The Maternal Environment Programs Postnatal Weight Gain and Glucose Tolerance of Male Offspring, but Placental and Fetal Growth Are Determined by Fetal Genotype in the Leprdb/+ Model of Gestational Diabetes

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Mice heterozygous for a signaling-deficient leptin receptor (Leprdb/+ [db/+]) are widely used as a model of gestational diabetes that results in poor fetal outcomes. This study investigated the importance of fetal genotype (db/+) relative to abnormal maternal metabolism for placental function and therefore fetal growth and offspring health. Wild-type (WT) and db/+ females were mated to db/+ and WT males, respectively, generating litters of mixed genotype. Placentas and fetuses were weighed at embryonic day 18.5; offspring weight, hormone levels, glucose tolerance, and blood pressure were assessed at 3 and 6 months. Pregnant db/+, but not WT, dams had impaired glucose tolerance. The db/+ placentas and fetuses were heavier than WT, but the maternal environment had no effect; WT placentas/fetuses from db/+ mothers were no bigger than WT placentas/fetuses carried by WT mothers. Postnatal weight gain, glucose metabolism, and leptin levels were all influenced by offspring genotype. However, maternal environment affected aspects of offspring health because WT male offspring born to db/+ dams were heavier and had worse glucose tolerance than the sex-matched WT offspring of WT mothers. Blood pressure was not affected by maternal or offspring genotype. These data reveal that studies using the db/+ mouse to model outcomes of pregnancy complicated by gestational diabetes should be mindful of the genetically predisposed fetal/postnatal overgrowth. Although inappropriate for dissecting the effect of maternal hyperglycemia on the contribution of placental function to macrosomia, the db/+ mouse may prove useful for investigating mechanisms underlying programming of suboptimal postnatal weight gain and glucose metabolism by an adverse maternal metabolic environment. (Endocrinology 156: 360–366, 2015)

The incidence of obesity among women of childbearing age has doubled over recent years (1). Prepregnancy weight is associated with the development of gestational diabetes mellitus (GDM), and the frequency of this condition has also increased (2).

Pregnancy complicated by GDM is associated with increased fetal mortality and morbidity (3). Fetal overgrowth (macrosomia) occurs in one third of babies born to such mothers (4). These infants are more likely to experience birth injuries, asphyxia, and postnatal metabolic

Abbreviations: BP, blood pressure; E, embryonic day; GDM, gestational diabetes mellitus; GLUT1, glucose transporter 1; WT, wild-type.
disturbances (5). Furthermore, in utero exposure to an adverse nutrient environment can perpetuate disease; long-term studies have demonstrated that macrosomic offspring have impaired glucose tolerance, increased adiposity, and raised systolic blood pressure (BP) as children and an increased risk of developing diabetes, obesity, and cardiovascular disease as adults (6, 7).

Maternal, and consequently fetal, hyperglycemia undoubtedly plays a role in fetal overgrowth. However, good maternal glucose control does not abolish macrosomia (8), suggesting that increased maternal-to-fetal transfer of other nutrients, for example, lipids and amino acids, may contribute to fetal overgrowth and, importantly, that instead of merely reflecting an increase in nutrient supply, macrosomia may be a consequence of abnormal placental function. Indeed, numerous studies have shown that nutrient metabolism and transport are altered in placentas from pregnancies complicated by GDM (9). Furthermore, placental mass is increased (10), exacerbating augmented nutrient transport by increasing the surface area of the transporting epithelium (syncytiotrophoblast).

Interventions aimed at modulating placental function and thereby preventing fetal macrosomia could be used to halt the transgenerational cycling of diabetes and reduce the consequent global health burden. However, such advances are dependent upon the availability of appropriate models to aid understanding of the role of the placenta in GDM and to test potential therapies. Mice, like humans, have a hemochorial placenta, and previous studies have suggested that a strain that is heterozygous for a signaling-deficient leptin receptor (C57BL/KSJ-Leprdb/+ ) is a good experimental model of GDM. Dams develop diabetes (impaired glucose tolerance and elevated hemoglobin A1c) only during pregnancy (11, 12), and the offspring have significantly greater birth weights (11, 13–15) and deranged metabolism (15) compared with the offspring of wild-type (WT) mothers. These poor outcomes have been attributed to the adverse maternal environment; however, the relative contribution and importance of the fetal genotype (db/+ ) to placental function, and therefore fetal growth and programming, have not been evaluated. This study aimed to determine the usefulness of the db/+ mouse as a model for investigating placental function in pregnancies complicated by the abnormalities in maternal metabolism that occur in GDM.

