Developmental adaptations to increased fetal nutrient demand in mouse genetic models of Igf2-mediated overgrowth

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ABSTRACT The healthy development of the fetus depends on an optimal balance between fetal genetic drive for growth and the maternal ability to provide nutrients through the placenta. Nothing is known about fetal-placental signaling in response to increased fetal demand in the situation of overgrowth. Here, we examined this question using the H19Δ13 mouse model, shown previously to result in elevated levels of Igf2. Fetal and placental weights in H19Δ13 were increased by 23% and 45%, respectively, at E19, when compared with wild-type mice. Unexpectedly, we found that disproportionately large H19Δ13 placentas transport 20–35% less (per gram placenta) glucose and system A amino acids and have similar reductions in passive permeability, despite a significantly greater surface area for nutrient exchange and theoretical diffusion capacity compared with wild-type mice. Expression of key transporter genes Slc2a3 and Slc38a4 was reduced by ~20%. Decreasing the overgrowth of the H19Δ13 placenta by genetically reducing levels of Igf2P0 resulted in up-regulation of system A activity and maintenance of fetal overgrowth. Our results provide direct evidence that large placentas can modify their nutrient transfer capacity to regulate fetal nutrient acquisition. Our findings are indicative of fetal-placental signaling mechanisms that limit total demand for maternal nutrients.—Angiolini, E., Coan, P. M., Sandovici, I., Iwajomo, O. H., Peck, G., Burton, G. J., Sibley, C. P., Reik, W., Fowden, A. L., Constância, M. Developmental adaptations to increased fetal nutrient demand in mouse genetic models of Igf2-mediated overgrowth. FASEB J. 25, 1737–1745 (2011). www.fasebj.org

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Fetal overgrowth is associated with an increased risk of complications both for the mother and infant at birth (1, 2). High birthweight in human populations is also associated with development of metabolic syndrome in childhood, as well as with an increased risk of obesity and metabolic disease in later life (3, 4). Large-for-gestational-age (LGA) infants are becoming more common in many parts of the world as a result of improved maternal nutrition, higher prepregnancy BMI, and an increasing prevalence of gestational diabetes (4, 5). While it is clear that the maternal environment plays an important role in determining fetal size, intrauterine growth is determined ultimately by the fetal genetic drive for growth (demand for nutrients) and the ability of the placenta to supply oxygen and nutrients to the fetus. Interactions between the fetal genome and maternal environment controlling fetal nutrient acquisition are thought to occur, in part, via adaptations in placental phenotype (6–8), although little is known about the mechanisms involved in the fetal-placental signaling of nutrient demand.

Imprinted genes are important regulators of the balance between supply and demand systems that are crucial to fine-tune mammalian growth (9–11). The distinctive features of imprinted genes are their monoallelic parental-specific expression, and their selective roles in key mammalian physiological pathways related to maternal resource acquisition (12, 13). Recent studies of murine knockouts for imprinted genes have shown that the capacity of the placenta to supply nutrients to the fetus varies according to fetal demand (14–17). The small Igf2-deficient P0 placenta compensates for its growth deficiency by up-regulating nutrient...
transporters in response to a normal fetal growth demand (14, 15). These placentas are highly “efficient” because they are able to support normal fetal growth until near term, when fetal growth restriction finally ensues. When the fetal demand is reduced, as seen in the total Igf2 knockout, this up-regulation is no longer observed, which strongly suggests that fetal demand influences placental phenotype (15). Similar adaptations in the nutrient transfer capacity of the placenta are seen when fetal demand exceeds the placental supply of nutrients during natural variations in placental growth (18). So far, these studies have been focused on models in which the potential for fetal growth is compromised by placental growth restriction. However, nothing is known about fetal-placental signaling in response to genetically determined overgrowth, despite the importance of these mechanisms to fully understanding the causes and consequences of growth-related complications of human pregnancy.

