Diet-Induced Obesity in Female Mice Leads to Offspring Hyperphagia, Adiposity, Hypertension, and Insulin Resistance

A Novel Murine Model of Developmental Programming


Abstract—Maternal obesity is increasingly prevalent and may affect the long-term health of the child. We investigated the effects of maternal diet-induced obesity in mice on offspring metabolic and cardiovascular function. Female C57BL/6J mice were fed either a standard chow (3% fat, 7% sugar) or a palatable obesogenic diet (16% fat, 33% sugar) for 6 weeks before mating and throughout pregnancy and lactation. Offspring of control (OC) and obese dams (OO) were weaned onto standard chow and studied at 3 and 6 months of age. OO were hyperphagic from 4 to 6 weeks of age compared with OC and at 3 months locomotor activity was reduced and adiposity increased (abdominal fat pad mass; \( P < 0.01 \)). OO were heavier than OC at 6 months (body weight, \( P < 0.05 \)). OO abdominal obesity was associated with adipocyte hypertrophy and altered mRNA expression of \( \beta \)-adrenoceptor 2 and 3, 11\HSD-1, and PPAR-\gamma 2. OO showed resistance artery endothelial dysfunction at 3 months, and were hypertensive, as assessed by radiotelemetry (nighttime systolic blood pressure at 6 months [mm Hg] mean±SEM, male OO, 134±1 versus OC, 124±2, \( n = 8, P < 0.05 \); female OO, 137±2 versus OC, 122±4, \( n = 8, P < 0.01 \)). OO skeletal muscle mass (tibialis anterior) was significantly reduced (\( P < 0.01 \)) OO fasting insulin was raised at 3 months and by 6 months fasting plasma glucose was elevated. Exposure to the influences of maternal obesity in the developing mouse led to adult offspring adiposity and cardiovascular and metabolic dysfunction. Developmentally programmed hyperphagia, physical inactivity, and altered adipocyte metabolism may play a mechanistic role. (Hypertension. 2008;51:383-392.)

Key Words: obesity | pregnancy | developmental programming | metabolic syndrome | appetite | blood pressure | mouse

Obesity among women of reproductive age is presenting a critical challenge to health care. 29% of USA women aged 20 to 39 years are reported to be clinically obese\(^1\) and there is serious concern in many European countries over the increasing obesity among young women.\(^2\)

While obesity is associated with increased risk of almost every common complication of pregnancy, obesity in the mother may play a direct role in transmission of an obesogenic and diabetogenic trait from generation to generation. Increasing evidence suggests that children born of pregnancies complicated by either obesity or related gestational diabetes mellitus (GDM) are at increased risk of obesity, impaired glucose tolerance, and other facets of the metabolic syndrome.\(^3\)

Animal models have proven invaluable in interrogation of associations between maternal diet and body composition and offspring phenotype.\(^4\) Those studies which have addressed effects of maternal calorific excess, including several from our laboratory, have generally fed rats diets rich in animal fat.\(^4-7\) Because young women of reproductive age often consume excessive amounts of sugars as well as fats,\(^8\) the relevance of a diet rich in fat alone is limited. In this study, we induced obesity by feeding mice a highly palatable diet rich in sugars and animal fat, and addressed the hypothesis that diet-induced obesity during pregnancy can transmit a propensity for adiposity, glucose intolerance, and cardiovascular dysfunction to the offspring. Obesity was induced in female mice and offspring cardiovascular and metabolic function...
was assessed at 3 and 6 months of age using radio-telemetry, small vessel myography, and assessment of glucose tolerance. Because it has been suggested that offspring adipocyte metabolism may be permanently influenced by maternal nutritional status, and contribute to adiposity,9,10 adipocyte morphology and expression of relevant adipocyte genes was determined. In view of the potential role of capillary rarefaction in hypertension and metabolic syndrome, and previous implication in developmental programming,11 capillary density in skeletal muscle was also investigated.