Materials and Methods

All experimental procedures were conducted in accordance with the Home Office Animals (Scientific Procedures) Act 1986 of the United Kingdom. All animals were maintained with free access to food and water. WT and db/+ females were mated at 12 weeks of age to db/+ and WT males, respectively, to generate litters of mixed genotype; the day a plug was observed was counted as embryonic day 0.5 (E0.5). Some dams (10 WT, 9 db/+ ) were euthanized on E18.5 to enable collection of placentas and fetuses (140 in total), which were weighed and then genotyped, using DNA extracted from tail snips, by sequencing PCR products using primers flanking the Lepr mutation (forward, 5'-CCTTCCCTCCTCCTAAAGTG-3'; reverse, 5'-CAGCAA CGTACACCATTA-3') (16). This analysis revealed that 58 of the placenta/fetus pairs were WT (28 from WT dams; 30 from db/+ mothers) and 82 were db/+ (40 and 42 from WT and db/+ mothers, respectively). WT and db/+ placentas were fixed in 4% paraformaldehyde and then wax-embedded and sectioned (5 μm) for immunohistochemical analysis of leptin (rabbit antimalouse leptin, 1/100; Abcam, UK) and glucose transporters (rabbit antimalouse glucose transporter 1 [GLUT1] 1/500; Millipore, UK) and rabbit antimalouse GLUT3 1/100; Biorbyt, UK). Other dams (6 WT; 10 db/+ ) were allowed to deliver, and pup genotype was determined by analysis of DNA extracted from ear punches obtained at weaning (21 days of age). The F1 offspring were maintained for up to 6 months.

Dams (day 18.5 of pregnancy) and F1 offspring (3 and 6 months) were subjected to a glucose tolerance test (fasted overnight, injected with 2 g glucose/kg ip, and tail vein blood samples collected at 0, 20, 30, 60, 90, and 120 minutes) before being killed. Glucose concentrations were measured using a glucometer (OneTouch Vita), and the 0-minute sample was also used to measure insulin (mouse-specific ELISA; intra-assay coefficient of variation was <10%; Millipore) and leptin (mouse-specific ELISA; intra-assay coefficient of variation was <5%; R&D Systems) levels.

The systolic and diastolic arterial BP of the F1 offspring was measured at 6 months of age by tail-cuff volume pressure recording (CODA system; Kent Scientific Corporation) as previously described (17), ensuring that mice were accustomed to the procedure before collecting the BP readings (average of 5 per animal).

Data are presented as mean ± SEM. Maternal parameters were compared using an independent t test. Data from individual pups (fetal and placental weights) were analyzed using a generalized linear mixed-models approach, with each litter used as a random effect, followed by a sequential Sidak multiple-comparisons test to assess whether there was a significant effect of maternal and/or fetal genotype. Litter data (mean fetal and placental weights) were compared using an independent t test. Data from different litters were analyzed using an independent t test; P < .05 was considered significant. Statistical analyses were performed using IBM SPSS Statistics software (IBM).

Results

The db/+ dams have impaired glucose tolerance

The db/+ dams had lower fasting insulin levels than WT mothers (0.16 ± 0.04 vs 0.31 ± 0.03 ng/mL; P < .05), and analysis of glucose levels confirmed impaired glucose tolerance during pregnancy (area under curve 1339 ± 85 vs 987 ± 16); P < .05). The db/+ mothers had higher circulating leptin levels
(760 ± 50 ng/mL) than WT mothers (148 ± 15 ng/mL; \( P < .05 \)), and both had significantly higher levels than their nonpregnant counterparts (33- and 19-fold, respectively).

