**Objective**—We have hypothesized that variation in imprinted growth-promoting fetal genes may affect maternal glucose concentrations in pregnancy. To test this hypothesis we evaluated the effects of fetal disruption of murine H19Δ13 on maternal glucose concentrations in pregnancy.

**Research Design and Methods**—Experimental mice were pregnant females that had inherited the disrupted H19Δ13 from their fathers and were therefore phenotypically wild type due to imprinting; approximately half of their litters were null for H19Δ13 through maternal inheritance of the disrupted gene. In control mice approximately half the litter paternally inherited the disrupted H19Δ13, so the pups were either genetically wild type or phenotypically wild type due to imprinting. Blood glucose concentrations were assessed by intraperitoneal glucose tolerance tests on days 1, 16, and 18 of pregnancy.

**Results**—There were no differences in the glucose concentrations of control and experimental pregnant mice at day 1. However, at day 16 mothers carrying H19Δ13-null pups had a significantly higher area under the glucose tolerance test curves than controls (1,845 ± 378 vs. 1,386 ± 107 mmol·min·1−1 [P = 0.01]) in association with increasing pregnancy-related insulin resistance. Although this difference lessened toward term, overall, mothers of maternally inherited H19Δ13 mutants had significantly higher glucose concentrations during the last trimester (1,602 ± 321 [n = 17] vs. 1,359 ± 147 [n = 18] mmol·min·1−1 [P = 0.009]).

**Conclusions**—This study provides evidence that maternal glucose concentrations in pregnant mice can be affected by targeted disruption of fetal H19Δ13. This implies that variable fetal IGF2 expression could affect risk for gestational diabetes.

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**Gestational diabetes mellitus (GDM) has traditionally been considered a condition originating through combined effects of maternal genetics (1,2) and her environment, including effects associated with maternal obesity. The current increasing incidence of GDM (3) is thought to result from the increasing prevalence of obesity, with effects of the associated insulin resistance combining with those of the physiological insulin resistance of pregnancy to impair glucose tolerance.**

Following Haig’s suggestion (4) that maternal metabolism during pregnancy could be influenced by variation in the fetal genome, we recently proposed that risk of GDM could also be modified by variation in the fetal genome, particularly in fetal growth genes (5). This might occur as part of the genetic conflict of pregnancy where maternal genes limit fetal growth and paternally inherited fetal genes enhance it by increasing the amount of metabolic fuel supplied by the mother to the fetus (6). Candidates include imprinted genes, where expression of transmitted alleles depends on their parent of origin (7,8). Preliminary evidence for this phenomenon comes from our studies of contemporary birth cohorts that have shown that both third-trimester maternal glucose tolerance and offspring birth weights are associated with common polymorphic variation in the H19 gene (9), which is reciprocally imprinted with respect to IGF2 and regulates its imprinting and expression.

The following study was therefore performed to test the hypothesis that changes in maternal glucose concentrations during pregnancy could be mediated by genetic variation in the murine fetal H19 region (5), which is usually expressed only from the maternally inherited fetal allele, using mice with targeted disruption of H19Δ13 that are born 30% heavier than controls (10,11).

**Research Design and Methods**

**Animals.** All experiments were performed under the Animals (Scientific Procedures) Act 1986 after Cambridge University Animal Ethics Committee approval. The mice were kept under controlled conditions with a 12-h light/dark cycle and had free access to food and water throughout (except during the glucose tolerance test [GTT] starvation period when only water was available). H19Δ13/+ mice (in which insertion of a neomycin resistance cassette originally replaced the entire 3-kb coding region of the gene and the 10-kb 5′ flanking region including the Igf2 control region [10,12]) were bred on C57BL/6 backgrounds and their offspring genotyped by PCRs of ear biopsy DNA (extracted using DNeasy kits; Qiagen, Crawley, U.K.). The 20-μl reaction mix contained 2 units of BIO-X-ACT polymerase (Bioline Ltd., London, U.K.), 10 μl Buffer A (Bioline), 8 pmol of forward (5′-TCCACAGGGAGAAAC-CAG-3′) and reverse (5′-AGTCAATGCAGATAATGC-3′) oligonucleotide primers, and 25 ng DNA. The reaction mix was incubated at 94°C for 5 min and then 20 cycles of 94°C (30 s), 40°C (30 s) dropping 0.5°C each cycle, and 72°C (1 min). After this, the mix underwent 30 cycles of 94°C (30 s), 30°C (30 s), and
72°C (1 min) and a final incubation at 72°C (10 min). Knockout mice produced a 895-bp band when separated electrophoretically on a 1% (wt/vol) agarose gel. Presence of the wild-type H19 gene was tested using similar reaction conditions but a different reverse primer (5'-TTCAGCTCTCTCCCTCCG-3') and increasing the annealing temperatures by 10°C throughout to produce a 494-bp band.

