DEXAMETHASONE TREATMENT OF PREGNANT F0 MICE LEADS TO PARENT OF ORIGIN-SPECIFIC CHANGES IN PLACENTAL FUNCTION OF THE F2 GENERATION

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ABSTRACT

Dexamethasone (dex) treatment of F0 pregnant rodents alters F1 placental function and adult cardiometabolic phenotype. The adult phenotype is transmitted to the F2 generation without further intervention but whether F2 placental function is altered by F0 dex treatment remains unknown. In this study, F0 mice were untreated or received dex (0.2 µg $g^{-1}day^{-1}$ *s.c.*) over days (D) 11-15 or D14-18 of pregnancy (term D21). Depending on the period of F0 dex treatment, F1 offspring were lighter at birth or grew more slowly until weaning ($P<0.05$). Glucose tolerance (1g kg^{-1} *ip*) of adult F1 males was abnormal. Mating F1 males dex exposed prenatally with untreated females had no effect on F2 placental function on D19 of pregnancy. Contrastingly, when F1 females were mated with untreated males, F2 placental clearance of the amino acid analogue, ^{14}C-methylaminoisobutyric acid, was increased by 75% at D19 specifically in dams prenatally dex exposed at D14-18 ($P<0.05$). Maternal plasma corticosterone was also increased but F2 placental *Slc38a4* expression was decreased in these dams ($P<0.05$). F0 dex treatment had no effect on F2 fetal or placental weights irrespective of lineage. Effects of F0 dex exposure are, therefore, transmitted intergenerationally to the F2 placenta via the maternal but not paternal line.

INTRODUCTION

Antenatal treatment with the synthetic glucocorticoid, dexamethasone (dex) is used clinically to induce fetal maturation in women threatened with preterm delivery. Despite the benefits to neonatal survival, antenatal dex exposure is associated with reduced size at birth, especially when glucocorticoid dosage is repeated or inappropriately timed (Khan *et al.* 2011). In turn, low birth weight leads to increased cardiometabolic disease risk in later life (Barker 1994). When dex is given to (F0) pregnant rodents, fetal and placental growth is restricted (Smith 2003; Ain 2005; Baisden *et al.* 2007; Cuffe *et al.* 2011; Xu *et al.* 2011; Vaughan *et al.* 2013) and the offspring (F1) develop high blood pressure, hyperglycaemia and insulin resistance (Benediktsson *et al.* 1993; Nyirenda *et al.* 1998; Sugden *et al.* 2001; O'Sullivan *et al.* 2013). More recent animal studies have demonstrated that the phenotype programmed in the F1 offspring by prenatal glucocorticoid overexposure can be transmitted to *their* offspring (F2), via either the mother or father, in the absence of further treatment (Drake *et al.* 2005; Crudo *et al.* 2012; Iqbal *et al.* 2012; Long *et al.* 2013a; Long *et al.* 2013b; Radford *et al.* 2014). Depending on whether it is transmitted through the male or female line, passage of F1 phenotypic traits to the F2 and subsequent generations may be a consequence of germ-line independent changes in maternal intrauterine environment or stable epigenetic changes to the chromatin inherited at fertilisation (Radford and Ferguson-Smith 2011). As the placenta is both zygote-derived and in direct contact with the maternal environment, changes in placental phenotype may mediate the physiological effects of grandparental environmental insults on the F2 fetus.

In the F1 generation, placental structure is altered as a direct consequence of dex treatment of the F0 pregnant mother (Hahn *et al.* 1999; Hewitt *et al.* 2006; Cuffe *et al.* 2011; O'Connell *et al.* 2013; Vaughan *et al.* 2013). Often, dex causes a reduction in the proportion of the labyrinthine zone (Lz) responsible for materno-fetal exchange, along with changes in the size and density of Lz blood vessels (Hewitt *et al.* 2006; O'Connell *et al.* 2011; Vaughan *et al.* 2013). Amino acid transport by the F1 placenta is also altered near term in pregnant mice given dex orally or by injection (Audette *et al.* 2011; Vaughan *et al.* 2013). In the F2 progeny of F1 rats exposed to dex *in utero*, there is altered placental expression of genes including the amino acid transporter genes, along with parent of origin-specific changes in feto-placental growth (Drake *et al.* 2011). Similarly, F1 prenatal exposure to nutritional manipulations that are known to raise endogenous glucocorticoid concentration, alters expression of nutrient

transporters in the F2 mouse placenta (Isganaitis *et al.* 2011; Radford *et al.* 2012). However, whether there are structural and functional changes in the actual nutrient transport capacity of the F2 placenta as a result of dex treatment of the pregnant grandmother remains unknown.