**Effect of maternal diabetes on placental and fetal growth**

There was no significant difference in the average litter size of WT and \( db/+ \) dams (6.8 ± 0.6 vs 8.0 ± 0.6, respectively) or in the number of WT and \( db/+ \) fetuses within each litter. Consequently, the total fetal and placental weight carried by WT dams (7375 ± 583 and 558 ± 45 mg, respectively) was similar to that carried by \( db/+ \) mothers (fetal weight, 8534 ± 610 mg; placental weight, 653 ± 46 mg). However, after accounting for the weight of the fetal/placental unit, \( db/+ \) mothers were significantly heavier than their WT counterparts (33.06 ± 0.75 vs 30.37 ± 0.72 g; \( P < .05 \)), which is in keeping with their increase in energy intake reported by other investigators (14). The \( db/+ \) fetuses (\( n = 40 \)) carried by WT dams exhibited a significantly higher (5%) birth weight than their WT littersmates (\( n = 28 \), \( P = .05 \); see Figure 1A for individual pup data and Table 1 for mean litter weights). The \( db/+ \) fetuses (\( n = 42 \)) from \( db/+ \) mothers were also bigger (3%) than their WT counterparts (\( n = 30 \), \( P = .05 \); Figure 1A and Table 1). Surprisingly, maternal genotype had no effect on progeny birth weight. The \( db/+ \) fetuses carried by \( db/+ \) mothers were of similar size to those from WT dams (Figure 1A and Table 1). Moreover, WT pups from \( db/+ \) mothers (offspring/dam combination that most closely models human GDM) were no bigger than WT fetuses carried by WT mothers (Figure 1A and Table 1). Similarly, placenta from \( db/+ \) fetuses (\( n = 82 \)) were larger (\( P < .05 \)) than those of WT fetuses (\( n = 58 \)) irrespective of maternal genotype (Figure 1B and Table 1). Consequently, the fetal to placental weight ratio, commonly used as an indicator of placental efficiency, was the same in all animals (Figure 1C). Immunohistochemical analysis of placental leptin expression revealed that within the labyrinth, the zone of maternal/fetal exchange, the syncytiotrophoblast but not the fetal endothelial cells, stained positively for leptin, whereas expression by the giant cells was heterogeneous. However, there was no striking or consistent difference in the level or pattern of expression between WT or \( db/+ \) placentas from either WT or \( db/+ \) dams (Supplemental Figure 1). Similarly, neither pup nor maternal genotype affected trophoblast or giant cell expression of GLUT1 and GLUT3 (Supplemental Figure 1).

**Effect of maternal diabetes on offspring weight gain, metabolic parameters, and BP**

Initial analysis of F1 offspring weight suggested no difference between those from normal pregnancy (23.72 ± 0.97 and 27.86 ± 1.05 g at 3 and 6 months, respectively) and those born to dams with GDM (24.83 ± 0.78 and 28.56 ± 0.88 g at 3 and 6 months). However, analysis of data accounting for offspring genotype and sex revealed that WT males born to \( db/+ \) mothers are heavier than WT males born to WT mothers (\( P < .05 \)), but the postnatal weight gain of female offspring is not affected by

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**Table 1. Placental and Fetal Weights of E18.5 Litters**

<table>
<thead>
<tr>
<th></th>
<th>WT Mothers (( n = 10 ))</th>
<th>( db/+ ) Mothers (( n = 9 ))</th>
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<tbody>
<tr>
<td></td>
<td>Offspring</td>
<td>+ Offspring</td>
</tr>
<tr>
<td>Placental weight, mg</td>
<td>78.61 ± 1.30</td>
<td>83.80 ± 1.91^p</td>
</tr>
<tr>
<td>Fetal weight, mg</td>
<td>1037 ± 39.6</td>
<td>1122 ± 35.8^b</td>
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\(^{a}\) The mean placental and fetal weight of all the WT or \( db/+ \) offspring within each litter was calculated from 10 WT and 9 \( db/+ \) mothers and are presented as mean ± SEM.

\(^{b}\) \( P < .05 \) vs WT pups from WT dam, paired t test.

\(^{c}\) \( P < .05 \) vs WT pups from \( db/+ \) dam, paired t test.
the maternal environment (Figure 2A). In keeping with our observations of the effect of the \( db / + \) genotype on fetal weight, male and female \( db / + \) offspring, from both normal and complicated pregnancies, are heavier (Figure 2A) than their WT littermates at both 3 and 6 months of age.

A comparison of all offspring born to WT and \( db / + \) mothers showed that at 6 months of age, those from mothers with GDM had significantly lower fasting insulin levels (0.16 ± 0.02 vs 0.21 ± 0.02 ng/mL in WT; \( P < .05 \)) and worse glucose tolerance (area under the curve 1603 ± 69 vs 1420 ± 41) in WT; \( P < .05 \)). Again there was an influence of sex and genotype as the glucose tolerance of WT males born to \( db / + \) mothers was significantly worse than that of WT males from normal pregnancies at both 3 and 6 months (Figure 2B). The \( db / + \) offspring, both male and female, had impaired glucose tolerance, irrespective of the maternal environment, in comparison with their WT littermates (Figure 2B).