Methods

Experimental Animals

All studies were approved by Local Ethics Committee, and conducted under UK Home Office License. Female C57BL/6J mice, proven breeders (one previous litter) and approximately 100 days old (Charles River Laboratories, UK) were maintained under controlled conditions (25°C, 12-hour light/dark cycle) and fed either a standard chow diet (7% simple sugars, 3% fat, 50% polysaccharide, 15% protein [w/w] RM1, Special Dietary Services, energy 3.5 kcal/g, n=20), or a semisynthetic energy-rich and highly palatable obesogenic diet (10% simple sugars, 20% animal lard 28% polysaccharide, 23% protein [w/w], Special Dietary Services, energy 4.5 kcal/g, n=30). The pelleted obesogenic diet was supplemented by ad libitum access to sweetened condensed milk (approx 55% simple sugar, 8% fat, 8% protein, w/w, Nestle) with added micronutrient mineral mix (AIN93G, Special Dietary Services). Macronutrient and calorific intake were calculated from measured daily intake of pellets and milk (approx 16% fat, 33% simple sugars, 15% protein, energy 4.0 kcal/g). After 6 weeks on the diet, animals were mated (day 0 pregnancy signified by the appearance of a copulation plug), and maintained on the obesogenic diet throughout gestation and suckling. Weight gain and food intake were measured throughout gestation. 48 hours after delivery litters larger than 6 pups were reduced to 6 (litters less than 4 not used), with equal sex ratios where possible. At
3 weeks, all pups were weaned onto standard chow. Samples of milk were obtained under anesthesia (isofluorane) without recovery from dams at weaning (for measurement of leptin) after injection of oxytocin. Body composition analysis was also performed in a subgroup of dams at weaning by dual energy X-ray absorptiometry (DEXA, Lunar PIXIImus; Integrity Medical Systems Inc).

A subgroup of dams (n = 9) were euthanized at gestational day 18 (G18) and nonfasted blood sampled for insulin, glucose, leptin, cholesterol, and triglyceride analysis.

Offspring body weight and food intake were recorded weekly, and metabolic and cardiovascular parameters determined at 3 and 6 months in one male and one female per litter. Heart, fat pad (inguinal), liver, and skeletal muscle (tibialis anterior) weights were determined in the same animals at necropsy.

**Plasm analysis**

Fasted plasma insulin, glucose, leptin, triglyceride, and cholesterol concentrations were assessed after cardiac puncture was performed on OO and OC (please see http://hyper.ahajournals.org).

**Radiotelemetry**

Cardiovascular function was assessed by remote radio-telemetry in conscious freely moving mice after surgical implantation of probes (TA11PA-C10, O.D 0.4 mm, Data Science International Inc) using carotid artery placement (see supplementary methods for details). Data are reported for one 24-hour period of 7 days recording.

**Vascular Function**

Small mesenteric arteries (approx 200 μm i.d.) were mounted on a myograph (MultiMyograph 610 mol/L, Danish Myo Technology) as previously described (please see the online supplemental material).

**Capillary Density**

Transverse sections (10 μm) were prepared from semitendinosus muscle, snap frozen at necropsy at 3 months. Capillary imaging was carried out by a modified ATPase staining method and visualized by light microscopy. Capillaries and muscle fibers from 3 random areas from each of 3 sections from each muscle were counted (Scion Image software).
Glucose Tolerance Test
Glucose tolerance was assessed after a single bolus of glucose (1g/kg, IP) after overnight fast and tail venous blood glucose concentration monitored at 0, 15, 30, 60, 90, and 120 minutes in conscious semirestrained animals (GlucoMen Sensor, A.Menarini Diagnostics).

Pancreatic Insulin Content
The snap frozen whole pancreas was homogenized in acid ethanol. Insulin content was determined by RIA14 and expressed as insulin content/mg tissue weight.

Adipose Cell Size
Adipocyte size was estimated in inguinal fat tissue from 3-month mice. Adipocytes were fixed as described by Etherton et al15 (see supplementary methods). Cell size was determined using ZEISS KS 400 3.0 software using an Axioskop2 Mot Plus Zeiss microscope (Carl Zeiss GmbH).