Here, we investigate placental adaptations in response to increased fetal growth demands in the imprinted H19Δ13 mouse model of overgrowth (19). H19 is a noncoding RNA, which is exclusively expressed from the maternal allele. Deletion of 13 kb, including the H19 gene and the H19/Igf2-imprinting control region IC1 (also known as H19 DMD), when transmitted maternally, leads to increased levels of Igf2 and fetal overgrowth (19, 20). Excess of Igf2 caused by disruption of IC1 has been implicated in the etiology of the overgrowth disorder Beckwith-Wiedemann syndrome (BWS), which is characterized by macroglossia, organomegaly, predisposition to embryonal tumors, and endocrine dysfunction (10, 21). Placentalig is commonly observed in mothers carrying BWS fetuses (22). In the H19Δ13 mouse model, placental weight is increased as a result of the doubling of all Igf2 transcripts, including the placental-specific P0 transcript (20, 23). The H19Δ13 placentas are more overgrown than the fetus (20) and relatively “inefficient” as they produce fewer grams of fetus per gram of placenta compared to wild type (WT). We set out to test the hypothesis that H19Δ13 placentas are less efficient because nutrient transfer to the fetus is reduced, perhaps in response to mechanisms that avoid excess drainage of maternal resources that might otherwise compromise fetal viability and future reproductive success.

**MATERIALS AND METHODS**

**Mice**

H19Δ13 and Igf2P0 mutant mice were generated as described previously (19, 24), and bred into an inbred C57BL/6J line for >10 generations. In experiments involving the single H19Δ13 knockout, the mutant alleles were transmitted by a heterozygous mother, giving the genotypes H19Δ13 (−/+ ) and WT (+/ +); further information in Supplemental Fig. S1). In experiments involving crosses between H19Δ13 and Igf2P0 mice, the mutant alleles were transmitted by a homozygous H19Δ13 mother and a heterozygous Igf2P0 father, giving the genotypes H19Δ13−/−Igf2P0−/− (H19Δ13/Igf2P0 double mutant) and H19Δ13Igf2P0−/− (H19Δ13 single mutant; further information in Supplemental Fig. S1). Pregnant females were killed by cervical dislocation, and the fetuses were dissected at embryonic day (E)16 and E19 (E1 was defined as the day of vaginal plug detection).

**Genotyping**

Transmission of the H19Δ13 allele was identified by PCR. The primer pair used to amplify an 895-bp fragment across the deletion was as follows: H19F, 5′-TGCGCAAGAGAA-GAAAACAG-3′; H19R, 5′-AGTCTAGCCGAATAGCC-3′. A third primer was used as an internal positive control for the PCR reaction amplifying a 494-bp fragment: H19WT, 5′-TCTAGTCACCTCCCTGACATCAC-3′. The transmission of the Igf2P0 allele was identified by PCR, as described previously (15).

**Placental transport assays of radiolabeled solutes**

We performed placental transfer assays according to our previous publications (14, 15). Briefly, radiolabeled solutes were injected into the jugular vein of H19Δ13 females either crossed with C57BL6/J males or heterozygous Igf2P0 males. Radioactive counts in each fetus were then used to calculate the amount of radioisotope transferred per gram of placenta or per gram of fetus. Average values for WT and mutant fetuses within a litter were then calculated and expressed as a ratio of mutant to WT for that litter. These values could then be used to calculate a mean for all litters at E16 and E19. The fetal accumulation of radioisotope expressed relative to placental weight and plotted as a ratio of mutant to WT gives a relative measure of placental transfer of solute; when expressed relative to fetal weight gives a relative measure of the amount of solute received by the fetus.

**Stereology**

Stereological analysis on H19Δ13 placentas from E16 and E9 mice were performed according to Coan et al. (25). Briefly, placentas were weighed and hemisected, and corresponding halves were fixed and embedded for generating paraffin wax or resin sections. Measurements were performed using the Computer Assisted Stereology Toolbox (CAST) 2.0 system from Olympus. For the analysis of Jz/Lz proportions in H19Δ13/Igf2P0 placentas, a simple grid system was employed, and in excess of a total of 700 points/midline placental section was counted. The percentage of points hitting each of the placental component layers was taken as an estimate of the fraction that each layer comprised of the total placenta.

**mRNA expression studies**

mRNA expression levels of nutrient transporter genes were analyzed by Northern blotting as described previously (15). Igf2P0 expression levels were measured using SYBR Green JumpStart Taq ReadyMix (Sigma-Aldrich, St. Louis, MO, USA) with the following primers: Igf2P0F, 5′-CTTCAGGAACTACGAGGACT-3′; and Igf2P0R, 5′-GTCGTCAGTGCGTCTCCTCTC-3′ (which yield a 101-bp PCR product spanning the intron between U1 and U2 exons). Results were normalized against the reference gene Gapdh, which was measured using the TaqMan Gene Expression Assay Mm09999915_g1 (Applied Biosystems, Foster City, CA, USA). All qPCR reactions were performed on an ABI 7000HT system (Applied Biosystems), according to the manufacturer’s recommendations.
TABLE 1. Fetal and placental weight and fetal-placental weight ratio of H19Δ13 and WT mice