Therefore, this study quantified F2 placental clearance of the non-metabolisable amino acid analogue, 14C-methylaminoisobutyric acid (MeAIB), and the volume of the placental Lz and junctional zone (Jz) in pregnant mice that were either exposed prenatally to dex and mated to untreated males or were untreated themselves and mated with males exposed to dex *in utero*. The System A/SNAT family of transporters that transfer MeAIB across the placenta are known to be essential for normal fetal growth (Cramer *et al.* 2002). Dex was administered to F0 dams at a dose similar to that given to pregnant women threatened with preterm delivery (Brownfoot *et al.* 2008). When given to pregnant animals in previous studies, this dex dosage is known to program abnormal cardiometabolic phenotype in the first-generation offspring (Benediktsson *et al.* 1993; Dodic *et al.* 1998; Nyirenda *et al.* 1998; Woods and Weeks 2005; de Vries *et al.* 2007). F0 females were injected with dex either over the period of maximal placental or fetal growth (Coan *et al.* 2004), whilst controls were untreated in order to remove the known stress of injection *per se* (Meijer *et al.* 2006; Drude *et al.* 2011; Vaughan *et al.* 2013). The study was designed to test the hypothesis that F2 placental phenotype would depend on the timing of F0 dex treatment and on whether treatment was of the maternal or paternal grandmother.

Methods

Animals

All procedures were conducted under the Animals (Scientific Procedures) Act 1986 by UK Home Office license holders. C57BL6/J mice (Harlan, UK) were housed in 12hr:12hr dark:light conditions with *ad libitum* access to food and water throughout. Sixteen female mice were mated with stud males and the day of copulatory plug detection designated as day (D) 1 of pregnancy. Pregnant females (F0) were given a subcutaneous injection of dex (0.2 µg g^{-1} day^{-1} in 0.2 ml sterile saline vehicle) on the morning of each of five days, from D11 to D15 (*n*=6) or from D14 to D18 (*n*=5). Control females (*n*=5) were left untreated. Females were allowed to litter normally at term and to suckle their own offspring. Neonates (F1) were weighed and sexed, and litter size reduced to four, balanced by sex where possible. Pups were weaned at three weeks of age, ear-notched for identification then housed in groups of the same sex but from mixed litters and maternal treatment groups. Body weight was recorded weekly before and after weaning. Upon reaching maturity, 6-8 week old *F1* females (control *n*=7; D11-15 dex *n*=6, D14-18 dex *n*=6) were mated with stud males. Twelve to fourteen week old *F1* male mice (control *n*=7, D11-15 dex *n*=9, D14-18 dex *n*=14) were mated with virgin untreated females, which received no further treatment during pregnancy (control *n*=10, D11-15 dex *n*=13, D14-18 dex *n*=18). Between 1 and 3 pregnancies were successfully sired by each *F1* male.

Glucose tolerance test

After mating, a glucose tolerance test (1 g kg^{-1}, 10% w/v in sterile saline *i.p.*) was carried out on the F1 males after an overnight fast at 14 weeks of age. Glucose concentrations were measured before and at 15, 30, 45, 60, 90 and 120 minutes after glucose injection in a small quantity of blood taken from the tail vein (OneTouch glucometer, Orthoclinical Diagnostics). Two weeks later, males were anaesthetized (fentanyl-fluanisone:midazolam:water, 1:1:2, 10µl g^{-1} body weight) and a blood sample collected for corticosterone measurement (ELISA, IBL International, Hamburg, Germany) before euthanasia by cervical dislocation for tissue collection.