Adipose Gene Expression
Real-time polymerase chain reaction (PCR) in adipose tissue was performed using standard laboratory techniques in RNA extracted from inguinal fat tissue from 3-month-old mice (for details and primer sequences of genes investigated please see the online data supplement).

Statistical Analysis
Data are expressed as mean±SEM and compared using ANOVA or Student t test unless otherwise stated. P<0.05 was regarded as significant. In all cases “n” refers to the numbers of litters in each group with one male and one female from a litter used for each experiment.

Results
Maternal
Calorific intake and weight gain were significantly greater in dams fed the obesogenic diet compared with chow fed controls (Figure 1A and 1B). Although maternal weights converged at the end of gestation, subscapular brown adipose tissue mass was increased in late pregnancy (BAT weight [g] OC, 0.10±0.005 versus OO, 0.15±0.02, P<0.05) and abdominal fat mass was 4-fold greater relative to controls (WAT weight, G18 [g] OC, 1.02±0.08 versus OO, 4.00±0.42, P<0.0001). At G18, obese dams were normoglycaemic but were hyperinsulinaemic and hyperleptinaemic (Figure 1C). After suckling and despite a decline in WAT mass from pregnancy in both groups (WAT weight at weaning [g] OC, 0.28±0.07 versus OO, 1.43±0.04, P<0.0001) obese dams demonstrated hyperleptinaemia, hyperinsulinaemia with hyperglycaemia, and hypercholesterolaemia (Figure 1D). The milk leptin concentration was significantly raised at weaning (Leptin [ng/mL] OC, 1.9±0.2 versus OO, 4.7±1.5, P<0.05, n=3). Maternal obesity was confirmed by DEXA at weaning (2.7-fold increase in fat mass, Figure 1E). Conception rates were reduced in the obese group (75% versus 90% in control), but 48-hour litter size was similar (OC, 5.9±0.4 versus OO, 5.6±0.3, PNS). OO neonates were heavier than OC at 48 hours (males and females combined, weight [g] OC, 1.31±0.04 versus OO, 1.66±0.05, P<0.01, n=16) and at weaning (male, weight [g] OC, 9.06±0.2 versus OO, 12.6±0.6, P<0.001, n=8, OO, P<0.001; females P=NS).

Offspring
Energy intake and body composition calorific intake was greater in OO versus OC from 4 to 6 weeks, before a significant increase in body weight in OO (males at 12 weeks; females transiently 5 weeks; Figure 2A and 2B). Male and female OO showed a marked increase in abdominal inguinal fat pad mass at 3 and 6 months (Figure 2C; Table). There was a significant reduction in mass of the tibialis anterior muscle at 3 and 6 months (Table).

Plasma Analytes
At 3 months, OO males and females had significantly elevated plasma triglyceride, insulin, and leptin concentrations relative to OC. By 6 months, OO were hyperglycaemic and plasma insulin was significantly reduced. Leptin remained significantly elevated and cholesterol was also raised. Triglycerides were not different from OC (Figure 2D).

Blood Pressure, Heart Rate, and Activity
At 3 months, nighttime (active phase) systolic blood pressure (SBP) was significantly higher in male and female OO versus OC (Figure 3A). Diastolic blood pressure (DBP) was increased in male OO only (data not shown). Daytime SBP and DBP did not differ significantly in either group. Nighttime mean arterial pressure (MAP) was significantly raised in males and female OO versus OC (male OO, MAP [mm Hg] 111±2 versus OC, 98±1, P<0.001, female OO, 115±2 versus OC, 107±3, P<0.02, n=6 to 8). Male and female nighttime heart rate was reduced in OO versus OC (Figure 3B). Locomotor activity during the nighttime was signifi-
cantly reduced in 3-month male and female OO versus OC (Figure 3C). At 6 months, nighttime, but not daytime, SBP was significantly higher in male and female OO versus OC (Figure 4A). ANOVA showed an association with gender and systolic blood pressure in OO, with a greater effect in female OO. Nighttime mean arterial pressure (MAP) was significantly raised in males and female OO versus OC (male OO, MAP [mm Hg] 120±3 versus OC 111±2, *P<0.05, female OO 119±2 versus OC 113±1, **P<0.01, n=6 to 8). Male and female OO heart rate was increased during day and night (Figure 4B). At 6 months only female OO were less active (Figure 4C). Because estrus cycle stage has been related to activity in mice we examined activity over the full week’s recording and found a sustained reduction in activity.