The aim of our breeding program was to produce experimental dams, in which approximately half of their litter was H19+/- null due to inheriting the disrupted gene from their mothers, and control dams, in which approximately half of their genetically matched litter inherited the disrupted H19+/- from their fathers and were therefore phenotypically wild type due to imprinting. To achieve this, experimental females were genotypically heterozygous knockout mice (H19+/+/-) that were phenotypically wild type due to paternal inheritance of the disrupted gene and were mated to wild-type C57BL/6 male mice (Charles River Ltd., Margate, U.K.) (Fig. 1). Controls were wild-type C57BL/6 females mated to genotypically heterozygous H19+/-/+ males. Due to imprinting, the genotype, but not the gene expression distribution among the pups, was therefore the same for both groups. Pregnancy was assumed at the expulsion of a vaginal plug (day 0), although for the studies on days 16 and 18 of pregnancy only those animals who gained weight suggestive of pregnancy were assessed.

Glucose tolerance tests. For each day, 8–10 different experimental and control mice had their glucose concentrations assessed on day 1, 16, or 18 of pregnancy (day 1 was used as baseline; the other days were used because in previous experiments using an alternative mouse model in which placental Igf/2 expression was manipulated, there were differences in effects on placental nutrient transport among these days [13]). The animals were starved for 15 h and were then tail bled to produce samples for the measurement of blood glucose and other analytes. The mice were then injected intraperitoneally with 1 g/kg body wt glucose (administered as a 10% [wt/vol] solution). Further blood glucose measurements were then taken 15, 30, 60, 120, and 180 min later.

Blood glucose, serum insulin, and Igf-ii concentrations. Blood glucose was measured using a 201+ glucose meter (Hemscoe Ltd, Sheffield, U.K.). Fasting serum Igf-ii (following standard acid-ethanol extraction and neutralization; RayBiotech Mouse Igf-ii ELISA; Innovate Biotechnology, London, U.K.) and insulin (Linco Rat & Mouse Insulin ELISA; Millipore, London, U.K.) concentrations were measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer’s instructions. Fasting insulin sensitivity was assessed indirectly by homeostasis model assessment (HOMA) (http://www.dtu.ox.ac.uk/index.php?maindoc=+homa/index.php). Although its underlying principles are invalid in rodents (14), the values produced by this model do correlate with hyperinsulinemic-euglycemic clamp measures of insulin sensitivity in mice (15) without the need for prior surgery or general anesthesia.

Statistical analysis. Glucose concentrations were assessed as areas under the (0–180 min) glucose curves (AUCs), calculated using the trapezoid rule. When comparing normally distributed data from two groups on a particular day of pregnancy, Student t tests were used (having performed the Levine test for equality of variances); otherwise, comparison was by Mann-Whitney U test. Where overall comparisons were made using all 3 days of pregnancy in the same model, two-way ANOVA was used with the experimental group and day of pregnancy as the fixed variable and glucose AUC as the dependent variable. All statistical analysis was performed using SPSS, version 14.0 (SPSS, Chicago). Data are presented as mean ± SD for the GTT data and median (interquartile range) for everything else. P < 0.05 was considered statistically significant.

RESULTS

On day 1 of pregnancy there was no detectable difference in glucose AUCs during the GTT between experimental and control groups: 1,478 ± 197 (n = 10) vs. 1,708 ± 318 (n = 10) mmol · min⁻¹ (P = 0.07). There were also no detectable differences in fasting serum insulin or Igf-ii concentrations or in insulin sensitivity (Table 1).

On day 16 of pregnancy, H19+/-/-+ pups weighed on average 118% that of wild-type pups (supplementary Table 1), mice carrying litters containing H19+/-/-+ mice still had higher mean glucose AUCs, but the difference was not statistically significant (P = 0.2, Fig. 3). There was no difference in either day 18 fasting insulin or Igf-ii concentrations or in insulin sensitivity (Table 1). Combining data from across the third trimester, the experimental mice still had significa...
significant higher glucose AUCs than the controls: 1,602 ± 321 (n = 17) vs. 1,359 ± 147 (n = 18) mmol · min⁻¹ (P = 0.009).

There were no differences in the number of pups carried by experimental and control mice (data not shown). Nor was the number of pups carried correlated with any measure of glucose concentrations in either of the groups. No difference was made to the results of analyzing the area under the GTT curves when adjusting for the numbers of pups being carried (data not shown). When comparing glucose AUCs on all the different days in the same statistical model, there were significant effects of both the day of pregnancy (P = 0.02) and the interaction between the day of pregnancy and the group (P = 0.001). Also, a lowering of glucose AUCs between days 16 and 18 of pregnancy was observed that was independent of group (P = 0.014; supplementary Table 2).

**DISCUSSION**

This study provides direct evidence that changes in murine maternal glucose concentrations during pregnancy can be mediated by alteration in a fetal growth gene, in this case *H19Δ13*..

