

# Maternal and fetal genomes interplay through phosphoinositol 3-kinase(PI3K)-p110 $\alpha$ signalling to modify placental resource allocation

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**Pregnancy success and life-long health depend on a cooperative interaction between the mother and the fetus in the allocation of resources. As the site of materno-fetal nutrient transfer, the placenta is central to this interplay; however, the relative importance of the maternal versus fetal genotypes in modifying the allocation of resources to the fetus is unknown. Using genetic inactivation of the growth and metabolism regulator, *Pik3ca* (encoding PIK3CA also known as p110 $\alpha$ ,  $\alpha$ /+) we examined the interplay between the maternal genome and the fetal genome on placental phenotype in litters of mixed genotype generated through reciprocal crosses of wildtype and  $\alpha$ /+ mice. We demonstrate that placental growth and structure were impaired and associated with reduced growth of  $\alpha$ /+ fetuses. Despite its defective development, the  $\alpha$ /+ placenta adapted functionally to increase the supply of maternal glucose and amino acid to the fetus. The specific nature of these changes however, depended on whether the mother was  $\alpha$ /+ or wildtype and were related to alterations in endocrine and metabolic profile induced by maternal p110 $\alpha$  deficiency. Our findings thus show that the maternal genotype and environment programmes placental growth and function and identify the placenta as critical in integrating both intrinsic and extrinsic signals governing materno-fetal resource allocation.**

resource allocation | fetus | placenta | PI3K | nutrient transport

The successful outcome of mammalian pregnancy depends on balancing resource allocation between the genetically-determined fetal drive for growth and the maternal nutrient requirements to support pregnancy and lactation. Failure to achieve the right balance can lead to pregnancy complications and abnormal fetal development with long-term consequences for maternal and offspring health (1, 2). As the interface between the mother and fetus, the placenta is central to this tug-of-war over nutrient allocation. Genetic and dietary manipulations during pregnancy as well as reciprocal crosses between breeds of different sizes indicate that the placenta can adapt dynamically to both fetal signals of nutrient demand and maternal signals of nutrient availability to ensure appropriate allocation of available resources (3-9). The fetus and mother therefore, have to cooperate to optimise both offspring and maternal fitness but, to date, little is known about the relative importance of fetal versus maternal genomes in balancing resource allocation at the placental level.

The phosphoinositol 3-kinase (PI3K) pathway is highly conserved and evolved to regulate growth in relation to nutrient supply (10). In adult tissues, the PI3K p110 $\alpha$  isoform is primarily responsible for mediating the metabolic effects of insulin and insulin-like growth factors (IGFs); which are major feto-placental growth factors (11-14). Altered placental PI3K signalling, particularly downstream of p110 $\alpha$  accompanies adaptations in placental phenotype when mismatches occur between maternal resource availability and the fetal genetic drive for growth during mouse development (15-18). These findings suggest that p110 $\alpha$  may be a critical sensor integrating the various signals involved in pla-

cental adaptation. In mice, inactivating p110 $\alpha$  kinase genetically causes embryonic lethality of p110 $\alpha$ <sup>D933A/D933A</sup> ( $\alpha$ / $\alpha$ ) homozygotes (13). In contrast, heterozygotes (p110 $\alpha$ <sup>D933A/+</sup>,  $\alpha$ /+) are viable although growth restricted near term (13, 19). However, nothing is known about the placenta in these  $\alpha$ /+ mutants. Postnatally,  $\alpha$ /+ mice remain lighter and are insulin resistant, glucose intolerant and obese, but not overtly diabetic, as adults (13). During pregnancy the consequences of these metabolic and endocrine alterations for placental phenotype and materno-fetal resource allocation remain unknown. Therefore, this study sought to answer the following three questions; 1) what role does fetoplacental p110 $\alpha$  inactivation have in determining placental phenotype and resource allocation to fetal growth? 2) what effect does maternal  $\alpha$ /+ phenotype have on fetoplacental development? and 3) do fetal and maternal genomes interact at the level of the placenta to modify resource allocation during late mouse pregnancy? To achieve this, we established reciprocal crosses of wildtype (WT) and  $\alpha$ /+ mice bearing litters of mixed genotype.

## Results

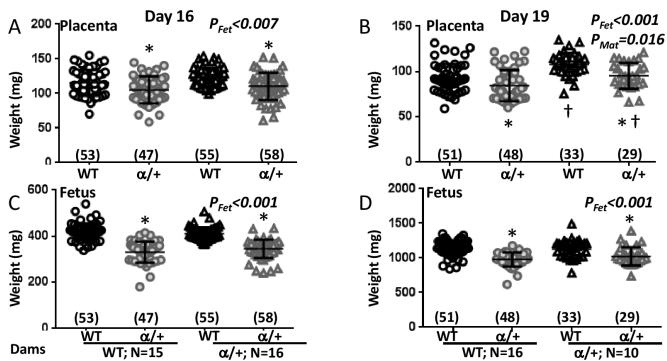
### *p110 $\alpha$ deficiency affects fetoplacental growth*

To unravel the role of p110 $\alpha$  at the fetomaternal interface, conceptus growth and placental phenotype were assessed on day (D) 16 and D19 of pregnancy in WT and  $\alpha$ /+ dams that had been mated with  $\alpha$ /+ and WT males, respectively. Both crosses, therefore, generated two fetal genotypes within a litter (i.e. WTs