Offspring leptin levels were affected by genotype (17.5 ± 2.2 ng/mL in 6-month-old \( db / + \) animals vs 5.4 ± 0.81 ng/mL in WT mice; \( P < .05 \)) rather than sex or maternal environment (Table 2).

At 6 months of age, the systolic, diastolic, and mean arterial BP of the male and female WT offspring from \( db / + \) mothers was similar to that of the sex-matched WT offspring from uncomplicated pregnancies (mean arterial pressure 132 ± 7 vs 148 ± 5 mm Hg, respectively); none of the parameters measured were affected by offspring genotype (Table 3).

**Discussion**

This study shows that although the pregnant \( db / + \) mouse has impaired glucose tolerance, other aspects of its metabolic profile (hypoinsulinemia and severe hyperleptinemia) do not mimic that of women with GDM and that the model is inappropriate for investigating the effect of an adverse metabolic environment on placental function and contribution to fetal growth. However, the model may be useful for dissecting mechanisms underlying maternal programming because the male, but not female, offspring from \( db / + \) mothers were heavier and had impaired glucose tolerance at 6 months of age.

**Figure 2.** Effect of maternal diabetes on postnatal weight gain and glucose tolerance. Offspring (male and female) from WT female/\( db / + \) male or \( db / + \) female/WT male crosses were weighed (A) or subjected to a glucose tolerance test (B) at 3 and 6 months of age. Data are shown as mean ± SEM; \( n \) is the number of offspring analyzed, *, \( P < .05 \) (independent t test). Abbreviation: AUC, area under the curve.
We demonstrate that fetal genotype influences both placental and fetal growth as the weight of db/+ placentas and fetuses, carried by either WT or db/+ mothers, was significantly greater than that of WT placentas. In adipose tissue, leptin mRNA is regulated by the level of leptin receptor expression (18); thus, it is possible that lepr heterozygosity affects placental leptin production and consequently placental development and function, leading to increased fetal growth. Our qualitative assessment of leptin and glucose transporter expression by cells in the area of maternal/fetal exchange did not support differences between WT and db/+ placentas, but we did not investigate fetal leptin levels, the activity of GLUT1 and GLUT3, or the activity of other nutrient transporters such as system A, which is activated by leptin in human placenta (19).

Crucially, however, our data suggest that in this model, fetal genotype is more important than the maternal environment in determining placental and fetal growth as the placental and birth weight of WT fetuses carried by db/+ and WT dams were similar. These data contrast with that of other studies that report that db/+ mothers bear offspring with greater placental (13) and birth (13–15, 20, 21) weights than WT mothers. Differences in experimental design likely explain these discrepant findings. Previous studies either set up matings such that db/+ pups were absent from WT pregnancies (20, 21) or compared all pups born from db/+ vs WT pregnancies without knowledge of pup genotype (13), or commented, without detailing the results, that there were no significant differences in placental and birth weight between WT and db/+ fetuses from the same litter; thus, data from each litter were grouped (14, 15).

Interestingly, the weight of fetuses from db/+ mothers is reported to be greater than that of pups from WT pregnancies even when maternal hyperglycemia was reduced by overexpression of GLUT4 (14). Increased placental growth, and therefore transfer of nutrients, was thought to explain this observation, but the current study does not support this hypothesis. Moreover, administration of leptin to db/+ mothers during late pregnancy reduced their adiposity and circulating glucose levels, but fetal growth was not affected (20). In that study (20), placental and fetal leptin levels were higher in db/+ compared with WT pregnancies, which also points toward fetal genotype as the dominant regulator of placental and fetal growth in this model.

The fact that the db/+ model does not mimic the placental/fetal overgrowth often associated with GDM in women (4) is interesting and suggests that impaired maternal glucose tolerance is not necessarily detrimental to placental function. A study of trophoblasts isolated from normal human placentas at term found that unlike elevated levels of nonesterified fatty acids, raised glucose levels had little effect on placental structure, metabolism, and inflammation (22), suggesting that maternal dyslipidemia is a key determinant of placental dysfunction in pregnancies complicated by diabetes. In our study, db/+ dams were heavier than WT dams at E18.5, which is in keeping with their reported hyperphagia (15, 20), although we did not assess maternal adiposity or profile circulating lipids.

