weaning weight, g (n)	10.31 ± 0.26 (11)	9.97 ± 0.39 (10)	9.83 ± 0.25 (13)	10.28 ± 0.041 (10)
Weight at 6 months, g (n)	34.76 ± 0.63 (11)	35.32 ± 0.95 (10)	37.05 ± 0.72 (13)	34.43 ± 1.15 (10)
Mesenteric fat weight, mg/g (n)	0.82 ± 0.05 (7)	0.90 ± 0.07 (8)	0.95 ± 0.07 (9)	0.73 ± 0.07 (9)
Subcutaneous fat weight, mg/g (n)	0.79 ± 0.08 (7)	1.23 ± 0.14[b] (8)	1.11 ± 0.11[b] (9)	0.86 ± 0.09 (9)
Retroperitoneal fat weight, mg/g (n)	0.31 ± 0.05 (7)	0.43 ± 0.05 (8)	0.41 ± 0.04 (9)	0.31 ± 0.04 (9)
Epididymal fat weight, mg/g (n)	0.97 ± 0.14 (7)	1.19 ± 0.11 (8)	1.27 ± 0.07 (9)	1.08 ± 0.12 (9)
Plasma TG, mmol/L (n)	0.54 ± 0.02 (8)	0.47 ± 0.03 (9)	0.65 ± 0.04 (9)	0.63 ± 0.05 (9)
Plasma cholesterol, mmol/L (n)	2.5 ± 0.33 (8)	2.35 ± 0.16 (9)	1.82 ± 0.16[b] (9)	1.54 ± 0.24[b] (9)
Hepatic TG (nmol/mg) (n)	17.0 ± 1.4 (6)	17.6 ± 1.3 (5)	16.7 ± 1.8 (6)	18.2 ± 3.1 (5)
Females				
Birth weight, g (number of pups)	1.31 ± 0.02 (42)	1.23 ± 0.02[b] (28)	1.31 ± 0.02 (30)	1.27 ± 0.02 (29)
Weaning weight, g (n)	9.97 ± 0.33 (10)	10.18 ± 0.22 (9)	9.79 ± 0.27 (11)	9.72 ± 0.41 (8)
Weight at 6 months, g (n)	27.10 ± 0.94 (10)	27.08 ± 0.80 (9)	26.66 ± 0.75 (11)	26.10 ± 0.62 (8)
Mesenteric fat weight, mg/g (n)	0.65 ± 0.06 (8)	0.68 ± 0.05 (8)	0.65 ± 0.07 (8)	0.56 ± 0.06 (8)
Subcutaneous fat weight, mg/g (n)	1.15 ± 0.17 (8)	0.94 ± 0.08 (8)	1.05 ± 0.14 (8)	0.88 ± 0.09 (8)
Retroperitoneal fat weight, mg/g (n)	0.34 ± 0.06 (8)	0.34 ± 0.09 (8)	0.29 ± 0.07 (8)	0.19 ± 0.04 (8)
Plasma TG, mmol/L (n)	0.55 ± 0.08 (8)	0.59 ± 0.08 (9)	0.55 ± 0.04 (9)	0.48 ± 0.05 (8)
Plasma cholesterol, mmol/L (n)	1.35 ± 0.40 (8)	1.99 ± 0.28 (9)	1.76 ± 0.24 (9)	1.35 ± 0.22 (8)
Hepatic TG (nmol/mg) (n)	28.1 ± 2.8 (6)	18.8 ± 2.3 (6)	25.9 ± 5.3 (6)	25.7 ± 3.6 (6)

[a] Organ weight is expressed relative to total body weight. Analysis was performed using 1-way ANOVA.
[b] Significant differences compared with mCON/pCON.

The control diet was matched for protein and micronutrient content to minimize variation in maternal nutritional intake between groups and additionally to avoid low protein intake in the cafeteria diet group during pregnancy, which is important given the known programming effects of prenatal exposure to a low-protein diet (33). Cafeteria-fed females were heavier, with increased fat pad weights, hyperinsulinemia, hyperglycemia, and hyperlipidemia before pregnancy. Although the weights and metabolic status of control and cafeteria-fed females are consistent with those in previous studies using different diets, which report effects on offspring body weight, appetite, glucose-insulin homeostasis, and blood pressure (12), we observed few effects on F1 offspring phenotype in this model. Future studies are required to determine the role of different dietary constituents and the time at which the maternal diet is introduced in determining offspring outcome.