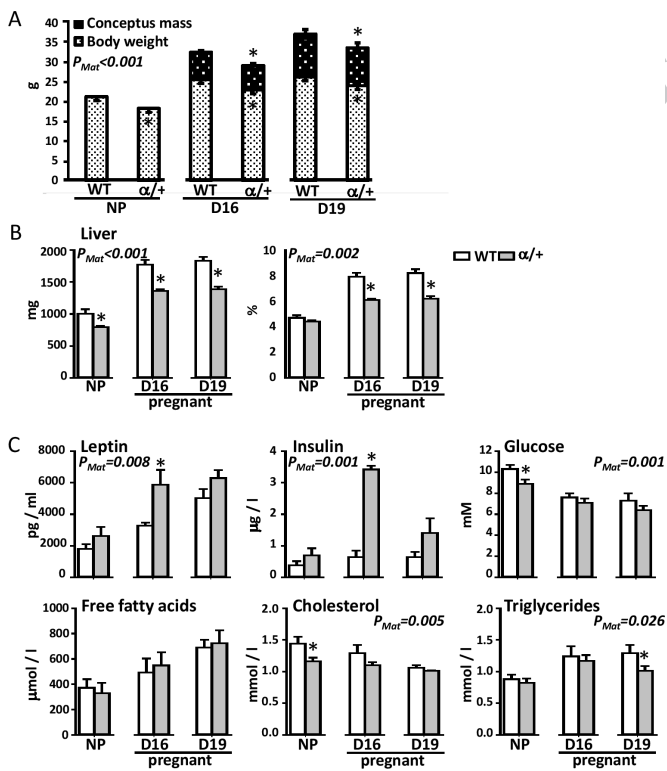
## Significance

**During pregnancy, nutrients are required for fetal growth and for the mother to maintain the pregnancy. The placenta is central to this tug-of-war over nutrients as it is responsible for materno-fetal resource allocation. Failure to allocate resources appropriately can lead to pregnancy complications and abnormal fetal development with long-term health consequences for both mother and baby. Here we use manipulation of a growth-regulatory protein, p110 $\alpha$ , in mice to compare the importance of the maternal environment and genotype, with fetal genotype, in determining placental resource allocation. This study shows that the placenta fine-tunes the supply of maternal resources to the fetus via p110 $\alpha$  in accordance with both the fetal drive for growth, and the maternal ability to supply the required nutrients.**

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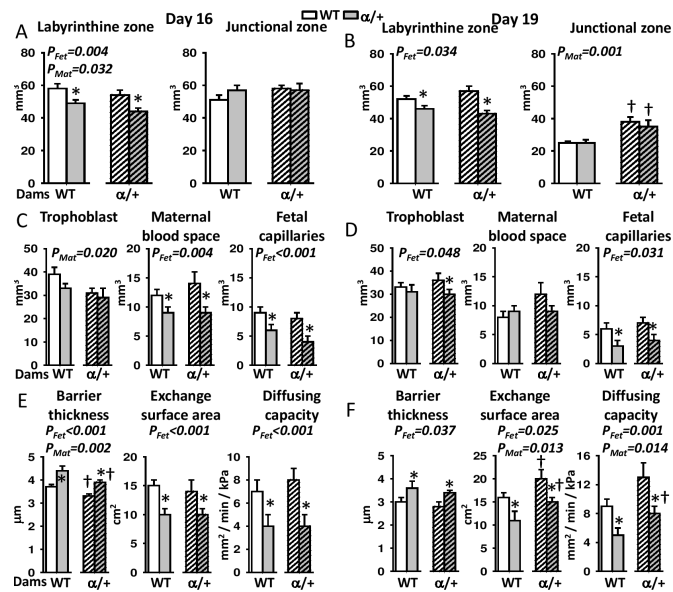
**Fig. 1. Feto-placental growth in response to p110 $\alpha$  deficiency.** Placental (A, B) and fetal weights (C, D) on D16 (A, C) and D19 (B, D) of pregnancy. Data from crosses where the mother is WT (circle symbols) and  $\alpha/+$  (triangle symbols) with mean denoted as a horizontal line with SEM. Number of fetuses in parentheses. \*Significantly different to WT littermates ( $P < 0.05$ , pairwise comparison). †Significantly different to WT dams ( $P < 0.05$ , pairwise comparison).



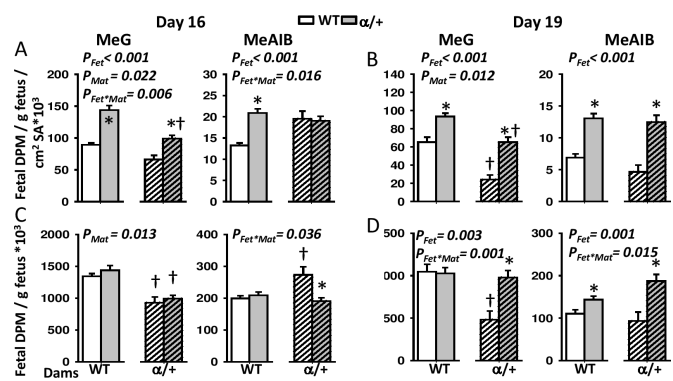
**Fig. 2. The effect of p110 $\alpha$  deficiency on maternal body weight (A), hepatic weight (B) and circulating metabolic indicators (C).** Mean $\pm$ SEM values from age-matched non-fasted WT and  $\alpha/+$  littermates. \*Significantly different to WT dams ( $P < 0.05$ , t test comparison). Numbers of mice used for body composition and blood glucose were 7-11 and for plasma metabolites and hormones were 5-8 samples per genotype, per age.

and heterozygous p110 $\alpha$  mutants,  $\alpha/+$ ; Fig. S1). This breeding strategy allows assessment of the effects of the p110 $\alpha$  mutation in the feto-placental unit (13), compared to WT littermates (referred to as fetal genotype and statistically as  $P_{Fet}$ ) as well effects of the p110 $\alpha$  mutation in the mother, compared to WT dams ( $P_{Mat}$ ). Effects of both maternal and fetal genotypes are referred throughout as fetal and maternal interaction ( $P_{Fet*Mat}$ ).

There was no significant difference in the litter size of WT and  $\alpha/+$  dams at either D16 or D19 (mean $\pm$ SEM: D16,  $6.5 \pm 0.3$  and  $6.7 \pm 0.4$ ; D19,  $6.3 \pm 0.4$  vs  $6.2 \pm 0.3$ , respectively) or in the



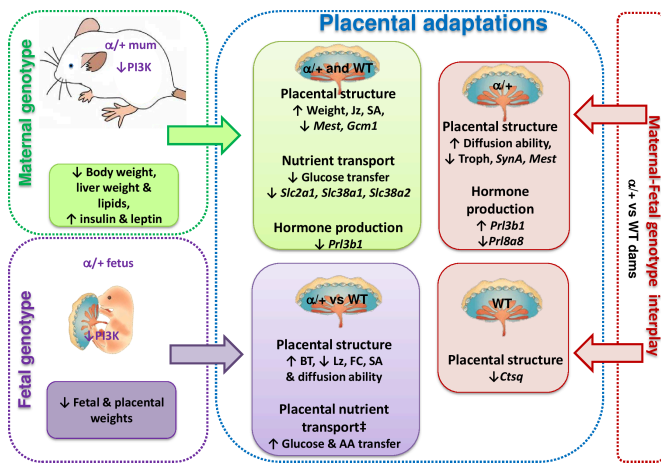
**Fig. 3. Placental morphology in response to p110 $\alpha$  deficiency.** Placental structure (A, B), labyrinthine composition (C, D) and substrate exchange characteristics (E, F) on D16 (A, C, E) and 19 (B, D, F) of pregnancy. Mean $\pm$ SEM values for >5 litters per maternal genotype per gestational age. From each litter, 1-2 placentas per genotype were analysed for gross structure. Across 4-5 litters, a representative placenta per genotype, per cross was taken for labyrinthine zone analysis. \*Significantly different to WT littermates ( $P < 0.05$ , pairwise comparisons). †Significantly different to WT dams ( $P < 0.05$ , pairwise comparisons).