<table>
<thead>
<tr>
<th>Gestational age</th>
<th>Genotype</th>
<th>n</th>
<th>Fetal wet weight (g)</th>
<th>Placental wet weight (g)</th>
<th>Fetal-placental weight ratio</th>
</tr>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>56</td>
<td>0.414 ± 0.011</td>
<td>0.097 ± 0.002</td>
<td>4.363 ± 0.133</td>
<td></td>
</tr>
<tr>
<td>H19</td>
<td>51</td>
<td>0.463 ± 0.012</td>
<td>0.126 ± 0.003</td>
<td>3.721 ± 0.101</td>
<td></td>
</tr>
<tr>
<td>E16 H19/WT (%)</td>
<td></td>
<td>112***</td>
<td>130***</td>
<td>85***</td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>71</td>
<td>1.143 ± 0.016</td>
<td>0.084 ± 0.001</td>
<td>13.766 ± 0.243</td>
<td></td>
</tr>
<tr>
<td>H19</td>
<td>88</td>
<td>1.410 ± 0.022</td>
<td>0.122 ± 0.001</td>
<td>11.658 ± 0.192</td>
<td></td>
</tr>
<tr>
<td>E19 H19/WT (%)</td>
<td></td>
<td>123***</td>
<td>145***</td>
<td>85***</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± se. ***P < 0.001.

Statistical analysis

Differences in mRNA expression levels between group means were evaluated by the 2-tailed unpaired t test. All other data were analyzed by means of 2-way analyses of variance, with “litters” and “genotype” as the two factors. Data are expressed as means ± se. For data representing radioactive counts, a logarithmic transformation was carried out before statistical analysis. The summary data from these experiments were then represented as ratios, together with 95% confidence limits.

RESULTS

Fetal and placental overgrowth in H19Δ13 mutants

As described previously (19, 20), maternal inheritance of the H19Δ13 mutation results in fetal and placental overgrowth. Fetal wet weight was increased in H19Δ13 mutants by 12% at E16 and 23% at E19 compared with WT littermates (Table 1). We found that the overgrowth was more pronounced in the placenta at both gestational ages. Accordingly, H19Δ13 placental wet weights were increased by 30% at E16 and 45% at E19 compared to WT. As a result of the disproportionate overgrowth of the placenta, H19Δ13 mutants demonstrated a decreased fetal-placental weight ratio (85% of WT for both E16 and E19) (Table 1). This relative inefficiency of the large H19Δ13 placenta in supporting fetal growth may have either a morphological and/or functional origin.

Increased surface area for nutrient exchange in the H19Δ13 placenta

To investigate the possible morphological causes of H19Δ13 placental inefficiency, we studied the structural basis of maternal-fetal nutrient transfer using stereological analyses of mutant vs. WT littermate placentas. We found that the placental overgrowth in H19Δ13 was global, affecting both the labyrinthine zone (Lz) and junctional zone (Jz) (Supplemental Fig. S2 and Table 2). The absolute volumes of the different components of the H19Δ13 placenta and WT litters are summarized in Table 2. The absolute volume of the Lz was significantly increased to 198 and 167% of WT littermate values at E16 and E19, respectively, similar to the increases of 178 and 179% in Jz volume at E16 and E19, respectively. Within the Lz, further measurements were made of the surface area of trophoblast and blood vessels to establish whether the morphology of the transport surface was normal (Table 3). At both gestational ages, expansion of the H19Δ13 Lz was associated with significant increases in the volume and surface areas of maternal blood spaces, fetal capillaries, and trophoblast (Tables 2 and 3). The harmonic mean thickness of the H19Δ13 Lz exchange barrier did not differ significantly from WT (Table 3). As a result of the increased surface area, the average theoretical diffusing capacity (TDC) in H19Δ13 placentas was dramatically increased to 208 and 158% of WT, at E16 and E19, respectively (Table 3). Thus, the changes in morphology of the H19Δ13 placenta are unlikely to explain its decreased efficiency.