In late gestation, glucose and insulin concentrations increased in both control and cafeteria-fed females compared with nonpregnant females, consistent with the decline in insulin sensitivity that normally takes place during pregnancy (34). However, this occurred to a lesser extent in cafeteria-fed females, so that although they remained hyperinsulinemic relative to controls, they were no longer hyperglycemic or hypertriglyceridemic. Additionally, whereas cafeteria-fed females consumed more calories per day than controls in the nonpregnant state, during pregnancy, there was no difference in calorie intake between groups and gestational weight gain in cafeteria-fed females was less than that in controls. This reduction in maternal weight gain was not solely due to the reduced litter size in the DIO group because there were no persistent differences in maternal weight or fat pad mass when the uteri were removed. We propose that the lack of difference in calorie intake in pregnancy and the decreased gestational weight gain in DIO females may account, at least in part, for the improvement in metabolic phenotype of the DIO females and may be of critical importance in limiting the metabolic consequences for the offspring. Indeed, in human studies, gestational weight gain has been identified as an important determinant of cardiometabolic outcome in young adult offspring, independent of maternal body mass index (35). Further studies are required to dissect the precise mechanisms accounting for the reduced gestational weight gain in cafeteria-fed females and the apparent protection against metabolic consequences in the offspring.

Birth weight was unchanged in F1 DIO males but decreased in F1 DIO females; however, there was evidence of catch-up growth because both male and female offspring of obese mothers were heavier than controls at weaning. Offspring remained with their mothers until weaning so that exposure to the cafeteria diet occurred both pre- and postnatally. The increased postnatal weight gain in F1 DIO offspring may therefore be due to ingestion of milk that is higher in calorie content and/or increased intake, since a recent study in rats showed that high-fat–fed mothers produce milk with a higher fat content than controls and that their offspring consume more milk (36). The early postnatal period is an important developmental window for the later programming of disease risk; in animal studies, early postnatal overnutrition results in obesity, hypertension, and hyperinsulinemia (37–39), and in humans, rapid weight gain in early infancy is associated with metabolic risk factors in young adulthood (40, 41). Nevertheless, despite both pre- and postnatal exposure and this rapid early postnatal growth in F1 DIO males, there were no persistent differences in weight after weaning in male offspring. There was, however, a sex difference, and although F1 DIO females were heavier at weaning, they were lighter than F1 CON females at 6 months of age. Although there were no differences in organ weight, it is possible that programmed differences in body length (25) could explain the observed weight differences. There were no effects on metabolic parameters in adulthood in either sex; whether programmed effects on metabolism might become evident after a second hit, such as postweaning exposure to a cafeteria diet (42) or with aging, which has been associated with a more pronounced programmed phenotype (43, 44), remains to be explored in this model.

Despite the minimal effects noted in the F1 generation, there were clear impacts on the F2. Grand-maternal DIO acting through the maternal line was associated with reduced birth weight and with increased fat pad weight, increased insulin levels, and altered hepatic gene expression in F2 male offspring, whereas grand-maternal DIO acting through the paternal line reduced birth weight in F2 females. Additionally, there were marked effects on hepatic gene expression in F2 offspring, notably with complex sex-specific effects on the expression of enzymes involved in lipid metabolism. In males, parental exposure to obesity in early life resulted in a decrease in the expression of enzymes involved in de novo lipogenesis (*ACC* and *FAS*) but also in a decrease in the expression of *PPARα*, which would predict increased hepatic lipid accumulation. In females, although parental exposure to obesity in early life was also associated with decreased *PPARα* expression, there was also a decrease in the expression of *LPL*, predicting reduced hepatic lipid accumulation. Additionally, there was an effect of parental exposure to obesity in early life to reduce the expression of *PEPCK*; this would predict reduced hepatic gluconeogenesis and is an intriguing finding given that insulin levels were also reduced in these groups. The expression of *PGC1α* was

