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Short communication

The effects of an obesogenic diet during pregnancy on fetal growth and placental gene expression are gestation dependent

V. King [a], N. Hibbert [a], J.R. Seckl [b], J.E. Norman [a], A.J. Drake [b, *]

[a] MRC/University of Edinburgh Centre for Reproductive Health, Queen's Medical Research Institute, 47 Little France Crescent, Edinburgh EH16 4TJ, UK
[b] Endocrinology Unit, University/BHF Centre for Cardiovascular Science, University of Edinburgh, Queen's Medical Research Institute, 47 Little France Crescent, Edinburgh EH16 4TJ, UK

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ABSTRACT

Exposure to overnutrition *in utero* may increase offspring cardiometabolic disease risk. A mouse model of maternal exposure to an obesogenic diet (DIO) was used to determine effects on fetal and placental weight and gene expression in mid- and late gestation. DIO altered placental gene expression in mid-gestation without differences in fetal or placental weights. Weight gain was attenuated in DIO dams in late gestation and male pup weight was reduced, however there were no persistent changes in placental gene expression. Differences in maternal weight gain and/or specific dietary components may impact on fetal and placental growth and later disease risk.

1. Introduction

Maternal obesity may lead to an increased risk of cardiovascular disease in the offspring, via a phenomenon termed 'developmental programing' [1]. Given the increasing prevalence of obesity [2], understanding how obesity in a mother might impact on her children's health is of major public health importance. In animal models designed to dissect the mechanisms linking maternal obesity and offspring health, the extent to which programmed effects occur in the offspring is highly variable [3]. We have previously reported very few effects on offspring physiology in offspring of dams maintained on a high-fat, high-sucrose 'cafeteria' diet prior to and during pregnancy (DIO) compared to the offspring of mothers fed a control diet (CON) which was otherwise matched in terms of micro- and macronutrients [4]. In this model, although DIO females were heavier, hyperglycaemic and hyperinsulinaemic before pregnancy, we showed reduced maternal weight gain and improvements in metabolic parameters in DIO dams in late gestation which may be one explanation for the lack of offspring phenotype [4].

Changes in placental nutrient supply may mediate adverse effects on fetal development [5]. Studies in humans and in animal offspring of high-fat fed mothers have reported altered placental growth, gene expression and nutrient transport [6–9]. We hypothesised firstly that maternal exposure to a high-fat high-sucrose diet would affect fetal and placental growth and the expression of placental genes important in growth and nutrient transport in mid-gestation, when the definitive placenta is functional [10] and DIO dams are still heavier than controls, and secondly, that these effects would no longer be present in late gestation as a consequence of reduced maternal weight gain and the improvement in insulin resistance and glucose intolerance [4].

2. Methods

2.1. Animal model

Animal studies were conducted as previously reported [4] under approval by the UK Home Office, under the Animals (Scientific Procedures) Act. From 5 weeks, female C57BL/6 mice were allowed free access to cafeteria (DIO: 58 kcal% fat, 25.5 kcal % carbohydrate as sucrose, 16.4% protein, 5.56 kcal/g) or matched control diets (CON: 10.5 kcal% fat and 73.1 kcal% carbohydrate as corn starch, 16.4% protein, 4.07 kcal/g) (Diets D12331 and D12328, Research Diets, New Brunswick, USA). At 17 weeks, females were time-mated with chow-fed C57BL/6 males (RMI 801002, Special Diets Services, Witham, UK). Females remained on experimental diets through pregnancy. Pregnant females were killed at E14.5 or E18.5 and pups and placentas weighed. Placentas were dissected and placental labyrinth stored at −80 °C. Tissues were PCR typed for gender using an assay for the *sry* gene [11].

2.2. Quantification of mRNA by real-time PCR

RNA was extracted from placental labyrinth and real-time PCR performed as previously described [4]. We studied the expression of candidate genes known to be important in placental function and which have previously been shown to be sensitive to the prenatal environment, including maternal consumption of an obesogenic diet, glucocorticoid overexposure and undernutrition [6,7,12,13]. These included key nutrient transporters: the glucose transporter *glut1* and the system A amino acid transporters *slc38a1*, *slc38a2* and *slc38a4*; the imprinted genes: insulin like growth factor 2 (*igf2*) and insulin like growth factor 2 receptor (*igf2r*) and genes

* Corresponding author. Tel.: +44 131 2426748; fax: +44 131 2426779.
E-mail address: mandy.drake@ed.ac.uk (A.J. Drake).

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involved in glucocorticoid metabolism and action – the glucocorticoid receptor (*gr*) and 11β-hydroxysteroid dehydrogenase type 2 (*11βhsd2*). Primer sequences are available on request. Results were normalised to the housekeeping gene *tbp*.

2.3. Statistics

Data are expressed as mean ± SEM. Groups were compared by independent *t*-tests using Statistica (Statsoft).

3. Results and discussion

DIO females were heavier than CON at mating and at E14.5 (Table 1). At E14.5 there were differences in the expression of placental genes (Fig. 1A and B) with increased expression of *slc38a2* in males and *slc38a4* in females consistent with previous studies reporting increased expression of placental amino acid transporters with maternal exposure to an obesogenic diet [6,13]. There was also a >2-fold increase in *gr* expression in both sexes comparing DIO with controls. Since glucocorticoid exposure is associated with increased placental amino acid transport [12] increased glucocorticoid signalling through placental *gr* may be one mechanism driving the altered amino acid transporter expression. *igf2* and *igf2r* expression was also increased in placentae of male pups in association with maternal obesity at E14.5. Although increased *igf2* expression seen in males would predict increased placental and/or fetal growth there were no differences in placental or fetal weight (Table 1). The increased *igf2* expression may be counteracted by increased expression of *igf2r* which acts to reduce *igf2* signalling [14]. In females neither gene changed. Sex differences in offspring responses to early life environmental influences have been reported [15,16] and potential mechanisms include sex chromosome complement, sex steroid exposure, sex differences in epigenetic regulation and different rates of growth and development between males and females (reviewed in Ref. [15]).