TABLE 2. Absolute volume of components in the placenta of H19Δ13 and WT mice at 2 gestational ages

<table>
<thead>
<tr>
<th>Component</th>
<th>E16</th>
<th>E19</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>WT H19 H19/WT (%)</td>
<td>WT H19 H19/WT (%)</td>
</tr>
<tr>
<td>Placenta</td>
<td>86.19 ± 1.834</td>
<td>148.82 ± 7.028</td>
</tr>
<tr>
<td>Lz</td>
<td>37.29 ± 1.365</td>
<td>73.83 ± 2.562</td>
</tr>
<tr>
<td>Jz</td>
<td>31.26 ± 2.048</td>
<td>55.61 ± 5.027</td>
</tr>
<tr>
<td>Db</td>
<td>16.52 ± 1.909</td>
<td>15.67 ± 4.130</td>
</tr>
<tr>
<td>Jz/Lz</td>
<td>0.85 ± 0.083</td>
<td>0.76 ± 0.062</td>
</tr>
<tr>
<td>Trophoblast</td>
<td>22.85 ± 0.414</td>
<td>46.95 ± 2.359</td>
</tr>
<tr>
<td>MBS</td>
<td>8.00 ± 1.112</td>
<td>15.59 ± 1.007</td>
</tr>
<tr>
<td>FC</td>
<td>6.45 ± 0.557</td>
<td>11.49 ± 1.207</td>
</tr>
</tbody>
</table>

Values are mean ± se volume (mm³); n = 6 from 3 litters/group. Lz, labyrinthine zone or trophoblast; Jz, junctional zone or spongiotrophoblast; Db, decidua basalis; Jz/Lz, ratio of junctional to labyrinthine volume; MBS, maternal blood spaces; FC, fetal capillaries. *P < 0.05; **P < 0.01; ***P < 0.001.
The H19Δ13 overgrown placenta down-regulates nutrient supply

Despite the observed expansion in volume and surface area of the Lz trophoblast and the increase in TDC of the H19Δ13 placenta, we found a pronounced reduction in its passive permeability relative to WT controls. The amounts of 14C-mannitol and 14C-inulin transferred per gram of placenta were significantly reduced to 65–80% of the WT values at both E16 and E19 (Fig. 1A). This, combined with the larger size of the placenta, resulted in H19Δ13 mutant fetuses receiving the same actual amount of the solute as WT littermates, except for 14C-mannitol at E19 (Fig. 1A).

Next, we investigated the capacity of the placenta to transfer nutrients by facilitated diffusion (14C-glucose) and active transport (14C-MeAIB) in vivo in relation to placental expression of the glucose and system A amino acid transporters. We found that transfer of 14C-glucose was significantly reduced per gram of placenta compared to WT at both gestational ages (Fig. 1B). Accumulation of 14C-glucose per gram of fetus was significantly reduced in H19Δ13 mutants, i.e., fetuses were receiving less of the solute than WT. We found that mRNA levels of glucose transporter Slc2a3 were reduced significantly in H19Δ13 compared to WT placenta at E16 but not E19 (Fig. 2). There were no significant changes in mRNA levels of the glucose transporter Slc2a1 at either gestational age. 14C-MeAIB transfer per gram of placenta was also less in H19Δ13 mutants than in their WT littermates (Fig. 1B). However, in contrast to 14C-glucose, mutant fetuses were receiving an appropriate amount of 14C-MeAIB for their size. We found that the levels of mRNA of the Slc38a4 2 kb isoform were reduced in H19Δ13 relative to WT placenta at E19 but not at E16 (Fig. 2). None of the other isoforms of the system A amino acid transporters were altered in expression in the H19Δ13 placenta.

Feto-placental signaling of nutrient demand in H19Δ13/Igf2 P0 double mutants

Large placentas can, therefore, respond to resource allocation signals and down-regulate the nutrient transfer capacity when placental supply and fetal demand are genetically matched at a high level. To test whether large placentas can respond to feto-placental signals when the genetic drive for fetal growth is higher than that for placental growth, we aimed to genetically reduce the degree of placental overgrowth. This was achieved by crossing H19Δ13 homozygous females (−/−) with Igf2 heterozygous P0 males (+/−) and examining the progeny at E19. Two possible combinations of H19Δ13 and Igf2P0 mRNA expression were represented among the conceptuses: H19Δ13 single mutant (H19Δ13−/Igf2P0+) and H19Δ13/Igf2P0 double mutant (H19Δ13−/Igf2P0−; see Supplemental Fig. S1). H19Δ13/Igf2P0 placentas showed a specific reduction in P0 levels (~65% of H19Δ13; Fig. 3A), as expected from the deletion of one active allele. As a result of reducing P0 levels, the H19Δ13/Igf2P0 double-mutant placentas