**Fig. 4. Placental nutrient transfer in response to p110 $\alpha$  deficiency.** Materno-fetal transfer of MeG and MeAIB on D16 (A and C) and 19 (B and D) of pregnancy expressed relative to the exchange surface area (A, B) and/or per gram of fetus (C, D). Mean $\pm$ SEM for 8 and 10 litters on D16 ( $n = 23-36$  per fetal genotype, per cross) and 5 and 5 litters on D19 ( $n = 15-19$ ), for WT and  $\alpha/+$  dams, respectively. \*Significantly different to WT littermates ( $P < 0.05$ , pairwise comparisons). †Significantly different to WT dams ( $P < 0.05$ , pairwise comparisons). SA, exchange surface area.

ratio of WT to  $\alpha/+$  fetuses per litter (mean $\pm$ SEM:  $0.49 \pm 0.03$  versus  $0.51 \pm 0.03$  for litters in WT and  $\alpha/+$  dams at both days of pregnancy).  $\alpha/+$  placentas and fetuses were significantly lighter than WT at both ages (Fig. 1;  $P_{Fet} < 0.01$ ). The average growth restriction of  $\alpha/+$  placentas across maternal genotypes was 9% and 12% of WT, at D16 and D19, respectively, with corresponding fetal growth restriction of 19% and 11% of WT. Consequently, placental efficiency (measured as weight ratio of fetus to placenta) in  $\alpha/+$  fetuses was reduced at D16 but not D19 (Fig. S2;  $P_{Fet} < 0.001$ ). Both placental weight and efficiency, but not fetal weight, were significantly influenced by maternal genotype at D19 (Fig. 1B and Fig. S2; both  $P_{Mat} < 0.05$ ). Accordingly, placentas of

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**Fig. 5. Summary illustration showing the effects of maternal genotype (green text boxes), fetal genotype (purple text boxes) and their interplay (pink text boxes) in determining placental phenotype at D19 of pregnancy.** ‡transfer per unit SA. AA, amino acid; BT, barrier thickness; FC, fetal capillaries; Jz, junctional zone; Lz, labyrinthine zone; SA, surface area for exchange; Troph, trophoblast.

$\alpha/+$  dams were heavier ( $\sim+15\%$ , Fig. 1B;  $P_{Mat} < 0.05$ ) and less efficient relative to WT dams (Fig. S2;  $P_{Mat} < 0.05$ ), irrespective of fetal genotype. These findings reveal for the first time that fetal p110 $\alpha$  deficiency causes placental growth restriction and that maternal p110 $\alpha$  genotype affects placental weight and efficiency but not fetal weight, particularly at D19. The latter suggest a role for maternal genotype in programming placental phenotype.

#### p110 $\alpha$ deficiency alters female endocrine and metabolic profile

We therefore determined whether p110 $\alpha$  genotype affected the metabolic and endocrine profile of non-pregnant (NP) and pregnant female mice. Age-matched  $\alpha/+$  NP females were 14% lighter than WT (Fig. 2A), in line with previous observations of postnatal growth for these mice (13). Consistent with lower starting D1 weights (mean $\pm$ SEM: 21.8 $\pm$ 0.3g and 19.4 $\pm$ 0.3g for WT and  $\alpha/+$  dams,  $P < 0.05$ , respectively), age-matched, time-mated  $\alpha/+$  females were  $\sim 10\%$  lighter than their WT counterparts at D16 and D19, in whole body and hysterectomised weight (Fig. 2A). Gravid uterus mass, however, was similar in the two maternal genotypes (Fig. 2A). Absolute liver weight was 20-25% less in  $\alpha/+$  than WT females. In NP females, this reduction was in line with the reduced body weight, whereas during pregnancy, the liver accounted for a smaller proportion of hysterectomised weight in  $\alpha/+$  than WT dams (Fig. 2B). At D16,  $\alpha/+$  females were hyperinsulinemic, with concentrations 5.3-fold higher than WT values (Fig. 2C, trends only observed for NP and D19 pregnant dams). Compared to WTs, NP  $\alpha/+$  females were hypoglycaemic (glucose concentration -14%), however, during pregnancy they were normoglycaemic, suggesting insulin resistance of the  $\alpha/+$  dam particularly at D16 (Fig. 2C). Whilst there was no difference in plasma leptin with genotype prior to pregnancy, D16  $\alpha/+$  pregnant females were hyperleptinemic (+80%), with concentrations returning to WT values by D19 (Fig. 2C). Plasma concentrations of cholesterol in NP  $\alpha/+$  females were 19% lower than WT but did not differ between genotypes during pregnancy (Fig. 2C).  $\alpha/+$  females had lower plasma triglycerides specifically on D19 (-22%) although free fatty acid concentrations were unaffected by genotype either before or during pregnancy (Fig. 2C). Thus, p110 $\alpha$  deficiency altered body composition and the endocrine and metabolic state in both NP and pregnant states but with more pronounced effects during pregnancy. These metabolic changes are likely to impact on placental phenotype which we interrogated next.

#### p110 $\alpha$ deficiency impairs the formation of the placenta

We assessed whether changes in placental growth with fetal and maternal p110 $\alpha$  genotype were accompanied by morphological alterations using stereology. With respect to fetal genotype, the placental exchange labyrinthine zone (Lz) was 18% smaller in  $\alpha/+$  than WT littermates (Fig. 3A-B;  $P_{Fet} < 0.05$ ) and related to reductions in the fetal capillary volume (-41% and -47%), exchange surface area (-22% and -27%) and in diffusion capacity (-47 and -36%), at D16 and D19, respectively (Fig. 3C-F;  $P_{Fet} < 0.01$ ). These changes were accompanied by a  $\sim 20\%$  thicker barrier between the fetal and maternal blood spaces and a  $\sim 25\%$  reduction in fetal capillary length, at both ages (Fig. 3E-F and Fig. S3).  $\alpha/+$  placentas had 30% less maternal blood space volume at D16 and 12% less trophoblast at D19 compared to WT littermates (Fig. 3C-D;  $P_{Fet} < 0.05$ ).