Although DIO females were heavier than CON at mating and at E14.5, they gained less weight during late gestation, so that by E18.5 there were no differences in maternal body weight between groups (Table 1) and we have previously also shown that there are no persistent differences in fat mass between DIO and CON females at E18.5 [4]. We have proposed that the reduced late gestation weight gain in DIO dams and the relative improvement in metabolic parameters compared to pre-pregnancy, including normalisation of glucose tolerance and triglyceride levels, might be important in limiting offspring effects [4] and consistent with this, the differences in placental gene expression did not persist into late gestation (Fig. 1C and D). In a recent detailed study the maintenance of female mice specifically during pregnancy on an obesogenic diet previously shown to programme cardiovascular and metabolic dysfunction in offspring [17] resulted in altered expression and activity of placental nutrient transporters and altered *igf2* expression at E16. These differences were no longer present at E19, although there were persistent changes in the expression of genes important in fat metabolism and insulin signalling [13]. Our results add to this study and to others using other dietary manipulations which report dynamic changes in placental gene expression including *igf2* and nutrient transporters [18,19] by showing that such changes may be sex-specific.

At E18.5, male weight was marginally lower in DIO litters but there were no differences in the weights of females or placentas between groups (Table 1). The reduced male pup weight is consistent with rodent studies reporting reduced fetal weight with maternal obesity/overnutrition [20,21] and in humans, although maternal obesity is generally associated with increased birthweight [22], there is also an increased incidence of fetal growth retardation [23]. Studies in sheep suggest that fetal adaptation to nutrient excess may result in a period of fetal *undernutrition* in later gestation as a consequence of alterations in placental vascularity and nutrient transporter activity [24,25] and our results suggest that the preceding, diet-induced, sex-specific changes in *igf2* and *igf2r* may also influence the trajectory of fetal growth.

Our study adds to the literature showing dynamic effects on placental gene expression with maternal exposure to an obesogenic diet. Whilst placental development and transplacental nutrient transport are clearly sensitive to dietary manipulations [13,19,26,27], studies aimed at dissecting the effects of exposure to maternal obesity or overnutrition *in utero* differ in terms of the diets used and the length of time animals are maintained on the diet. Additionally, the obesogenic and control diets used within studies are often not matched in terms of micro- and macronutrient content. Such studies report different patterns of maternal weight gain and adipose tissue deposition [4,13] and result in different short- and long-term effects on offspring development [4,13,17]. We suggest that differences in maternal weight gain, maternal physiology and/or specific dietary components consumed during pregnancy may be important determinants of fetal and placental growth and, ultimately, later disease risk. Further work is needed to understand the effects of specific dietary components on offspring growth and development, how this differs at specific times during pregnancy and how these effects differ between sexes. Studies are

Table 1
Maternal, fetal and placental weights during pregnancy. Data are mean ± SEM. *For Dams*: 16 CON and 21 DIO dams were mated and data on body weight are included for all females at plugging and E14.5. Nine CON and 14 DIO dams were killed at E14.5 and 7 DIO and 7 Con at E18.5 allowing measurement of maternal body weight without uterus. *For fetuses and placentas*: Data was collected from offspring from 9 CON and 14 DIO litters at E14.5 and 7 CON and 7 DIO litters at E18.5.

	CON dams	DIO dams	*p* value
Weight (g) at plugging	21.35 ± 0.26	28.00 ± 0.69	<0.0001
Weight (g) at E14.5 with uterus	29.24 ± 0.56 g	33.40 ± 1.26	<0.0001
Weight (g) at E14.5 without uterus	24.33 ± 0.24 g	27.88 ± 0.63	<0.001
Weight (g) at E18.5 with uterus	32.98 ± 0.53 g	35.85 ± 1.26	0.057
Weight (g) at E18.5 without uterus	26.10 ± 1.35	27.76 ± 0.91	0.33
Litter size (combined E14.5 and E18.5)	6.75 ± 0.49	7.12 ± 0.58	0.63

	F1 CON Males	F1 DIO Males	*p* value	F1 CON females	F1 DIO females	*p* value
E14.5 no. of pups	*n* = 10	*n* = 43		*n* = 14	*n* = 13	
E14 fetal weight (g)	0.20 ± 0.01	0.20 ± 0.01	0.60	0.19 ± 0.01	0.19 ± 0.01	0.93
E14 placental weight (g)	0.074 ± 0.004	0.075 ± 0.009	0.74	0.076 ± 0.004	0.079 ± 0.003	0.66
E14 fetus:placenta ratio	2.77 ± 0.13	2.64 ± 0.10	0.55	2.54 ± 0.15	2.43 ± 0.11	0.61
E18.5 no. of pups	*n* = 27	*n* = 24		*n* = 8	*n* = 18	
E18 fetal weight (g)	0.76 ± 0.02	0.70 ± 0.01	0.049	0.66 ± 0.06	0.67 ± 0.03	0.90
E18 placental weight (g)	0.091 ± 0.003	0.104 ± 0.008	0.15	0.086 ± 0.012	0.089 ± 0.004	0.83
E18 fetus:placenta ratio	8.72 ± 0.39	7.65 ± 0.53	0.11	8.69 ± 1.36	7.82 ± 0.49	0.46