Multiparity leads to obesity and inflammation in mothers and obesity in male offspring

Sandra L. Rebholz, Thomas Jones, Katie T. Burke, Anja Jaeschke, Patrick Tso, David A. D’Alessio, and Laura A. Woollett

Department of Pathology and Laboratory Medicine, University of Cincinnati Medical School, Cincinnati, Ohio

Submitted 15 September 2011; accepted in final form 23 November 2011

Rebholz SL, Jones T, Burke KT, Jaeschke A, Tso P, D’Alessio DA, Woollett LA. Multiparity leads to obesity and inflammation in mothers and obesity in male offspring. Am J Physiol Endocrinol Metab 302: E449–E457, 2012. First published November 29, 2011; doi:10.1152/ajpendo.00487.2011.—Multiparity is an independent risk factor for obesity in parous females. In addition to being a health issue for the mother, offspring of multiparous females may also be at risk for obesity later in life. The aim of the current study was to establish a mouse model that mimics the human pathology of multiparity and determine the effects of multiparity-induced obesity (MIO) on offspring in adulthood. C57BL/6 mice were mated and studied when primiparous (1st pregnancy) or multiparous (4th pregnancy). Dams became obese with multiparity, an effect that was independent of the age of the dam. Multiparous dams also had increased markers of inflammation (JNK activation, cytokine expression) in adipose tissue and liver that was greater than inflammation in nulliparous females made obese with a high-fat diet. Placental inflammation was prevalent in multiparous vs. primiparous dams as well. Male offspring of the multiparous dams developed increased adiposity by 24 wk of age relative to the progeny of primiparous dams, although food consumption was similar in both groups. Lipid metabolism was altered in liver and fat in that mRNA levels of regulatory genes (PGC-1α) as well as metabolic genes (CPT I) and Akt phosphorylation were decreased in offspring of multiparous dams. Thus, in mice, as in humans, multiparity increases adiposity and is associated with hepatic and placental inflammation and abnormal glucose tolerance. Importantly, MIO leads to increased body fat and metabolic dysfunction in the offspring, suggesting a role in the propagation of obesity.

Obesity has become an epidemic in developed countries and is on the rise in underdeveloped countries. Consequently, a significant number of women in their reproductive years, including those that are pregnant, are obese or overweight (30, 31). Obesity during gestation poses a significant clinical problem since it is linked to a spectrum of maternal complications, including gestational diabetes, exaggerated inflammation, hypertension, thromboembolism, preeclampsia, and delivery complications (26, 31, 55, 62, 69).

Multiparity is a cause of weight gain in women during their reproductive years and is in fact an independent predictor of obesity (12, 20). The increase in multiparity-induced obesity (MIO) has followed overall trends in obesity, since gestational weight gain is additive to prepregnancy weight and the inability to lose weight gained postpartum (51, 53, 54). Moreover, overweight women are more likely than leaner women to retain the weight gained during pregnancy (19). Thus, as the population becomes heavier, the effect of multiparity on body weight is amplified. Similar to obesity, pregnancy has been described as an inflammatory state, with the placenta contributing increased levels of cytokines from both the trophoblasts and resident placental macrophages (reviewed in Ref. 22) and with an increase in the numbers of activated monocytes (39). Thus multiparous women may be subjected to repeated episodes of this placental inflammation with adverse consequences. In fact, it is known that women who have had multiple pregnancies are at an increased risk to develop diabetes, independent of visceral adiposity (1), and diabetes has been associated with chronic inflammation (5, 48, 66). Since obese pregnant women have increased inflammation compared with lean pregnant women (10, 59), obese multiparous women could have an even greater inflammatory state.