Maternal genotype affected placental Lz morphology at both ages ( $P_{Mat} < 0.04$ ), but with opposite effects at the two ages. At D16 in  $\alpha/+$  dams, the volumes of the placental Lz and trophoblast were reduced (-9% and -17%, respectively), fetal capillaries were 14% shorter and exchange barrier was 11% thinner compared to WT dams (Fig. 3A and E and Fig. S3A). At D19 in  $\alpha/+$  dams, the volume of the placental endocrine junctional zone (Jz) was 46% greater, the fetal capillaries were 18% larger in diameter, the diffusing capacity 52% greater and the exchange surface 34% greater (Fig. 3B and F and Fig. S3B).

There were significant interactions between maternal and fetal genomes in determining fetal capillary length and diameter on D16 ( $P_{Fet \times Mat} < 0.03$ ). Placental capillaries were significantly shorter (-29%) in WT fetuses of  $\alpha/+$  dams specifically, whilst diameter was reduced (-18%) in  $\alpha/+$  fetuses of  $\alpha/+$  dams only (Fig. S3A). There were also interactions between fetal and maternal genotypes at the level of placental nutrient storage at D16. There was no difference in glycogen content between WT and  $\alpha/+$  placentas in WT dams (mean $\pm$ SEM: 9.7 $\pm$ 0.4mg/g vs 9.9 $\pm$ 0.7mg/g) whereas, in  $\alpha/+$  dams, glycogen content was reduced by 38% in  $\alpha/+$  placentas compared to their WT littermates (mean $\pm$ SEM: 8.7 $\pm$ 0.7mg/g vs 5.7 $\pm$ 0.4mg/g for WT and  $\alpha/+$ , respectively,  $P < 0.001$ ). Collectively, these data indicate that fetal and maternal genotypes interact to modify the morphology and composition of the placenta.

#### p110 $\alpha$ deficiency affects placental nutrient transport

To assess whether placental transport function was altered by lack of p110 $\alpha$  in either the fetus and/or mother, we determined the *in vivo* uni-directional, materno-fetal transfer of the non-metabolisable analogues of glucose and a neutral amino acid using  $^3\text{H}$ -methyl D-Glucose (MeG) and  $^{14}\text{C}$ -methyl amino-isobutyric acid (MeAIB), respectively at D16 and at D19. Since the mutant placentas were smaller relative to their WT littermates at these ages, we measured absolute accumulation of radioisotopes by the fetus and also expressed fetal counts relative to estimated surface area for transport and/or to fetal weight, which provide indices of the placental capacity for nutrient transfer and fetal growth relative to supply.

Placental transfer of both MeG and MeAIB relative to estimated surface area shows that  $\alpha/+$  mutant placentas transferred more of both solutes compared to WT at both gestational ages (+56% and +107% for MeG and +30 and +122% for MeAIB at D16 and D19, respectively, Fig. 4A-B;  $P_{Fet} < 0.001$ ). This suggests the  $\alpha/+$  placenta transfers the solutes with increased efficiency in compensation for the reduced surface area. In agreement with these findings, mutant fetuses received either normal or increased amount of solutes for their size (Fig. 4C-D;  $P_{Fet} < 0.004$ ), even though the absolute amount of MeG and MeAIB solute received by the  $\alpha/+$  fetus is reduced at D16 (Fig. S4). The increase in solute transfer per surface area is most evident at D19 (Fig. 4B) and is related to less severe growth restriction of  $\alpha/+$  fetuses than at D16 (-19% D16 vs -11% D19).



Maternal genotype also exerted a significant effect on placental MeG transfer (Fig. 4;  $P_{\text{Mat}} < 0.05$ ). Overall placental transfer of this solute estimated per unit surface area was reduced in  $\alpha/+$  compared to WT dams at both ages (63% average of WT, Fig. 4A-B), with  $\sim 30\%$  less fetal accumulation of MeG irrespective of fetal genotype at D16 ( $P_{\text{Mat}} < 0.05$ ; Fig. 4C and Fig. S4). At D19, reduced fetal accumulation of MeG was only seen in WT fetuses of  $\alpha/+$  dams ( $-55\%$ , Fig. 4D;  $P_{\text{Fet}^* \text{Mat}} < 0.01$ ). There were also fetal-maternal genotype interactions modulating fetal MeAIB accumulation at both ages ( $P_{\text{Fet}^* \text{Mat}} < 0.05$ , Fig. 4C and D). In particular, the WT fetus accumulated more MeAIB in  $\alpha/+$  dams on D16, when compared to WT (Fig. 4C). Furthermore, in  $\alpha/+$  but not WT dams,  $\alpha/+$  fetuses accumulated less MeAIB than their WT littermate on D16 (Fig. 4C). Thus, placental transfer capacity is modified by both fetal and maternal genotypes, however the specific nature of the changes depends on gestational age and the nutrient.

#### *p110 $\alpha$ deficiency alters placental expression of nutrient transporters and lineage genes*

Finally, we sought to identify the molecular mechanisms altering placental MeG and MeAIB transport in  $\alpha/+$  fetuses in WT and  $\alpha/+$  dams. We assessed placental expression of genes encoding the glucose (SLC2A; *Slc2a1* and *Slc2a3*) and System A amino acid (SLC38A; *Slc38a1*, *Slc38a2* and *Slc38a4*) transporter proteins. We also measured expression of placental cell lineage genes, which in some cases have been implicated in regulating materno-fetal nutrient allocation (20-22).

Maternal genotype had the strongest effect on placental expression of the transporter and cell lineage genes, although fetal genotype determined the specific nature of the effect (Table S1). In particular, at D16, the placental expression of *Slc38a1* and the giant cell gene *Hand1* were reduced in  $\alpha/+$  dams by  $\sim 40\%$  and  $\sim 35\%$ , respectively, compared to WT dams (Table S1;  $P_{\text{Mat}} < 0.01$ ). In  $\alpha/+$  dams, *Slc38a1* continued to be expressed at lower levels at D19, with expression of *Slc2a1* and *Slc38a2* transporter genes also now reduced by  $\sim 18\%$  ( $P_{\text{Mat}} < 0.001$ ). There was no effect of fetal genotype on placental expression of any of the nutrient transporters at either age studied (Table S1).