**Resistive Artery Function**

At 3 months, increased reactivity to NA was observed in male and female OO versus OC (Figure 5A). ACh-induced relaxation was significantly impaired (Figure 5B). Endothelium-independent responses to NO were similar in OO and OC (RM ANOVA, P=NS). At 6 months, resistance artery function was similar between groups (data not shown), because of deterioration of the ACh-induced relaxation with age in controls (max relaxation to ACh [%NA-induced tension] male and female combined, OC, 40.3±6.4 n=10 versus OO, 41.4±6.6 [n=12] P=NS).

**Capillary Density**

Capillary:fiber density was similar in OO and OC when examined across the whole muscle area (capillaries per muscle fiber mean±SEM, OC, 1.13±0.07 n=10 versus OO, 1.14±0.09, n=9, P=NS).

**Glucose Tolerance and Pancreatic Insulin Content**

In addition to abnormal fasting glucose and insulin, glucose intolerance was evident at 3 and 6 months in male and female

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**Figure 3.** Cardiovascular parameters at 3 months. Systolic blood pressure (A), heart rate (B), and locomotor activity (C) in 3-month-old male and female offspring of obese dams (OO, closed circles) compared with offspring of control dams (OC, open circles). **P<0.01, *P<0.05 RM ANOVA for dark phase (bar), n=6 to 8 per group.
OO versus OC (Figure 6A and 6B), with a prolonged recovery phase in OO, although only male OO showed a significantly different area under the glucose concentration curve. Pancreatic insulin content was raised in male and female OO versus OC at 3 months. At 6 months female OC (P<0.05), but not male OC insulin content, had declined with age. Male OO insulin content was significantly lower than OC (Figure 6C).

**Adipose Cell Size Distribution**
At 3 months, adipocyte hypertrophy in OO was evident from a rightward shift in cell size distribution compared with OO in adipocytes of inguinal fat tissue (Figure 7A), and a significant increase in mean adipocyte diameter (mean cell size [microns] OC, 48.1±1.60 versus OO, 55.7±1.65, n=3, P<0.05).

**Adipose Gene Expression**
At 3 months mRNA expression of β2 (P<0.05) and β3 (P<0.05) adrenoreceptors and 11-βHSD type 1 (P<0.01) was significantly reduced in OO versus OC mice. PPARγ type 2 expression was significantly increased in OO (P<0.05, Figure 7B).