<table>
<thead>
<tr>
<th>Measurement</th>
<th>WT E16</th>
<th>H19 E16</th>
<th>H19/WT (%)</th>
<th>WT E19</th>
<th>H19 E19</th>
<th>H19/WT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBS SA (cm²)</td>
<td>18.81 ± 0.374</td>
<td>39.77 ± 3.051</td>
<td>211**</td>
<td>14.16 ± 1.018</td>
<td>25.08 ± 1.405</td>
<td>177**</td>
</tr>
<tr>
<td>FC SA (cm²)</td>
<td>15.88 ± 1.084</td>
<td>30.97 ± 3.306</td>
<td>195*</td>
<td>16.17 ± 1.100</td>
<td>24.98 ± 1.871</td>
<td>154*</td>
</tr>
<tr>
<td>IMT (μm)</td>
<td>4.20 ± 0.187</td>
<td>4.18 ± 0.152</td>
<td>99</td>
<td>3.11 ± 0.148</td>
<td>3.29 ± 0.356</td>
<td>105</td>
</tr>
<tr>
<td>TDC (mm²min⁻¹kPa⁻¹)</td>
<td>7.2 ± 0.3</td>
<td>15.0 ± 1.9</td>
<td>208*</td>
<td>8.6 ± 0.8</td>
<td>13.6 ± 1.6</td>
<td>158*</td>
</tr>
</tbody>
</table>

Values are means ± se; n = 6 from 3 litters/group. MBS SA, maternal blood space surface area; FC SA, fetal capillary surface area; IMT, labyrinthine intermembrane thickness; TDC, theoretical diffusion capacity of the interhemal membrane. *P < 0.05; **P < 0.01.

Figure 1. Placental transfer of passive diffusion markers, 14C-mannitol and 14C-inulin (A) and facilitated and active transport markers, 14C-glucose and 14C-MeAIB (B) in H19Δ13, calculated as a ratio of mutant to WT transfer, expressed either per gram of placenta or per gram of fetus at 2 gestational ages (E16 and E19). Ratios <1 indicate reduced transfer by the mutant placenta with respect to either placental or fetal weight. (E16: mannitol, n = 31 H19Δ13 and n = 34 WT from 10 litters; inulin, n = 30 H19Δ13 and n = 28 WT from 9 litters; MeAIB, n = 24 H19Δ13 and n = 22 WT from 8 litters; and glucose, n = 20 H19Δ13 and n = 28 WT from 8 litters. E19: mannitol, n = 14 H19Δ13 and n = 17 WT from 6 litters; inulin, n = 65 H19Δ13 and n = 71 WT from 22 litters; MeAIB, n = 29 H19Δ13 and n = 16 WT from 8 litters; and glucose, n = 33 H19Δ13 and n = 30 WT from 9 litters.) Bars indicate 95% confidence limits. *P < 0.05; **P < 0.01; ***P < 0.001.
were significantly less overgrown (~15%) than $H19^{\Delta 13}$ single-mutant (0.110 ± 0.001 g, $n=80$ vs. 0.130 ± 0.001 g, $n=69$, respectively; $P<0.001$) but fetal weights of the $H19^{\Delta 13}$ single and $H19^{\Delta 13}/Igf2P0$ double mutants were similar (1.370 ± 0.017 g, $n=80$ vs. 1.330 ± 0.024 g, $n=69$, respectively; $P>0.05$). The $H19^{\Delta 13}/Igf2P0$ double-mutant placenta was thus significantly more efficient compared to $H19^{\Delta 13}$ single-mutant (115% of $H19^{\Delta 13}$).