At D19, the fetal endothelium *Mest* gene, Jz spongiotrophoblast genes *Prl3b1*, *Prl8a8*, Lz sinusoidal giant cell gene *Ctsq* and syncytial trophoblast genes *Gcm1* and *Syna*, were also lower in expression (ranging from  $-15\%$  to  $-60\%$ ) in litters carried by  $\alpha/+$  compared to WT dams (Table S1). *Mest* and *Prl3b1* expression were also affected by fetal genotype at D19, with lower and higher expression, respectively in  $\alpha/+$  than WT placentas ( $-12\%$  and  $+24\%$ , respectively,  $P_{\text{Fet}} < 0.05$ , Table S1). There was also fetal-maternal genotype interactions in regulating *Syna* gene expression at both ages ( $P_{\text{Fet}^* \text{Mat}} < 0.05$ , Table S1). Thus, fetal and maternal genomes interact to modulate placental expression of several cell lineage genes, particularly at D19 of pregnancy.

## Discussion

This study shows for the first time that: (i) signalling via PI3K-p110 $\alpha$  regulates the formation and function of the mouse placenta, (ii) placental phenotype is influenced by maternal p110 $\alpha$  genotype and (iii) there is an interplay between fetal and maternal genotypes in determining specific placental phenotypes. Using genetic manipulation of p110 $\alpha$  (13), this study demonstrates that development of the placental exchange region was impaired by D16 of pregnancy in association with reduced growth of  $\alpha/+$  fetuses. However, despite its compromised morphology, the  $\alpha/+$  placenta adapted functionally to increase the supply of maternal nutrients to the fetus, an effect that was more pronounced near term. The specific nature of these changes however, depended on whether the mother was WT or mutant, which probably relates, in part, to the altered endocrine and metabolic profile of  $\alpha/+$  dams. Because of these maternal genotype-driven adaptations

in placental phenotype, fetal growth was unaffected by maternal genotype. Thus, by acting on placental phenotype, signalling via p110 $\alpha$  kinase is critical in integrating intrinsic and extrinsic signals governing the balance of materno-fetal resource allocation to fetal growth.

#### *(i) Signalling via PI3K-p110 $\alpha$ regulates placental formation*

We found that the  $\alpha/+$  placenta was lighter at both D16 and D19 of pregnancy and there were defects in the formation of the Lz, irrespective of maternal genotype. At both ages, Lz vascularization was impaired; the fetal capillaries were reduced in volume and shorter in length in the  $\alpha/+$  placentas. Reduced vascularisation of the  $\alpha/+$  placenta is consistent with previous work showing impaired developmental angiogenesis and vascular patterning in these mutant fetuses (19).

At D16, but not D19, the volume of Lz maternal blood spaces was also decreased in  $\alpha/+$  placenta. This may reflect delayed trophoblast-induced dilation of the uterine spiral arteries, which normally occurs by D14.5 of pregnancy (23) as PI3K signalling also regulates the differentiation and invasive properties of trophoblast (24). At both ages, the trophoblast interhemal membrane was thicker and the surface area for exchange was reduced in the  $\alpha/+$  placenta, which resulted in a decreased theoretical diffusing capacity. Changes in Lz morphology of the  $\alpha/+$  mutants were similar to those reported previously for mice deficient in *Igf2*, as well as the PI3K effector protein, protein kinase b (*Akt1*) (25, 26). Together, these data suggest that p110 $\alpha$  is a main mediator of IGF2 actions, operating primarily via AKT1 to attain normal placental weight and Lz structure in mice.

As expected, the changes in Lz morphology of the  $\alpha/+$  placentas impaired the normal capacity to deliver nutrients from mother to fetus; the absolute amount of MeG (significant in WT dams) and MeAIB solute received by the  $\alpha/+$  fetus was reduced at D16, compared to WT littermates. However, estimating the placental transfer of these solutes per unit exchange surface area showed greater transport for  $\alpha/+$  fetuses at both ages, suggesting compensatory adaptive mechanisms of both facilitated and active transport in the  $\alpha/+$  placenta. This upregulation of placental transport achieved greater amounts of solute per gram fetal mass in  $\alpha/+$  mutants compared to their WT littermates at D19 and was associated with an improved fetal growth trajectory of  $\alpha/+$  compared to WT. Alterations in nutrient transport capacity were however, unrelated to expression of *Slc2a* and *Slc38a* glucose and amino acid transporter genes in the  $\alpha/+$  placenta at either age. Adaptive upregulation of placental transport function has been seen when the fetal demand for maternal resources exceeds the supply capacity of the placenta following deletion of the *Slc2a3* glucose transporter gene, in naturally small placentas within litters and when placental growth is impaired by a deficiency in the Lz-specific *Igf2* transcript or the 11- $\beta$  hydroxysteroid dehydrogenase gene (27-31). Moreover, System A amino acid transporter activity is adaptively up-regulated in the small human placenta of infants at the lower end of the normal birthweight range (32), but is diminished in pathological pregnancies with severe fetal growth restriction (33, 34). Further work is required to determine the mechanism by which the  $\alpha/+$  placenta adapts, largely independent of maternal genotype. Further work should also identify the whether fetal sex influences the observed alterations in placental phenotype with fetal genotype, and their possible interaction with the maternal environment (35).

#### *(ii) Placental phenotype is modified by maternal p110 $\alpha$ genotype*

Placental transport for both WT and  $\alpha/+$  fetuses were additionally influenced by the phenotype of the  $\alpha/+$  dam.  $\alpha/+$  female mice were lighter and exhibited an altered endocrine and metabolic profile prior to pregnancy. However, some of these changes were exacerbated (fractional liver weight, hyperinsulinemia, hyperleptinemia and hypotriglyceridemia) and others mitigated (hypcholesterolemia) by pregnancy in these mutants.