### Table 2. Serum Leptin Levels of Offspring From WT and db/+ Mothers Measured at 6 Months of Age

<table>
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<tr>
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<th>WT Mothers</th>
<th>db/+ Mothers</th>
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<tr>
<td></td>
<td>WT (n = 8)</td>
<td>db/+ (n = 12)</td>
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<tr>
<td>Offspring</td>
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<tr>
<td>Leptin, ng/mL</td>
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<tr>
<td>$\delta$ (n = 4)</td>
<td>4.5 $\pm$ 1.8</td>
<td>7.9 $\pm$ 3.0</td>
</tr>
<tr>
<td>$\gamma$ (n = 4)</td>
<td>16.6 $\pm$ 2.5$^c$</td>
<td>23.3 $\pm$ 6.5$^c$</td>
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</table>

$^a$ Results are shown as mean $\pm$ SEM.
$^b$ Number of offspring.
$^c$ $P < .05$ vs sex-matched WT littermates from WT dam, independent t test.

### Table 3. Systolic and Diastolic BP of Offspring Born to WT and db/+ Mothers Measured at 6 Months of Age

<table>
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<tr>
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<th>WT Mothers</th>
<th>db/+ Mothers</th>
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<tbody>
<tr>
<td></td>
<td>WT (n = 9)</td>
<td>db/+ (n = 12)</td>
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<tr>
<td>Offspring</td>
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<tr>
<td>Systolic BP, mm Hg</td>
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<tr>
<td>$\delta$ (n = 4)</td>
<td>186 $\pm$ 3</td>
<td>157 $\pm$ 4</td>
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<tr>
<td>Diastolic BP, mm Hg</td>
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<tr>
<td>$\delta$ (n = 8)</td>
<td>179 $\pm$ 8</td>
<td>169 $\pm$ 14</td>
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$^a$ Results are shown as mean $\pm$ SEM. Neither parameter was significantly affected by offspring sex, genotype, or the maternal environment.
$^b$ Number of offspring.
The db/db mice are known to have raised systolic, diastolic, and mean arterial BP in comparison with their db/+ littermates (23), but a comparison of db/+ and WT offspring, born to either WT or db/+ mothers, has not been reported. In this study, all measures of BP were similar between offspring. However, a more sensitive assessment, for example using radiotelemetry, might reveal subtle effects of the maternal environment and/or offspring genotype.

Male WT offspring from db/+ mothers were heavier and had poorer glucose tolerance than those from normal pregnancies in agreement with a previous study that reported differences in the weight of 8-week-old WT male, but not female, offspring from db/+ and WT pregnancies (21). However, differences observed in the leptin levels of such animals are not replicated herein; in our study, the levels of circulating leptin in 6-month-old animals are related to genotype rather than the maternal environment. It is possible that the adverse maternal influence resolves with increasing age. Others have reported that at 6 months, the weight of WT offspring born to db/+ and WT mothers is similar in both sexes but that female offspring have increased body fat and insulin resistance (15). Offspring adiposity was not assessed in our study, but we did not detect sex differences in fasting insulin levels. A sex difference in offspring outcomes, with males often faring worse, is a common observation in programming studies, especially in relation to glucose intolerance, and has been ascribed to differences in maternal investment of energy depending on fetal sex (24).

It will be interesting to uncover the mechanisms that can program the postnatal health in the absence of placental/fetal overgrowth. In vitro studies suggest that an adverse maternal metabolic environment could influence epigenetic programming (25, 26), and more recently, genes involved in appetite control and energy metabolism have been shown to be epigenetically modified in placenta and cord blood of infants from pregnancies complicated by GDM (27, 28). Furthermore, a mouse model of maternal hyperglycemia induced by streptozotocin found that although the birth weight of F1 offspring was not affected, male offspring had impaired glucose tolerance as adults and altered methylation of the imprinted genes Igf2/H19 that are important for pancreatic islet development (29). Altered nutrition in the perinatal period can also cause epigenetic changes that affect adult health (30, 31). Nothing is known about the quantity and quality of milk from db/+ dams; thus, cross-fostering experiments will be important to determine how maternal nutrient supply during early postnatal development contributes to the long-term health of the WT offspring born to db/+ dams.

In summary, our study highlights the need to genotype offspring when interpreting the effect of the maternal environment on placental and fetal weight in the db/+ model of maternal hyperglycemia. The db/+ mouse may be most useful for investigating mechanisms underlying the abnormalities in postnatal weight gain and glucose metabolism programmed by an adverse maternal metabolic environment.

Acknowledgments

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Disclosure Summary: The authors have nothing to declare.

References