Gestational obesity is known to cause acute and chronic consequences in the offspring as well as the female itself. Acute consequences range from shoulder dystocia, low Apgar score, macrosomia, congenital defects, and death (42, 46). Chronic consequences include developmental programming, leading to an increased risk of obesity and glucose intolerance in adulthood (reviewed in Refs. 2, 9, 23, and 44). The fact that obesity during pregnancy increases the incidence of obesity in the offspring raises the worrisome prospect of transgenerational perpetuation of obesity (8, 51). Although the mediators of developmental programming of metabolism are not clear, gestational diabetes, maternal overnutrition, circulating factors associated with maternal obesity, or factors associated with inflammation in the placenta or other tissues of obese pregnant females are all possible routes of regulation (2, 9, 23, 44). Although multiparity is a risk factor for obesity, there is little information about the risks of adverse metabolic outcomes in the offspring of these women.

Adiposity is the result of positive energy balance, and caloric intake greater than caloric expenditure. Hunger and satiety are controlled by a number of factors in specific regions in the brain, and changes in activity of any number of these pathways could affect food consumption and thereby lead to altered adiposity. A reduction in energy expenditure could also lead to accelerated fat deposition. As in the brain, a variety of proteins and pathways are involved in peripheral lipid metabolism and energy expenditure. However, one protein has been suggested to be key in the regulation of energy expenditure, and that protein is peroxisome proliferator-activated receptor (PPAR)γ coactivator-1α (PGC-1α; reviewed in Refs. 15, 21, and 25). PGC-1α is upstream of mitochondrial biogenesis and fatty acid oxidation, as well as oxidative phosphorylation and other mitochondrial functions, and as such plays a pivotal role in energy metabolism. PGC-1α coactivates...
mitochondrial biogenesis and fatty acid oxidation with other proteins, i.e., the estrogen-related receptor. Although processes are regulated by protein complexes, levels of PGC-1α are thought to be the driving force for gene transcription of the downstream targets (64).

Thus, the present study was designed in two parts. In the first part, we established a mouse model that mimics MIO in humans. Female parous mice became obese by the 4th pregnancy with exaggerated inflammation. In the second part, we demonstrated that male offspring of these multiparous females have altered metabolism, resulting in increased adiposity. The change in body composition was not the result of a change in input of energy intake but was due to a change in energy output (lipid catabolism). Specifically, we found marked decreases in the mRNA levels of carnitine palmitoyltransferase I (CPT I), the rate-limiting step for fatty acid oxidation. The decrease was likely mediated by decreased levels of direct upstream regulators of CPT I, those being PGC-1α and/or PPARα. Thus, multiparous females with marked obesity and inflammation induce developmental programming, leading to increased adiposity in adult male offspring.

RESEARCH DESIGN AND METHODS

Animals

Pregnant dams. All male and nonpregnant female C57BL/6 mice were purchased from The Jackson Laboratories and fed a pelleted chow (Harlan Laboratories); females were 10 wk old when they arrived. Animals were housed in a temperature- and humidity-controlled room and subjected to 12 h of light and 12 h of darkness. After ≈1 wk acclimation, females were fed a breeder chow that contained 9% fat (wt/wt; PMI Nutrition International) for 1–2 wk prior to mating. To mate females, one male was placed in a cage with three females. The presence of a postcopulatory plug was checked when the lights came on and denoted 0.5 days postconception (dpc). When a plug was detected, female mice were separated into their own cage. After 5 days of mating, males were separated from remaining nonpregnant females. After 2 days, the process was repeated. Females underwent mating until a plug was detected or until three mating cycles were completed. For multiparous females, pups were born and allowed to suckle. Pups were weaned at 21 days after parturition, and females remated within 5 days of the weaning. One group of females was maintained on breeder chow until the same age as the dams mated the fourth time (≈7 mos) and then mated (1st old pregnancy.

At 18.5 dpc of the 1st, 4th, or 1st old pregnancy, either glucose tolerance tests (GTT) were performed on one set of fasted dams or tissues (liver and retroperitoneal, inguinal, and ovarian adipose tissues) were collected from a different set of dams at the beginning of the light phase. At a later time, an additional group of females were fed a high-fat diet starting at 3 mo of age. Diets consisted of 58% fat (calories) primarily as hydrogenated coconut oil plus soybean oil with corn starch (Research Diets); soybean oil was used because this was the primary oil of the breeder chow. These females were never mated, and tissues were collected when nulliparous and multiparous females were the same age.