At D16 of pregnancy, Lz volume and its volume of trophoblast were less and fetal capillaries shorter particularly for WT placentas, in  $\alpha/+$  dams, even though absolute placental weight was not altered by maternal genotype at this age. Reduced placental vascularisation has been observed in embryos transferred to recipients of a smaller breed (8, 36) and, thus, the current findings suggest that the smaller-sized  $\alpha/+$  dam may be attempting to constrain fetal growth via actions on the placental supply capacity. Indeed, the placental ability to transport MeG per gram fetus was less for both WT and  $\alpha/+$  fetuses in  $\alpha/+$  dams at D16 compared to WT. These observations are also consistent with reduced placental glucose transport capacity in cows of small maternal size (9), sheep of young maternal age (37) as well as in mouse pregnancies where fetal demand for growth exceeds the maternal ability to supply nutrients via the placenta (38). Reduced MeG transfer may also be related to elevated insulin concentrations in  $\alpha/+$  dams, as chronic exposure to maternal hyperinsulinemia reduces placental uptake and fetal supply of glucose (39) and diminished placental glucose transfer occurs in late gestation in association with hyperinsulinemia and other changes in maternal-nutrient partitioning in pregnant mice treated with glucocorticoids (17). In contrast to MeG transport, there was no independent effect of maternal genotype on MeAIB transfer. This occurred despite reduced placental expression of the amino acid transporter gene, *Slc38a1* in litters of  $\alpha/+$  dams (also at D19 along with decreased *Slc38a2*). Similar disparity between *Slc38a* gene expression and transport of MeAIB *in vivo*, has been observed in placentas in which growth regulatory genes (15) or the maternal environment have been affected in rodents (3-7). These findings suggest that there are temporal differences between the expression, translation and membrane trafficking of amino acid transporters in the placenta, and that measuring placental expression of *Slc38a* genes provides little information with respect to *actual* transport function *in vivo*. Further work should investigate the contribution of other transporters that are responsible for facilitating amino acid accumulation, exchange and efflux in the placenta (40). In  $\alpha/+$  dams, the placental barrier to exchange was however, thinner at D16 irrespective of fetal genotype. This structural alteration would have optimised passive diffusion of molecules, such as oxygen, which could be beneficial for fetal growth, particularly given the compromised glucose transfer and maternal environment in  $\alpha/+$  dams.

There were also changes in placental morphology specific to  $\alpha/+$  dams on D19. Placentas were heavier and the exchange surface area larger for both fetal genotypes in  $\alpha/+$ , compared to WT dams. Expression of the *Gcm1* gene, which is involved in early patterning of the Lz vasculature was reduced and fetal capillaries were larger in calibre and showed reduced *Mest* expression, irrespective of fetal genotype, in placentas of  $\alpha/+$  dams, which taken together may indicate a more advanced Lz vascular phenotype (22, 23). Increased placental growth and maturation of the exchange area would have enhanced the placental capacity to supply nutrients and oxygen to the fetus and suggest that the placenta adapts morphologically in response to the altered maternal environment induced by maternal p110 $\alpha$  deficiency. Beneficial changes in placental morphology have also been observed in response to suboptimal maternal environments, including poor nutrition, hypoxia and glucocorticoid over-exposure in many species (3-7).

### (iii) Interplay between fetal and maternal genotypes in determining specific placental phenotypes

Fetal and maternal genomes also interacted at the level of placental nutrient storage and endocrine capacity. In  $\alpha/+$ , but not WT dams, glycogen storage in the  $\alpha/+$  placenta was diminished on D16. Placental glycogen content is reduced in dams fed diets high in sugar and fat and in dams carrying *Igf2PO* null litters, both conditions in which there are signs of maternal

insulin resistance and altered placental PI3K signalling (15, 16).  $\alpha/+$  dams were normoglycemic despite their hyperinsulinemia at D16, indicative of maternal insulin resistance in this mutant too. However, in contrast to the  $\alpha/+$  placenta, glycogen content of the WT placenta was not affected by maternal genotype, which suggests that signalling via p110 $\alpha$  in the mouse placenta is required to maintain placental glycogen under conditions of maternal insulin resistance. Future investigations should determine whether the differentiation and/or lysis of glycogen cells in the Jz (41), is altered in response to fetal and maternal p110 $\alpha$  deficiency especially since Jz volume was greater for both fetal genotypes in mutant dams at D19. Maternal genotype also affected the expression of different cell lineage genes in the placental Lz (*Syna*, *Mest*, *Gcm1* and *Ctsq*) and the Jz (*Prl8a8* and *Prl3b1*). In some instances, fetal genotype determined the extent of the effect. For instance, spongiotrophoblast *Prl8a8* expression was significantly decreased in  $\alpha/+$ , but not WT fetuses in mutant dams at D19. Moreover, depending on maternal genotype, the  $\alpha/+$  placenta exhibited increased expression of the *Prl3b1*/chorionic somatomammotropin-2 gene, compared to WT littermates at D19. Insulin secretion and  $\beta$ -cell proliferation are increased by placental production of the prolactin/chorionic somatomammotropin family of hormones (PRL/CSH) (42). Future work should determine the inter-relationship between changes in the endocrine Jz and the metabolic state of the  $\alpha/+$  dams.

Due to overall differences in placental weight, structure and functional capacity, the outcome for fetal growth was the same in  $\alpha/+$  and WT dams (i.e. there was no interaction of fetal and maternal genomes on fetal weight). These findings suggest that the placenta acts as a 'buffer' to fine-tune the supply of maternal resources to the fetus in accordance with both the genetically-determined fetal drive for growth, and the maternal ability to supply the nutrients required for fetal growth. However, the adaptive signals modifying placental transport capacity in  $\alpha/+$  mutants in late pregnancy remain to be identified, although they are likely to originate from both the mother and the growth restricted conceptus (Fig. 5). The relative contribution of the fetus and placenta to placental phenotype and adaptive responses could not be determined as both the fetus and placenta carry the mutation in  $\alpha/+$  conceptuses. Future work could use cell-selective manipulation of the p110 $\alpha$  gene to examine the specific contribution of these tissues in the regulation of placental phenotype.

In the present study, the fetal weight to placental weight ratio, which is often used as a proxy measure of placental efficiency (3), did not always track with nutrient transfer estimated per unit placental surface area. Numerous factors determine the ability of the placenta to support fetal nutrient provision and growth, beyond simply its wet weight; including surface area, vascularity and activity of nutrient transporters (43). This work therefore, highlights that caution is warranted when interpreting changes in the fetal weight to placental weight ratio, particularly if there is no information on the morphological or functional characteristics of the placenta.