Previously, there has been only indirect evidence from humans to support this hypothesis (5), partially due to the difficulty in differentiating effects of fetal genes on maternal metabolism from effects of maternal genes. Evidence implicative variation in fetal *IGF2* expression comes from the observed trend for an increased risk of developing GDM in mothers carrying offspring with Beckwith-Wiedemann syndrome (16). Additional indirect evidence for the fetal genome modifying the risk for maternal GDM comes from studies showing an increased risk in women carrying males rather than smaller females (17), and in multiple pregnancies (18).

Our studies provide further support for this hypothesis. On day 1 of pregnancy there was no change in glucose concentrations in mice carrying *H19Δ13−/+* pups. However by day 16 significant increases in maternal glucose concentrations were observed in dams carrying *H19Δ13−/+* pups despite overall similar decreases in HOMA-derived insulin sensitivity in both groups. These raised glucose concentrations could have resulted from reduced first-phase insulin secretion and/or insulin-stimulated glucose uptakes, neither of which would have influenced HOMA measurements.

Our mice also displayed a generalized lowering of glucose concentrations between days 16 and 18, as has recently been observed in another mouse model (19). This may have limited our power to detect continuing differences between groups, as there was no effect of fetal *H19Δ13−/+* on maternal glucose concentrations evident on day 18 of pregnancy. Whereas in humans it is known that

<table>
<thead>
<tr>
<th>Day 1</th>
<th>Experimental (n = 10)</th>
<th>Controls (n = 10)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (pmol/l)</td>
<td>46 (35–74)</td>
<td>35 (35–61)</td>
<td>0.4</td>
</tr>
<tr>
<td>Igf-ii (ng/ml)</td>
<td>All &lt;6</td>
<td>All &lt;6</td>
<td>1.0</td>
</tr>
<tr>
<td>Insulin sensitivity (HOMA %S)</td>
<td>113.9 (64.3–147.8)</td>
<td>140.0 (78.6–145.1)</td>
<td>0.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Day 16</th>
<th>Experimental (n = 8)</th>
<th>Controls (n = 9)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (pmol/l)</td>
<td>137 (92–237)</td>
<td>112 (52–154)</td>
<td>0.3</td>
</tr>
<tr>
<td>Igf-ii (ng/ml)</td>
<td>All &lt;6</td>
<td>All &lt;6</td>
<td>1.0</td>
</tr>
<tr>
<td>Insulin sensitivity (HOMA %S)</td>
<td>40 (23–61)</td>
<td>50 (40–119)</td>
<td>0.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Day 18</th>
<th>Experimental (n = 9)</th>
<th>Controls (n = 9)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (pmol/l)</td>
<td>111 (63–279)</td>
<td>141 (83–1,472)</td>
<td>0.7</td>
</tr>
<tr>
<td>Igf-ii (ng/ml)</td>
<td>All &lt;6</td>
<td>All &lt;6</td>
<td>1.0</td>
</tr>
<tr>
<td>Insulin sensitivity (HOMA %S)</td>
<td>52 (21–102)</td>
<td>40 (6–65)</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Data are median (interquartile range).
Glucose tolerance generally deteriorates as pregnancy progresses (20), data relating to the very last weeks of pregnancy corresponding to day 18 in the mouse are less certain. Women with preexisting type 1 diabetes have a fall in insulin requirements in the last 2 weeks of pregnancy (21), suggesting a possible improvement in insulin sensitivity. If effects on maternal glucose metabolism in our mice were mediated by placental hormones (5), the generalized fall in maternal glucose concentrations at the end of pregnancy may therefore have resulted from a preparum decrease in placental metabolic activity similar to that which has been observed in other model systems (22).

H19 disruption affects Igf2 imprinting and expression, and fetal growth in mice (10,11). We hypothesize that our results therefore relate to increased placental and fetal Igf2 expression. However we cannot rule out a role for the disruption of H19 per se or changes in 9IH (antisense H19) RNA expression (23). We propose that the increased placental Igf2 expression, rather than causing changes in maternal Igf2 concentrations that were not detectably raised in our mice, affects the expression and release of metabolically active placental hormones into the maternal circulation that worsens their glucose tolerance (5). Indirect support for this comes from the enhanced lowering of glucose concentrations we observed in H19−/− mice between days 16 and 18 of pregnancy, as at equivalent stages in human pregnancies at least, placental protein concentrations in the maternal circulation fall (24). One candidate hormone for this process is mouse placental lactogen II because its placental expression has been linked to that of Igf2 (25), it regulates pancreatic β-cell expansion in pregnancy (26), and pregnant mice without functional prolactin receptors, for which it is a ligand, become glucose intolerant (19). These animal experimental data may be important in the understanding of the pathogenesis of human GDM. Previously, we found that in tal data may be important in the understanding of the pathogenesis of human GDM. Previously, we found that in tal data may be important in the understanding of the pathogenesis of human GDM.

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No potential conflicts of interest relevant to this article were reported.

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