Male offspring. In a separate set of studies using different dams, pups were born to dams of their first pregnancy and dams of their fourth pregnancy; all litters used had five to nine pups per litter. At weaning, one male pup from each litter was housed individually and fed chow; thus n equals litter. Each week, animal weights and the amounts of food consumed were measured. At 14 wk of age, GTT were performed, followed by body composition measurements by MRI after animals had time to recover. At 24 wk of age, GTT were performed again. After recovery, body composition was determined by MRI. Approximately 1 wk after the MRI, blood and tissues were collected after a 6-h fast. All animal protocols were approved by the Institutional Animal Care and Use Committee at the University of Cincinnati.

GTT

GTT were performed similarly in fasted pregnant females and male offspring (6-h fast). Animals were injected intraperitoneally (ip) with glucose (2 mg/g). Blood was collected from the tail, and glucose was measured in triplicate with a glucometer using glucose strips (Abbott Laboratories) at 0, 15, 30, 60, 120, and 180 min postinjection.

Real-Time PCR

Livers and adipose tissues were collected rapidly in pregnant females (18.5 dpc) or adult offspring (~5 mos of age) after exanguination, snap-frozen in liquid nitrogen, and stored at −80°C until use. Tissue RNA was isolated using TRIzol and stored in FORMAZol at −80°C. RNA was treated with RNase-free DNase I and reverse transcribed to cDNA by SuperScript II reverse transcriptase using random hexamers. PCR assays were performed on a Bio-Rad iCycler iQ real-time PCR Detection System, using SYBR green as our fluorophore; primers will be given upon request. A serial dilution of a randomly picked sample was used to generate a standard curve for each gene examined. This standard curve was used to calculate the relative levels of mRNA for the gene of interest compared with the reference/housekeeping gene (cyclophilin).

Hormone Levels and Adiposity

Insulin concentrations were measured by LINCOpelx by the Mouse Metabolic Phenotype Center (MMPC) of the University of Cincinnati. Adiposity was measured by MRI (Echo MRI; Echo Medical Systems, Houston, TX).

Plasma and Liver Lipid Concentrations

Plasma lipids were measured enzymatically. Plasma was pooled and separated into lipoproteins by fast-protein liquid chromatography using two Sephadex columns in tandem as described (41), and cholesterol was measured in each fraction enzymatically. Lipids were extracted from livers with Folch, and triglyceride content was measured chemically by the MMPC of the University of Cincinnati (6). Cholesterol was extracted from saponified liver and measured by gas chromatography, using stigmastanol as an internal standard (63).

Western Blots

Expression levels of proteins or phosphorylation of proteins were determined by Western blot. Briefly, tissues were homogenized in a lysis buffer. Proteins were then separated by gel electrophoresis, and the relative amounts of phosphorylated (Cell Signaling Technology) and total JNK (Pharmingen), phosphorylated (Cell Signaling Technology) and total AKT (Cell Signaling Technology), and PGC-1α (Novus Biologicals) were determined by Western blotting using appropriate secondary antibodies and enhanced chemiluminescence plus, as described (68); similar gel loading was verified as needed after stripping membranes and reprobing with antibodies to tubulin or β-actin (33).

Statistics

Data are presented as means ± SE. Differences between dams of the 1st vs. 4th or offspring of 1st vs. 4th dams were determined by two-tailed Student’s t-tests (P < 0.05). When three groups of mice were compared, i.e., 1st, 4th, and 1st old pregnancy, animals were compared by one-way ANOVA, followed by Student-Newman-Keuls test to determine differences between groups (P < 0.05).
RESULTS

Effects of Multiparity on Maternal Adiposity and Inflammation

Previous studies have demonstrated that multiparous women become obese (12, 20) and likely have increased inflammation (1). To establish a mouse model that mimics the human pathology of multiparity, we mated C57BL/6 mice once (primiparous) or four times (multiparous). The body weights for multiparous and primiparous dams were 48.5 ± 2.2 and 33.7 ± 0.9 g, respectively. Both the retroperitoneal (RP) and ovarian (OV) fat depots weighed ∼6.5-fold more, and the inguinal (ING) depot weighed ∼3.5-fold more in the multiparous vs. primiparous dams (Fig. 1A). In addition, multiparous mice had increased hepatic lipid content and plasma cholesterol concentrations (Table 1); the increase in plasma cholesterol was due to increased LDL as well as HDL cholesterol (data not shown).