In summary, our data show that the WT and  $\alpha/+$  placenta adopt different strategies at the level of nutrient supply capacity, to cope with the altered metabolic state of the  $\alpha/+$  dam and, in a broader sense, emphasise the interplay between maternal and fetal genomes in determining the amount of materno-fetal resource allocation (Fig. 5). These findings are important in the context of human pregnancy as dysregulated expression of both up and down-stream components of the PI3K pathway have been reported in human placenta associated with abnormal fetal growth (44-50). Furthermore, our study highlights the usefulness of the PI3K  $\alpha/+$  mouse as a tool for investigating the role and mechanisms of maternal-fetal genomic interactions in determining fetoplacental phenotype and the programming of subsequent offspring health.



## Materials and Methods

Mice were housed in the University of Cambridge Animal Facility abiding by the UK Home Office Animals (Scientific Procedures) Act 1986 and local ethics committee. Virgin 10 week old C57BL/6 WT and *a/+* female mice were mated to *a/+* and WT males, respectively. *a/+* mice were backcrossed to C57BL/6 for >8 generations. On D16 or 19 of pregnancy, dams were anaesthetised (fentanyl-fluanisone:midazolam in sterile water at 1:1:2, Jansen Animal Health) and underwent a placental nutrient transfer assay or were used for maternal blood collection by cardiac puncture and liver retrieved following cervical dislocation. Virgin 8-10 week old WT and *a/+* female mice (littermates) were anaesthetised, blood collected and liver weighed as for pregnant mice. The *a/+* mice were previously generated as described in (13) and genotyped using primers 5'-TTCAAGCACTGTTTCAGCT-3' and 5'-TTATGTTCTTGCTCAAGTCTCA-3'. Blood glucose and plasma concentrations of leptin, insulin, triglycerides, cholesterol and free fatty acids were deter-

1. Barker DJ (2004) Developmental origins of adult health and disease. *J Epidemiol Community Health* 58(2):114-115.
2. Petry CJ, Ong KK, & Dunger DB (2007) Does the fetal genotype affect maternal physiology during pregnancy? *Trends in Molecular Medicine* 13(10):414-421.
3. Fowden AL, Sferruzzi-Perri AN, Coan PM, Constancia M, & Burton GJ (2009) Placental efficiency and adaptation: endocrine regulation. *J Physiol* 587(Pt 14):3459-3472.
4. Dimasuy KG, Bouef P, Powell TL, & Jansson T (2016) Placental responses to changes in the maternal environment determine fetal growth. *Front Physiol* 7:12.
5. Sferruzzi-Perri AN & Camm EJ (2016) The programming power of the placenta. *Front Physiol* 7:33.
6. Myatt L (2006) Placental adaptive responses and fetal programming. *J Physiol* 572(Pt 1):25-30.
7. Zhang S, et al. (2015) Placental adaptations in growth restriction. *Nutrients* 7(1):360-389.
8. Wilson ME, Biensen NJ, Youngs CR, & Ford SP (1998) Development of Meishan and Yorkshire littermate conceptuses in either a Meishan or Yorkshire uterine environment to day 90 of gestation and to term. *Biol Reprod* 58(4):905-910.
9. Ferrell CL (1991) Maternal and fetal influences on uterine and conceptus development in the cow: II. Blood flow and nutrient flux. *Journal of Animal Science* 69(5):1954-1965.
10. Engelman JA, Luo J, & Cantley LC (2006) The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. *Nat Rev Genet* 7(8):606-619.
11. Knight ZA, et al. (2006) A pharmacological map of the PI3-K family defines a role for p110[alpha] in insulin signaling. *Cell* 125(4):733-747.
12. Grey A, et al. (2010) Evidence for a role for the p110[alpha] isoform of PI3K in skeletal function. *Biochem Biophys Res Commun* 391(1):564-569.
13. Foukas LC, et al. (2006) Critical role for the p110[alpha] phosphoinositide-3-OH kinase in growth and metabolic regulation. *Nature* 441(7091):366-370.
14. Sferruzzi-Perri AN, Owens JA, Pringle KG, & Roberts CT (2010) The neglected role of insulin-like growth factors in the maternal circulation regulating fetal growth. *J Physiol* 589(Pt 1):7-20.
15. Sferruzzi-Perri AN, et al. (2011) Placental-specific Igf2 deficiency alters developmental adaptations to undernutrition in mice. *Endocrinology* 152(8):3202-3212.
16. Sferruzzi-Perri AN, et al. (2013) An obesogenic diet during mouse pregnancy modifies maternal nutrient partitioning and the fetal growth trajectory. *FASEB* 27(10):3928-3937.
17. Vaughan OR, et al. (2015) Corticosterone alters maternal-fetal glucose partitioning and insulin signalling in pregnant mice. *J Physiol* 593(5):1307-1321.
18. Higgins JS, Vaughan OR, de Liger EF, Fowden AL, & Sferruzzi-Perri AN (2015) Placental phenotype and resource allocation to fetal growth are modified by the timing and degree of hypoxia during mouse pregnancy. *J Physiol* 594(5):1341-1356.
19. Graupera M, et al. (2008) Angiogenesis selectively requires the p110-a isoform of PI3K to control endothelial cell migration. *Nature* 453(7195):662-666.
20. Simmons DG & Cross JC (2005) Determinants of trophoblast lineage and cell subtype specification in the mouse placenta. *Dev Biol* 284(1):12-24.
21. Simmons DG, Fortier AL, & Cross JC (2007) Diverse subtypes and developmental origins of trophoblast giant cells in the mouse placenta. *Dev Biol* 304(2):567-578.
22. Mayer W, et al. (2000) Expression of the imprinted genes MEST/Mest in human and murine placenta suggests a role in angiogenesis. *Developmental Dynamics* 217(1):1-10.
23. Adamson SL, et al. (2002) Interactions between trophoblast cells and the maternal and fetal circulation in the mouse placenta. *Dev Biol* 250(2):358-373.
24. Kent L, Konno T, & Soares M (2010) Phosphatidylinositol 3 kinase modulation of trophoblast cell differentiation. *BMC Dev Biol* 10(1):97.
25. Coan PM, et al. (2008) Disproportional effects of Igf2 knockout on placental morphology and diffusional exchange characteristics in the mouse. *Journal of Physiology* 586(20):5023-5032.
26. Yang Z-Z, et al. (2003) Protein kinase B{alpha}/Akt1 regulates placental development and fetal growth. *J Biol Chem* 278(34):32124-32131.
27. Coan PM, et al. (2008) Adaptations in placental nutrient transfer capacity to meet fetal growth demands depend on placental size in mice. *J Physiol* 586(18):4567-4576.
28. Constancia M, et al. (2005) Adaptation of nutrient supply to fetal demand in the mouse involves interaction between the Igf2 gene and placental transporter systems. *Proc Natl Acad Sci USA* 102(52):19219-19224.
29. Dilworth MR, et al. (2010) Placental-specific Igf2 knockout mice exhibit hypocalcemia and adaptive changes in placental calcium transport. *Proc Natl Acad Sci USA* 107(8):3894-3899.
30. Ganguly A, et al. (2007) Glucose transporter isoform-3 mutations cause early pregnancy loss and fetal growth restriction. *Am J Physiol Endocrinol Metab* 292(5):E1241-1255.
31. Wyrwoll CS, Seckl JR, & Holmes MC (2009) Altered placental function of 11[beta]-hydroxysteroid dehydrogenase 2 knockout mice. *Endocrinology* 150(3):1287-1293.
32. Godfrey KM, et al. (1998) Neutral amino acid uptake by the microvillous plasma membrane