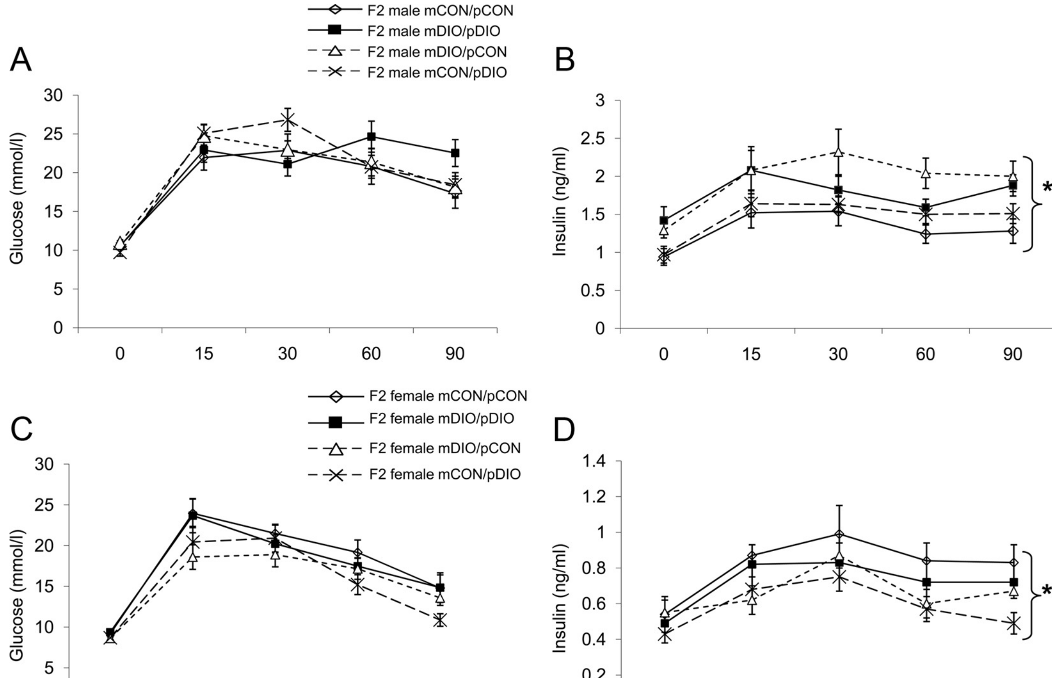

Figure 5. Glucose tolerance tests in F2 offspring at 6 months of age. A and C, There were no effects on plasma glucose concentrations in F2 males (A) or females (C). B and D, However, there was an effect of maternal early-life exposure to obesity to increase insulin concentrations in F2 male offspring (B) (*a, $P < .005$) and an interaction between maternal and paternal early life exposure to obesity to reduce insulin levels in F2 females (D) (*b, $P < .05$). Data are mean ± SEM and were analyzed by 2-way ANOVA for areas under the curve (n = 8–10 per group).