To assess age as a contributing factor for increased adiposity, dams were mated their first time at a similar age as those of the primiparous dams (Fig. 1). In addition, multiparous mice had increased hepatic lipid content and plasma cholesterol concentrations (Table 1); the increase in plasma cholesterol was due to increased LDL as well as HDL cholesterol (data not shown).

Table 1. Metabolite levels in plasma and livers of primiparous and multiparous dams

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1st Pregnancy</th>
<th>4th Pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma glucose, mg/dl</td>
<td>120 ± 11</td>
<td>103 ± 7</td>
</tr>
<tr>
<td>Plasma cholesterol, mg/dl</td>
<td>37.0 ± 2.1</td>
<td>58.9 ± 4.6*</td>
</tr>
<tr>
<td>Liver triglyceride, mg/g</td>
<td>2.55 ± 0.14</td>
<td>2.42 ± 0.12</td>
</tr>
<tr>
<td>Liver cholesterol, mg/g</td>
<td>17.6 ± 1.2</td>
<td>23.5 ± 2.4*</td>
</tr>
</tbody>
</table>

Data are means ± SE. *Significant differences between 1st and 4th dams (P < 0.03).

Fig. 1. Parameters of multiparous and primiparous dams. A: adipose tissue mass of different fat depots of primiparous and multiparous females; primiparous dams were younger (~3 mo of age; 1st pregnancy) or older and age-matched to multiparous dams (~7 mo of age; 1st old pregnancy). Retroperitoneal (RP), inguinal (ING), and ovarian (OV) depots were collected from dams at 18.5 days postconception (dpc). Data are presented as means ± SE (n = 9, 14, and 6 for dams of the 4th, 1st, and 1st old pregnancies, respectively). *Differences (P < 0.05) between the amount of fat in depots compared with primiparous younger dams; #difference (P < 0.05) between the amount of fat in 1st old dams compared with dams of 1st and 4th pregnancies. B: glucose tolerance tests of primiparous and multiparous dams at 18.5 dpc. Glucose tolerance tests were performed as described. Data are presented as means ± SE (n = 5 for dams in both groups). *Differences (P < 0.05) between the concentrations in the 1st vs. 4th pregnancies.

Effects of Multiparity on Offspring Glucose Homeostasis and Adiposity

Knowing that diet-induced obese females have offspring with increased adiposity (2, 9, 23, 44), we next set out to determine whether offspring of multiparous obese dams would be at an increased risk of becoming obese. Offspring of
multiparous dams weighed slightly more at weaning (12.5 ± 0.6 vs. 10.8 ± 0.3 g, \( P = 0.001 \); Fig. 3A), although the differences in neonatal mass disappeared within 1 wk of weaning and were similar during the remainder of the rapid growth phase postweaning. The difference at weaning was not due to litter size, since litters of primiparous dams culled to five pups were the same mass as those not culled (10.3 ± 0.9 g). However, body weight was indeed greater in offspring of multiparous dams than offspring of primiparous dams by 26 wk of age (Fig. 3A). Body composition between offspring of dams at either parity was similar at 14 wk of age (Fig. 3, B–D), but by 24 wk of age the offspring of multiparous dams fed chow had significantly more body fat than the primiparous offspring, without a change in lean mass (Fig. 3, B–D). Food consumption was similar in all offspring, regardless of the parity of the dams (Fig. 4). Together, these data indicate that offspring of multiparous females have increased body weights and elevated adiposity, and these changes are independent of major differences in energy intake.