mined as reported (51). Materno-fetal clearance of MeAIB (NEN NEC-671; specific activity 1.86GBq/mmol, Perkin Elmer, Massachusetts, USA) and MeG (NEN NEC-377; specific activity 2.1GBq/mmol) were measured two minutes after maternal tracer injection as described (52). Entire litters of placentas were collected for morphological analyses using the Computer Assisted Stereological Toolbox program as previously described (16), or snap frozen for quantification of gene expression by qPCR (Table S2), or tissue glycogen content using amyloglucosidase as reported (51). Data were analysed using SPSS 21 using t test (effect of genotype on maternal body composition) or 2-way ANOVA (effect of maternal and fetus genotype) with pairwise comparisons. Data from individual litters (fetal and placental weights and transport) were analysed using a 2-factor generalized linear mixed-model (maternal and fetus genotypes as factors), with litter identified as a random effect, followed by sequential pairwise comparisons to assess the effect of maternal and/or fetal genotype. Data were significant when  $P < 0.05$ .

of the human placenta is inversely related to fetal size at birth in normal pregnancy. *The Journal of Clinical Endocrinology and Metabolism* 83(9):3320-3326.

33. Glazier JD, et al. (1997) Association between the activity of the system A amino acid transporter in the microvillous plasma membrane of the human placenta and severity of fetal compromise in intrauterine growth restriction. *Pediatr Res* 42(4):514-519.
34. Jansson T & Powell TL (2006) IFPA 2005 Award in Placentalogy Lecture. Human placental transport in altered fetal growth: does the placenta function as a nutrient sensor? -- a review. *Placenta* 27 Suppl A:S91-97.
35. Clifton VL (2010) Review: Sex and the human placenta: mediating differential strategies of fetal growth and survival. *Placenta* 31 Suppl:S33-39.
36. Biensen NJ, Wilson ME, & Ford SP (1998) The impact of either a Meishan or Yorkshire uterus on Meishan or Yorkshire fetal and placental development to days 70, 90, and 110 of gestation. *Journal of Animal Science* 76(8):2169-2176.
37. Wallace JM, Bourke DA, Aitken RP, Milne JS, & Hay WW (2003) Placental glucose transport in growth-restricted pregnancies induced by overnourishing adolescent sheep. *J Physiol* 547(1):85-94.
38. Angiolini E, et al. (2011) Developmental adaptations to increased fetal nutrient demand in mouse genetic models of Igf2-mediated overgrowth. *FASEB* 25(5):1737-1745.
39. Aldoretta PW, Carver TD, & Hay WW, Jr. (1994) Ovine uteroplacental glucose and oxygen metabolism in relation to chronic changes in maternal and fetal glucose concentrations. *Placenta* 15(7):753-764.
40. Cleal J & Lewis R (2008) The mechanisms and regulation of placental amino acid transport to the human foetus. *J Neuroendocrinol* 20(4):419-426.
41. Coan PM, Conroy N, Burton GJ, & Ferguson-Smith AC (2006) Origin and characteristics of glycogen cells in the developing murine placenta. *Developmental Dynamics* 235(12):3280-3294.
42. Brejlec TC, et al. (1993) Effect of homologous placental lactogens, prolactins, and growth hormones on islet B-cell division and insulin secretion in rat, mouse, and human islets: implication for placental lactogen regulation of islet function during pregnancy. *Endocrinology* 132(2):879-887.
43. Hayward CE, et al. (2016) Placental adaptation: What can we learn from birth-weight:placental weight ratio? *Front Physiol* 7:28.
44. Laviola L, et al. (2005) Intrauterine growth restriction in humans is associated with abnormalities in placental insulin-like growth factor signaling. *Endocrinology* 146(3):1498-1505.
45. Yung H-w, et al. (2008) Evidence of placental translation inhibition and endoplasmic reticulum stress in the etiology of human intrauterine growth restriction. *Am J Pathol* 173(2):451-462.
46. Street ME, et al. (2011) Impairment of insulin receptor signal transduction in placentas of intra-uterine growth-restricted newborns and its relationship with fetal growth. *European Journal of Endocrinology* 164(1):45-52.
47. Abu-Amro SN, Ali Z, Bennett P, Vaughan JI, & Moore GE (1998) Expression of the insulin-like growth factors and their receptors in term placentas: a comparison between normal and IUGR births. *Molecular Reproduction and Development* 49(3):229-235.
48. Jansson N, et al. (2012) Activation of placental mTOR signaling and amino acid transporters in obese women giving birth to large babies. *The Journal of Clinical Endocrinology and Metabolism* 98(1):105-113.
49. Scioscia M, et al. (2006) Insulin resistance in human preeclamptic placenta is mediated by serine phosphorylation of insulin receptor substrate-1 and -2. *The Journal of Clinical Endocrinology and Metabolism* 91(2):709-717.
50. Iniguez G, et al. (2014) IGF-IR signal transduction protein content and its activation by IGF-I in human placentas: relationship with gestational age and birth weight. *PLoS One* 9(7):e102252.
51. Musial B, et al. (2016) Proximity to delivery alters insulin sensitivity and glucose metabolism in pregnant mice. *Diabetes* 65(4):851-60.
52. Constancia M, et al. (2002) Placental-specific IGF-II is a major modulator of placental and fetal growth. *Nature* 417(6892):945-948.

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