**Discussion**
In this study we explored the long-term effects of obesity during pregnancy and lactation on cardiovascular and metabolic function of the offspring. Although the detrimental consequences of maternal undernutrition have been extensively studied, little attention has been directed toward maternal overnutrition, with previous emphasis being placed on the consequences for the offspring of a maternal diet rich in fat. In contrast to man, rodents reduce their food intake when fed a fat-rich diet, and in these studies the dams have generally not become overtly obese.20–24 Mice also demonstrate strain differences in response to high-fat diets,25 and rodent models of fat feeding in pregnancy therefore have limited relevance to human obesity. Our current approach was to use a highly palatable diet to induce maternal obesity.
Mice were fed an obesogenic diet, in which 16% of calories were provided by saturated fat, and 30% from simple sugars, equivalent to high values within the normal dietary range in man and hence reflective of human diet and obesity.\textsuperscript{8} Offspring displayed hyperphagia, increased adiposity, and an adult metabolic syndrome–like phenotype. As we have reported previously in offspring of fat fed rat dams,\textsuperscript{12} gender differences were apparent, particularly in relation to glucose homeostasis. To our knowledge this is the first report of persistent effects of maternal diet-induced obesity in mice on offspring cardiovascular and metabolic function. Two previous studies, including some from our own laboratory,\textsuperscript{10} have shown increased fat mass in adult offspring of nonobese rats fed a fat-rich diet during pregnancy and suckling develop increased body fat mass\textsuperscript{6,12,19,22,23} and insulin resistance,\textsuperscript{16,23,24} despite eating a balanced diet from weaning. However, not all of these report an increase in offspring fat mass\textsuperscript{25} and the fatty acid composition of the fat enriched diet appears critical, with excessive maternal saturated fat intake being key.\textsuperscript{16,18} An influence of maternal obesity per se has been suggested by recent studies of Yamashita et al\textsuperscript{10} and Lambin et al,\textsuperscript{10} which have shown increased fat mass in adult wild-type progeny of obese and insulin resistant heterozygous leptin receptor–deficient (Lepr\textsuperscript{+/+}) mice. Complex interactions between dietary and obesity related metabolic sequelae are therefore likely to set in train events leading to offspring obesity. Genotypic susceptibility could play an additive role, as suggested by Levin and colleagues; using rats bred to be obesity prone or resistant, these authors have shown clear interactions between maternal adiposity and offspring genotype in the development of offspring adiposity.\textsuperscript{27}

Several mechanistic pathways may contribute to development of adiposity in the offspring, and we present evidence for both hyperphagia and reduced energy expenditure in the young animals with daily energy intake being increased before increased weight gain, suggesting hyperphagia as the driving factor. Bayol et al recently demonstrated increased energy intake in offspring of “junk food”–fed rats, but in contrast to the present study, this was only evident when offspring were presented with a highly palatable hypercalorific diet, indicative of programming of food preference for sugary and fatty items, rather than hyperphagia.\textsuperscript{28} The suckling period in rodents has been identified as a critical time window for development of hyperphagia. Postnatal overfeeding induced by restriction of litter size immediately postpartum\textsuperscript{29} or by cross-fostering growth restricted offspring to normal dams\textsuperscript{8} is associated with increased food intake in adult offspring, and offspring of diabetic rats exposed to transient neonatal hyperinsulinaemia develop hyperphagia and adult obesity.\textsuperscript{30} The mice dams in the present study were hyperinsulinaemic and hyperglycaemic at weaning, which would be likely to trigger a neonatal insulin response via milk ingestion. Leptin levels during the suckling period have previously only been implicated in developmental programming of appetite\textsuperscript{31} with exogenous administration of leptin to neonatal mice promoting an obese phenotype.\textsuperscript{32} Further studies using accurate methods of assessment will determine whether metabolic rate is also “programmed” in the OO offspring, particularly because the large increase in energy intake was associated with only a modest rise in body weight.

Male and female offspring of the obese mice were less physically active at 3 months of age. Severe maternal undernutrition in the rat results in offspring with reduced locomotor activity and obesity,\textsuperscript{33} but little attempt has been made to measure physical activity in hypernutritional programming models, although offspring of “junk food”–fed rat dams show no evidence of reduced activity as assessed during the light phase.\textsuperscript{28} The current findings suggest programmed inactivity is likely to contribute to the increased adiposity observed.

Adipocyte hypertrophy was also evident in offspring of obese dams, as previously reported in weanling rats from dams fed an obesogenic diet\textsuperscript{34} but not previously observed in adulthood. Persistent alteration in mRNA expression of PPAR-\gamma2 and the \beta2 and \beta3 adrenergceptors as reported here could increase adipocyte adipogenesis and decrease lipolysis, respectively.\textsuperscript{35,36} Whether acquired through permanent modulation of adipose metabolism in early development or secondary to increased fat mass, these changes could contribute to increased fat mass. The observed decrease in 11\betaHSD-1 would be anticipated to decrease adipocyte differentiation and is contrary to the observation that transgenic mice selectively overexpressing 11\betaHSD-1 are obese.\textsuperscript{37}