The male offspring of multiparous mothers with increased adiposity also had increased plasma triglyceride levels (Fig. 5, inset). However, there was no difference in plasma cholesterol levels or the distribution of cholesterol between various lipoprotein fractions (Fig. 5). Hepatic lipid content and liver weights were similar in all offspring as well (data not shown).

Since glucose metabolism and insulin resistance can be affected by adiposity and developmental programming, we next performed GTT. At 25 wk of age, fasting glucose levels were lower in the offspring of primiparous dams (\( P < 0.05 \); Fig. 6A), plasma insulin levels were similar (Fig. 6B), and there was evidence of reduced hepatic Akt phosphorylation, as reflected in lesser hepatic Akt phosphorylation (Fig. 6C). During ip GTT, plasma glucose concentrations had higher peaks in the offspring of multiparous dams (\( P = 0.051 \)), but glucose tolerance was otherwise similar compared with the mice born of primiparous mothers.

**Metabolism of Adipose Tissue and Liver of Offspring**

We used a targeted gene approach to determine the mechanism(s) responsible for the 40% increased adiposity independent of a change in food consumption. Initially, we studied genes involved in synthesis of fat [fatty acid synthase (FAS)] and oxidation of fat (CPT I) of adipose tissue. There was \( \approx 40\% \) decrease in CPT I and \( \approx 45\% \) decrease in FAS mRNA levels in male offspring of multiparous dams compared with male offspring of primiparous dams (Fig. 7, A and B). We next measured levels of genes responsible for the regulation of these genes (Fig. 7, A and B). PGC-1\( \alpha \) mRNA levels were decreased in adipose tissues offspring of multiparous vs primiparous dams, whereas mRNA levels of PGC-1\( \beta \), PPAR\( \gamma \), liver X receptor (LXR), and sterol regulatory element-binding protein-1 (SREBP-1) were not affected. Compared with expression in adipose tissues, CPT I mRNA levels were decreased almost 40% in liver extracts from offspring of multiparous dams, with only modest changes in FAS mRNA levels (Fig. 7B). PGC-1\( \alpha \) mRNA levels were decreased in the livers of offspring of multiparous dams, as occurred in adipose tissue; hepatic PGC-1\( \alpha \) protein levels paralleled mRNA levels (Fig. 7C) and were around twofold greater in the primiparous offspring, as noted by ImageJ software. Unlike adipose tissue, PGC-1\( \beta \) mRNA levels were also reduced significantly in the livers of offspring of multiparous dams, and PPAR\( \alpha \) mRNA levels tended to be less (\( P = 0.069 \)). LXR and SREBP-1 mRNA levels did not differ from the primiparous group. The expression of phosphoenolpyruvate carboxykinase and glucose-6-phosphatase, key enzymes for hepatic glucose pro-
Adverse Effects of Multiparity on Dams and Offspring

In this set of studies, we have demonstrated that multiparity causes weight gain in mice, independent of age. In addition, multiparous females have increased inflammation in liver, adipose tissue, and placenta, processes that are not accounted for by increased adiposity. Multiparity also has metabolic consequences, since these animals were glucose intolerant compared with primiparous dams; this response could be explained by either the greater adiposity, increased inflammation, or some combination of these. In addition to the detrimental affects of obesity and its comorbidities, exaggerated inflammation is detrimental to the multiparous female and increases its risk for additional age-related diseases. For example, atherosclerosis is known to be a disease associated with chronic inflammation. Initial steps in atherosclerotic plaque formation involve recruitment of monocytes to the vessel walls that are then differentiated into macrophages, thereby initiating lesion formation (36, 50). Inflammation is linked with diabetes as well in that some proinflammatory cytokines can blunt insulin signaling, thereby leading to insulin resistance and diabetes (24). It is likely that obesity and all of its comorbidities and inflammation in the multiparous female could be difficult to overcome if inflammation is prominent in the brain because inflammation in the hypothalamus disrupts satiety signals originating in the brain (13, 73), making weight loss difficult.