Next, we measured maternal-fetal transfer of $^{14}$C-MeAIB and $^{14}$C-mannitol at E19. We observed increased transfer of $^{14}$C-MeAIB per gram in the $H19^{\Delta 13}/Igf2P0$ double-mutant placenta as compared with the $H19^{\Delta 13}$ single-mutant (Fig. 3B), which may explain, in part, the relative increase in placental efficiency. No difference in transfer of $^{14}$C-mannitol was observed between the genotypes (Fig. 3B). These findings were

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**Figure 2.** Northern blot analysis of mRNA levels of system A amino acid transporter genes $Slc38a1$, $Slc38a2$, and $Slc38a4$ and glucose transporter genes $Slc2a1$ and $Slc2a3$ in $H19^{\Delta 13}$ placentas compared to WT littermates at E16 (A) and E19 (B). Graphs show mean expression levels, with WT levels normalized to 1; Northern blots are representative of total RNA obtained from WT and $H19^{\Delta 13}$ placentas. Gapd was used as an internal control for RNA loading ($n$ for each genotype and gestational age: $n=7$ for $Slc2a1$ and $Slc2a3$; $n=17$ for $Slc38a1$ and $Slc38a4$; $n=10$ for $Slc38a2$). Bars indicate means ± SE. **P < 0.01; ***P < 0.001.

**Figure 3.** mRNA expression analysis and maternal-fetal transfer of radio-labeled solutes in $H19^{\Delta 13}/Igf2P0$ double mutants at E19. A) Q-RT-PCR analysis for Igf2 mRNA transcripts, showing specific reduction of Igf2P0 transcript levels in $H19^{\Delta 13}/Igf2P0$. Graphs show mean expression levels, with Igf2 levels in $H19^{\Delta 13}$ placentas normalized to 1 ($n=5$ group). B) Placental transfer of $^{14}$C-MeAIB and $^{14}$C-mannitol in $H19^{\Delta 13}/Igf2P0$ at E19, calculated as a ratio of double-mutant to single-mutant transfer, expressed either per gram of placenta or per gram of fetus ($n=40$ $H19^{\Delta 13}/Igf2P0$ and $n=23$ $H19^{\Delta 13}$ from 8 litters). Bars indicate 95% confidence limits. *P < 0.05. C) Northern blot analysis of mRNA levels of system A amino acid transporter genes ($Slc38a1$, $Slc38a2$, and $Slc38a4$) in $H19^{\Delta 13}/Igf2P0$ placentas compared to $H19^{\Delta 13}$ littermates. ($H19^{\Delta 13}/Igf2P0$: $n=8$ for $Slc38a1$ and $Slc38a2$; $n=15$ for $Slc38a4$. $H19^{\Delta 13}$: $n=8$ for $Slc38a1$; $n=7$ for $Slc38a2$; $n=13$ for $Slc38a4$.) Graphs show mean expression levels, with $H19^{\Delta 13}$ single-mutant levels normalized to 1; Gapd was used as an internal control for RNA loading. Bars indicate means ± SE. *P < 0.05.
not the result of perturbations in the proportions of the Lz and Jz in H19Δ13/Igf2P0 double-mutant placentas, as these were identical to those in H19Δ13 single-mutant (Supplemental Fig. S3). We found that transcript levels of system A transporter Slc38a4 were also modestly increased relative to the H19Δ13 single mutant (Fig. 3C), which could explain, at least in part, the relative increase in placental transfer of 14C-MeAIB.

DISCUSSION

This study, using genetic models of overgrowth and fetal-placental mismatch, provides the first direct evidence that large placentas can modify their nutrient transfer capacity to regulate fetal nutrient acquisition. These adaptations are important to maintain the balance between the fetal genetic drive for growth and the maternal ability to provide these resources, particularly when fetal nutrient demands for growth are high. We have shown that H19Δ13 placentas, overgrown because of double dosage of Igf2, have a reduced passive permeability and transport less glucose and amino acids per gram, despite a greater surface area for nutrient exchange and an increased theoretical diffusion capacity, compared to their WT littermates. This reduction in the nutrient transfer capacity of the large H19Δ13 single-mutant placenta may limit fetal overgrowth and, thereby, reduce the total demand for maternal nutrients during the period of late pregnancy when the fetus is normally growing most rapidly in absolute terms. Our study also shows that decreasing the overgrowth of the large H19Δ13 placenta by genetically removing ~65% of the Igf2 placental-specific P0 transcripts results in relative up-regulation of system A activity and maintenance of fetal overgrowth.

Placental transport characteristics

The relative inefficiency of the large H19Δ13 single-mutant placenta is more likely to be due to the reduced transfer of substances by the three principal mechanisms of transplacental transfer, namely passive diffusion, facilitated diffusion, and active transport. We demonstrated a reduction in transfer of radiolabeled markers of passive diffusion across the H19Δ13 single-mutant placenta to 65–80% of their WT values at both E16 and E19, despite the increases in Lz surface area and TDC.