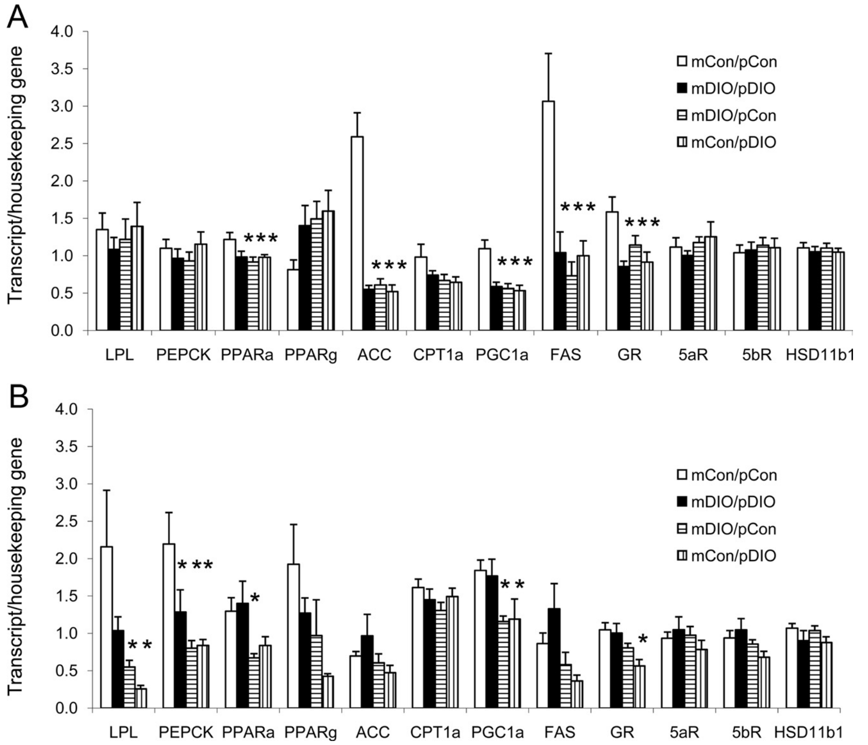

Figure 6. Gene expression analysis in liver from 6-month-old F2 offspring. There were effects on the expression of *GR* and *PPAR*α in F2 male offspring (A) and on the expression of *LPL*, *PPAR*α, and *GR* in females (B). *, Significant difference compared with mCON/pCON. Data were analyzed by one-way ANOVA (n = 7–8 per group).

affected in both male and female F2 offspring. Altered hepatic *PGC1*α expression has been reported in a number of animal models of programming including exposure to a high-fat diet and excess glucocorticoid prenatally (28, 45), and our results suggest that this is also important in the F2. Nevertheless, despite these changes in lipid-metabolizing enzymes, there were no changes in hepatic triglyceride content in either sex.

The transmission of programmed effects to a F2 through both maternal and paternal lines has now been reported in a number of animal models (14, 17, 18, 46); however, in most of these studies, there were also obvious programmed effects in the F1. Delineating multigenerational effects in humans is hampered by the difficulties in determining the relative importance of environmental/social/cultural and genetic effects. There is, however, some evidence for the transmission of effects on birth size and cardiometabolic risk factors through both maternal and paternal lines (15, 19, 47–50). Although many of these studies demonstrate transmission through the female line, in the Swedish Overkalix cohort, the effects of grandparental food availability on cardiovascular and diabetes risk in grand-offspring were transmitted through the paternal lineage (19).

A number of mechanisms may underlie the transmission of programmed effects to subsequent generations (51). Transmission through the maternal line may occur as a consequence of maternal effects (51), because females exposed to an adverse environment in utero may develop programmed effects such as hypertension (52, 53) or glucose-insulin dyshomeostasis (54, 55), which may influence the development of a fetus (51). In this study, although we found no overt phenotype in nonpregnant F1 female offspring, an inability of females to adapt to pregnancy could impact on the developing F2 fetus. This phenomenon has been reported in other animal models of programming (56, 57) and may contribute to the familial risk of type 2 diabetes and hypertension in humans (51). An alternative explanation for the transmission of effects through the maternal and/or paternal line is through direct effects on the developing germ cell (58). The germ cells that will form the F2 generation are present in the developing gonad of the F1 offspring during gestation and are therefore also exposed to, and potentially affected by, the abnormal environment of an obese mother maintained on a cafeteria diet. Indeed, recent studies have suggested that environmentally induced epigenetic modifications may be transmissible through the germ line and may be an important mechanism underlying intergenerational effects (17, 59–61). The mechanisms by which such effects are transmitted through the maternal line specifically to sons and through the paternal line to daughters remain unknown. Differences in the maturation rates of males and females may be important, so that exposure to an altered maternal environment during a critical developmental period may plausibly affect one sex more than the other (62). Alternatively, the F1 mothers may have invested differently in their offspring postnatally, through altered maternal care behavior, because maternal behavior such as pup retrieval may be influenced by pup gender and health (63, 64). However, these mechanisms do not account for the transmission of effects through the paternal line specifically to daughters, although a role for effects on sex chromosomes has been hypothesized (65).

In conclusion, we have shown that maternal obesity/perinatal exposure to a high-fat, high-sugar diet is not necessarily associated with programmed effects on metabolism in the F1 directly exposed offspring. Nevertheless, changes in prenatal growth, body composition, insulin and lipid levels, and gene expression were present in the F2, and these effects were both gender- and parent-of-origin–specific. Although the mechanisms remain to be determined, these findings, if translated into human studies, have profound implications for public health.

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