The metabolic detriments of multiparity were not limited to the mothers but extended to their male offspring. Importantly, male mice born of multiparous mothers had increased levels of body fatness in adulthood. These findings support a model whereby multiple pregnancies induce fixed metabolic stresses on females that have heritable consequences. Since there are...
parallels to some of these findings in humans, our results support yet another mechanism of cross-generational transmission of obesity risk. Interestingly, the differences became apparent when the mice were older, after 14 wk of age, suggesting that excess energy was stored as fat only after growth rate slowed down. Since food consumption was not different in offspring of dams with different parity, differences must have occurred in energy expended. Although we did not examine female offspring in these studies, some of the metabolic parameters measured may have been more exaggerated in the female mice based on previous reports by some (4, 34, 61), but not others (45).

We used a targeted gene approach to study metabolism of catabolic processes in two of the tissues where fatty acid oxidation occurs, the liver and adipose tissue (11, 67, 70). The lower CPT I mRNA levels in the offspring of multiparous dams in both tissues suggest that there is less fatty acid oxidation in the whole body, which has been shown to be related to obesity (38, 56). CPT I transcription was regulated differently in the multiparous offspring since expression of key metabolic regulators PGC-1α, PGC-1β, and PPARα was reduced as well. Since PGC-1α levels appear to be most important in mediating transcription compared with other proteins in the transcriptional complexes (64), reduction in its levels is likely enough to manipulate fatty acid oxidation. In addition to a direct effect on CPT I transcription, PGC-1α can manipulate fatty acid oxidation through affects on mitochondria (15, 21, 25). Although we did not measure mitochondrial activity or number in the current studies, we did find that mRNA levels for proteins expressed in the hepatic mitochondria of offspring of multiparous dams were reduced (cyp7a1: 2.72 ± 0.38 vs. 0.84 ± 0.08; citrate synthase: 4.47 ± 0.81 vs. 1.29 ± 0.11 for offspring of primi- vs. multiparous females, respectively), suggesting a change in mitochondrial function.

PGC-1α is one of the key regulatory proteins in mediating cellular metabolism (reviewed in Refs. 15 and 25), including mitochondrial biogenesis and CPT I transcription. PGC-1α is a coregulator that interacts with various DNA-binding factors, including PPARα/γ, estrogen-related receptors, and nuclear receptor factors, to coordinate the regulation of a variety of genes and processes. PGC-1α will form complexes with these various transcription factors and recruit additional proteins that modify histones and remodel nucleosomes (49). PGC-1α levels and phosphorylation (activation)/acetylation (inactivation) appear to be regulated by the energy and/or nutritional status of the cells and are regulated by PPARs, myocyte enhancer factor 2, forkhead box O, cAMP response element-binding protein, estrogen-related receptors, the adiponectin receptors (adipoR1/R2), AMPK, and sirtuin (15, 25, 27, 71). Results from the current studies parallel studies in which pregnant females were overfed in that offspring of overfed mothers had reduced adipoR1/R2 expression and AMPK activity (7, 57), which can lead to less expression and activation of PGC-1α (27, 71).

PGC-1α levels can also be regulated epigenetically (3, 58). Previous studies have shown that a variety of conditions can methylate the promoter and lead to reduced mRNA levels. Epigenetic changes in genes have been proposed to be involved in the passing on of traits from one generation to another, and methylation of specific genes at birth can predict adiposity later in life (17). Although most common routes by which the epigenome is affected include DNA methylation and histone modification (72), it is likely that histone modification plays a major role in developmental programming via epigenetic regulation (47). The study of epigenetic regulation is complex, since some epigenetic changes can be silent and their action becomes apparent with different diets, stressors, or stages of life (16, 47, 65).

**Fig. 5.** Plasma lipoprotein cholesterol levels in offspring of primi- (1st) or multiparous (4th) dams; animals are the same as those described in Fig. 3. Plasma was collected from offspring at the termination of the study when they were 26 wk of age, and lipoproteins were separated by fast-protein liquid chromatography. Two pooled samples were used, and the results were averaged. Inset: total plasma triglyceride (TG) levels are presented.