Facilitated diffusion of glucose across the H19Δ13 placentas was also only 63–77% of the WT values per gram of placenta, with the result that the H19Δ13 fetuses accumulated less glucose per gram of fetal weight. In part, this may result from the diversion of glucose from transplacental transfer to accumulation as placental glycogen within the placenta, as H19Δ13 placentas are known to have higher glycogen contents than their WT littermates during late gestation (30). At E16, the decreased glucose transfer across the H19Δ13 placentas may also reflect the reduced expression of the placental glucose transporter Slc2a3/Glut3. However, at E19, there were no changes in expression of either placental glucose transporter from WT values, which suggests there may be a simple diffusional component to glucose transfer across the mouse placenta in late gestation. Whatever the mechanisms involved, the decrease in placental glucose delivery per gram of H19Δ13 fetus will limit its growth relative to the size of its placenta.

Like simple and facilitated diffusion, active transport across the H19Δ13 mutant placenta, measured as maternal-fetal transfer of MeAIB per gram of placenta, was reduced at both E16 and E19. At E19, this may be related, in part, to the reduced expression levels of the 2-kb transcript of Slc38a4, one isoform of the system A family of amino acid transporters known to transfer MeAIB across the placenta (15, 31). However, since the H19Δ13 Lz was larger, total transfer of MeAIB to the H19Δ13 fetus still exceeded that of the WT at both ages, although this was less than would be predicted from placental size alone. When placental overgrowth was reduced in the double H19Δ13/Igf2P0 mutant placenta by decreasing the levels of Igf2P0 transcripts (Fig. 4), we observed that fetal overgrowth was maintained, at least in part, by up-regulation of placental Slc38a4 expression and MeAIB transport relative to the single mutant. The volume of the placental component layers remained unchanged in double compared to single mutants, ruling out morphological changes as the cause of the increased efficiency of the double-mutant placenta. Taken together, the findings on the two H19Δ13 mutants suggest that the large H19Δ13 placenta adapts its nutrient transfer capacity dynamically to regulate resource allocation to the fetus (see summary of findings in Fig. 4).
The nature of the adaptive signals influencing placental phenotype in the $H19^{Δ13}$ mutants remains unknown but may have a fetal and/or maternal origin. In the $H19^{Δ13}$/Igf2P0 double mutant, the fetal drive for nutrients produced by biallelic expression of all Igf2 transcripts in the fetus will exceed the supply of nutrient delivered by the less overgrown placenta expressing the Igf2P0 transcript monoallelically from the normally silent, maternal Igf2P0 promoter (Fig. 4). The concomitant up-regulation of system A activity in the $H19^{Δ13}$/Igf2P0 placenta is, therefore, likely to be an adaptive response to a fetal signal of this mismatch designed to help meet the fetal genetic drive for nutrients for growth. Indeed, fetal overgrowth was maintained in the $H19^{Δ13}$/Igf2P0 double mutant despite a 15% reduction in placental weight compared to the single $H19^{Δ13}$ mutant. Placental overgrowth is thus reduced compared to $H19^{Δ13}$ mutants, with levels of fetal demand unaffected. This reduction in placental supply mediated by loss of the Igf2 P0 isoform relative to the double mutant results in an adaptive compensatory response, e.g., up-regulation of system A activity, that helps maintain fetal overgrowth. These findings provide the first direct demonstration that overgrown placentas are responsive to fetal-placental signaling of nutrient demand and can adapt their phenotype to control nutrient allocation to the fetus in relation to the balance between maternal nutrient availability and fetal demands for growth.

Figure 4. Altered balance between fetal and placental growth in $H19^{Δ13}$ and $H19^{Δ13}$/Igf2P0 models of overgrowth. Growth is represented as a percentage of WT littermates (% WT) at E19. Relative levels of Igf2 promoters (P0-P3) are indicated for each genotype. Levels of the Igf2 placental-specific isoform P0 determine in part the degree of placental overgrowth and supply potential, whereas fetal Igf2 P1-P3 determine fetal demand. $H19^{Δ13}$ mutants show disproportionate growth of the placenta relative to the fetus and increased supply-potential relative to fetal demand. However, despite a dramatic increase in surface area for exchange of nutrients associated with overgrowth of the whole placenta, the passive permeability of the placenta and flux of nutrients to the fetus is dramatically reduced per gram of placenta compared to controls. We suggest that this reduction in supply of nutrients is controlled either by growth demand signals emanating from the fetus or by maternal constraint signals. Double $H19^{Δ13}$/Igf2P0 mutants differ from single $H19^{Δ13}$ mutants in that they carry only 1 active copy of the Igf2 placental-specific P0 allele (the maternal one) instead of 2 (maternal and paternal). Placental overgrowth is thus reduced compared to $H19^{Δ13}$ mutants, with levels of fetal demand unaffected. This reduction in placental supply mediated by loss of the Igf2 P0 isoform relative to the double mutant results in an adaptive compensatory response, e.g., up-regulation of system A activity, that helps maintain fetal overgrowth. These findings provide the first direct demonstration that overgrown placentas are responsive to fetal-placental signaling of nutrient demand and can adapt their phenotype to control nutrient allocation to the fetus in relation to the balance between maternal nutrient availability and fetal demands for growth.