The development of glucose intolerance with age, particularly in the male offspring of the obese dams, was
associated with altered plasma and pancreatic insulin content. Fasting plasma insulin was elevated in males and females at 3 months, suggesting insulin resistance. By 6 months the males showed frank diabetes with fasting hyperglycemia, reduced fasting plasma insulin, and reduced pancreatic insulin content, indicative of beta cell exhaustion, as reported previously in offspring of rats fed a lard-rich diet. It remains to be determined whether fetal

Figure 6. Glucose tolerance tests at 3 and 6 months. Blood glucose concentrations after administration of a bolus dose of glucose (1g/kg, i.p.) in 3-month-old male (left panel) and female (right panel) offspring (A); and male (left panel) and female (right panel) offspring of control (OC) and obese dams (OO) at 6 months (n=6 to 8) (B). Offspring of control (OC, open symbols) and obese dams (OO, closed symbols) * P<0.05 area under the glucose curve vs control. Pancreatic insulin content at 3 months (C) and 6 months (D) of age (n=5 to 10). * P<0.05 vs control, † P<0.05, 3 month vs 6 month within group comparison.

Figure 7. Adipose tissue in 3-month offspring. Adipose cell size distribution and gene expression in inguinal fat. Cell diameter expressed as numbers of cells for each size range (P<0.01, Kolmogorov-Smirnov nonparametric test) (A) and mRNA expression of $\beta_2$ and $\beta_3$ adrenoreceptors and 11-$\beta$ hydroxysteroid dehydrogenase ($\beta$HSD-1) and PPAR$\gamma$ type 2 (n=6 to 8) offspring of control (OC, open bars) and obese dams (OO, closed bars) (B). *P<0.05 vs control. mRNA expressed as copy number divided by the geometric mean of 3 reference genes (HPRT, Actin beta, TATA box), multiplied by 1000.
and neonatal pancreatic development is compromised, as shown by others, in neonates of dams fed a fat-rich diet or whether this occurs as a result of offspring adiposity.

To our knowledge this is the first study to demonstrate hypertension in offspring of obese mice. Using the same method of radiotelemetry we previously reported raised blood pressure in rats prenatally exposed to a maternal diet rich in fat. Endothelium-dependent relaxation to acetylcholine was impaired in offspring of obese mice suggesting endothelial dysfunction, and as suggested in other rodent models of developmental programming, this could contribute to the hypertension observed. However, raised blood pressure could also be a direct consequence of the increased fat mass. The observed fall in heart rate at 3 months relative to controls could reflect the normal baroreceptor response to increasing blood pressure, whereas at 6 months the abnormally high heart rate may be indicative of diminished baroreceptor sensitivity with age.

Impaired angiogenesis and microvascular rarefaction has been linked to hypertension in undernutrition models of developmental programming and may also contribute to the development of insulin resistance. Because we found no evidence for reduced capillary density in the skeletal muscle tissue in the offspring of the obese, vascular rarefaction is unlikely to play a role in this model, although the observed reduction in skeletal muscle mass could contribute to insulin resistance.

Perspectives

This study provides the first evidence that maternal diet–induced obesity in the mouse is associated with increased risk of metabolic and cardiovascular disease in the offspring. Mechanistically, persistent changes in hypothalamic energy balance regulatory centers, locomotor behavior, and adipocyte metabolism are likely to play a role in development of offspring adiposity, which in turn could contribute to the hypertension and insulin resistance observed. The underlying molecular basis, including the potential for persistent modulation of relevant gene expression through epigenetic mechanisms, remains to be determined. Whatever the underlying pathways, obesity in mouse pregnancy arising from consumption of a sugar- and fat-rich diet of the proportions often consumed by obese individuals can have persistent and deleterious consequences for the offspring, independent of genetic susceptibility. Corroboration in other mouse strains and species is important, but this model offers the potential for mechanistic and intervention studies relevant to obesity in pregnant women.

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Disclosures

None.

References