**Fig. 6.** Glucose tolerance tests and insulin levels and signaling in offspring of multi- and primiparous dams; animals are the same as those described in Fig. 3. A: glucose tolerance tests were performed on offspring of dams of different parity. *Differences (P < 0.05) between plasma glucose levels at zero time; †nearly significant differences (P = 0.051) at 30 min postinjection. B: plasma insulin levels in offspring of dams of different parity. Data are presented as means ± SE. C: phosphorylated (Akt-p) and total Akt in pooled liver samples of offspring of dams with different parity.
One surprising observation of these studies was that FAS mRNA levels were decreased in adipose tissues and livers with greater adiposity, and the decreases were independent of changes in SREBP-1 and LXR mRNA levels, the typical route of regulation. Contrary to conventional thinking, recent studies show that PGC-1β and PGC-1α can be coactivators of SREBP-1/LXR and thereby affect their downstream targets independent of a change in SREBP-1/LXR levels (37, 60). Thus, the PGCs can affect the sum of lipid metabolism via both catabolic and anabolic pathways, with the greater impact upon oxidation under the current conditions.

An important outcome of these studies is their implication for mouse research. When efficiently maintaining mouse colonies, a female that is a good breeder is often rebred multiple times. However, this female likely develops multiparity-induced obesity and inflammation. Importantly, the offspring of these multiparous females would accrue more fat independent of any dietary or genetic manipulation. Consequently, a difference in parity of the female could be part of the cause of scatter in data in some studies or lack of effects due to lack of significance. Thus, care should be taken when maintaining mouse colonies such that parity of breeding females should be kept equal to minimize scatter and thereby increase significance.

**Summary**

A question that arises from these studies, as well as others, is, “What is mediating the programming?” The mediator of developmental programming is currently unknown but likely includes circulating maternal factors associated with obesity, inflammation, and/or diabetes and/or the placental inflammatory status (2, 9, 23, 44). Some maternal factors that are associated with these diseases have been proposed as potential mediators because they manipulate nutrient transport across the placenta or placental function, thereby affecting fetal metabolism (14, 23, 28, 29, 32, 33). Another potential mechanism is based on the fact that placenta of obese females contain more inflammation (10, 52, 59, 74), and the inflammatory in utero state that the fetus is exposed to may be mediating the effect.

In the current study, the placentas of the multiparous dams were in a more severe proinflammatory state than placentas of the primiparous dams, as depicted by increased JNK activation, and had more macrophages, as do placentas of obese humans, as depicted by increased CD68 and F4/80. Since the studies in mice parallel those in humans and developmental programming did indeed occur, we can use this model to begin to dissect out the role of various maternal factors in mediating programming.

To summarize, these studies support the propagation of the obesity cycle in multiparous females. Since more women are obese at the start of pregnancy, more retain weight during and between pregnancies, thereby developing multiparity-induced obesity. Thus, offspring of the obese multiparous dams with increased inflammation will themselves become obese in adulthood. Interventions targeted to break the obesity cycle could occur at the level of the pregnant female as well as the offspring. One could target inflammation of the pregnant female with various anti-inflammatory treatments, i.e., fish oil or exercise or antiobesity treatments, i.e., food restriction or exercise; it should be noted that the ω-3 fatty acids in fish oil could have a direct impact upon the fetus itself (43). In the offspring, one could target mitochondrial biogenesis or fatty acid oxidation with various factors, such as lipic acid or carnitine (35, 40). The current studies are important in supporting a healthier, less obese population in that we have defined specific metabolic pathways that are likely involved in the programming of obesity and can be targeted in either the mother or her offspring.

**ACKNOWLEDGMENTS**

We thank Jodi Caldwell-Lawson for assistance with the various animal studies.

**GRANTS**

These studies were supported by a grant from the MICROMouse program of the MMPC and Grants HD-34089 and DK-059630 from the National Institutes of Health.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.
ADVERSE EFFECTS OF MULTIPARITY ON DAMS AND OFFSPRING