Demand signals

The nature of the adaptive signals influencing placental phenotype in the $H19^{Δ13}$ mutants remains unknown but may have a fetal and/or maternal origin. In the $H19^{Δ13}$/Igf2P0 double mutant, the fetal drive for nutrients produced by biallelic expression of all Igf2 transcripts in the fetus will exceed the supply of nutrient delivered by the less overgrown placenta expressing the Igf2P0 transcript monoallelically from the normally silent, maternal Igf2P0 promoter (Fig. 4). The concomitant up-regulation of system A activity in the $H19^{Δ13}$/Igf2P0 placenta is, therefore, likely to be an adaptive response to a fetal signal of this mismatch designed to help meet the fetal genetic drive for nutrients for growth. Indeed, fetal overgrowth was maintained in the $H19^{Δ13}$/Igf2P0 double mutant despite a 15% reduction in placental weight compared to the single $H19^{Δ13}$ mutant monoallelically expressing Igf2 in all fetal-placental tissues. Similar up-regulation of placental Slc38a4 expression and MeAIB transport is seen when nutrient demand and supply are mismatched by restricting placental relative to fetal growth in the single Igf2P0 mutant (15). In the single $H19^{Δ13}$ mutant, down-regulation of placental Slc2a3 and Slc38a4 transporters and transfer of glucose and MeAIB may also be a

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response to fetal signals designed to reduce the uptake of substrates in line with the reduced passive diffusion of other key substances required for intrauterine growth. Alternatively, maternal signals may be constraining placental nutrient allocation to the large H19g413 fetuses at the period of late gestation when the absolute demand for nutrients is at its greatest. By reducing expression of key transporters, these maternal signals may place an upper limit on nutrient transfer and avoid excess drainage of maternal resources into fetuses with an increased genetic drive for growth. However, little is known about the maternal metabolic or endocrine environment in these mutants.

**Importance for the human infant**

The adaptive regulation of nutrient supply by the large H19g413 placenta, identified here for the first time, has important implications for our understanding of placental biology and the control of intrauterine growth. Optimal fetal growth is of critical importance to pregnancy outcome, with as many as 15% of all human pregnancies complicated by aberrant fetal growth. The H19g413 mouse model recapitulates the fetal overgrowth and placentomegaly characteristics of the human disorder Beckwith-Wiedemann syndrome (20–22). Our work showing that placentomegaly is associated with a reduction in nutrient supply in response to constraint signals raises the hypothesis that similar mechanisms may apply to BWS.

Maternal nutrient excess and increased placental nutrient transfer are likely to play causal roles in the development of idiopathic LGA and macrosomic babies arising from maternal or gestational diabetes. Increased transfer of nutrients (e.g., system A and glucose) has been reported in fetal overgrowth in association with fetal macrosomia in human diabetic pregnancy and in mice fed high-fat diets (32–34). The H19g413 and H19g413/Igf2P0 mice described in this study are 2 mouse models of genetically determined LGA that differ in their degree of placental overgrowth and show that the trophoblast has an active, dynamic role in the regulation of fetal overgrowth. The absolute and relative amounts of nutrients transferred across overgrown placentas may differ between individual LGA infants with consequences for their morbidity and mortality rates both at birth and much later in life. Although the signals regulating the nutrient supply capacity of the placenta remain unknown, the results of this and our previous studies (15, 18) show that the placenta is responsive to mismatches between nutrient availability and the fetal genetic drive for nutrient acquisition. The placenta is, therefore, acting as a nutrient sensor and adapting its phenotype to optimize the nutrient supply to the fetus with respect to the various nutrient allocation signals.

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