F2 placental phenotype

Pregnant F1 females, and untreated females mated with F1 males were anaesthetized on D19 of pregnancy and unidirectional materno-fetal clearance of 14C-methylaminoisobutyric acid (100µl, NEN NEC-671, specific activity 1.86 GBq $mmol^{-1}$) determined as described previously (Vaughan *et al.* 2012). A cardiac blood sample was taken up to 4 minutes after injection of the tracer and the mother was killed by cervical dislocation. Plasma was separated by centrifugation for liquid scintillation counting and corticosterone measurement. The gravid uterus was removed and individual fetuses and placentae dissected and weighed. Fetuses were lysed (5ml Biosol, National Diagnostics, UK at 55°C) for liquid scintillation counting. The placenta closest in weight to the mean of each litter was halved and fixed in formaldehyde (4% in 0.1M HEPES) for histological processing and stereological determination of volumes of the Lz and Jz, responsible for the transport and endocrine functions of the placenta (Vaughan *et al.* 2012). The placenta second closest to the mean was snap frozen in liquid nitrogen for later gene expression analysis. Expression of System A amino acid transporter isoforms, *Slc38a1, Slc38a2* and *Slc38a4,* was determined relative to *Hprt1* and *Gapdh* using quantitative RT-PCR and the ΔΔCt method (Vaughan *et al.* 2012).

Statistics

All data are mean ± SEM. F1 glucose tolerance was assessed by repeated-measures two-way ANOVA, with F0 treatment and time from glucose injection as independent factors. The effect of F0 treatment on all other measured variables was determined by one-way ANOVA with Bonferroni post-hoc test. Linear correlations of the measured variables were computed by Pearson's product-moment coefficient.

Results

F1 neonatal weight and postnatal growth

All F0 females littered on D21 of pregnancy. Litter size was similar in all treatment groups (average 6.6 pups, range 4-8 pups); however, there was a significant effect of F0 dex treatment on F1 birth weight (Table 1). Newborn control and D11-15 dex treated pups were similar in weight whereas those exposed to dex from D14 to D18 were 8% lighter (Table 1). Conversely, during suckling, D11-15 dex pups gained weight more slowly (fractional growth rate, $P<0.05$ for males and females combined) such that by weaning at 3 weeks of age both males and females were lighter than controls and D14-18 dex pups (Table 1). Although both groups of dex offspring grew faster than controls after weaning ($P<0.05$), D11-15 dex treated animals remained lightest at week 6, when the females were mated (Table 1).

F1 male lineage

At 14 weeks of age, body weight was similar in the three groups of F1 males (control 30.3 ± 1.1, dex D11-15 30.7 ± 0.7, dex D14-18 31.0 ± 0.7 g, $P>0.05$). After glucose administration, the maximum increment in blood glucose concentration from baseline values was significantly less in both dex exposed groups than in the controls (Fig. 1). Moreover, blood glucose concentration remained elevated above baseline for longer in dex males (Fig. 1, $P<0.05$, one sample t-test of 120 min glucose versus zero), than in the controls ($P>0.05$). However, neither the area under the curve of blood glucose with time (Fig. 1) nor the basal fasting glucose concentrations differed with F0 treatment (control 6.9 ± 0.4, dex D11-15 7.4 ± 0.4, dex D14-18 8.2 ± 0.4 mM, $P>0.05$) . There was also no significant effect of F0 treatment on F1 plasma corticosterone concentrations at 16 weeks of age (control 233 ± 34, dex D11-15 170 ± 15, dex D14-18 189 ± 9 ng ml^{-1}). Heart weight was significantly greater in D14-18 dex treated males compared to the other two groups, both as a percentage of total body weight and an absolute value (control 142 ± 2, dex D11-15 144 ± 7, dex D14-18 159 ± 4 mg, $P<0.05$).

When F1 adult males were mated with untreated females, neither maternal weight nor F2 fetal and placental weights differed between the three groups at D19 of pregnancy (Table 2). Materno-fetal clearance of MeAIB across the F2 placenta and placental expression of the *Slc38a* genes were also similar in control and dex groups (Figure 2A and B). The Lz and Jz volumes of the F2 placentae, determined by stereology, did not differ with paternal prenatal treatment (Table 2). Neither did plasma corticosterone concentrations of the D19 pregnant

females differ with paternity of their offspring (control 603 ± 64 ng/ml, D11-15 dex 540 ± 42 ng/ml, D14-18 dex 687 ± 93 ng ml^{-1}, P>0.05).

F1 female lineage

Body weight of the F1 females at D19 of their pregnancy differed with F0 treatment: those exposed prenatally to dex from D11 to 15 were lightest whereas the controls were heaviest (Table 2). In particular, the weight of the gravid uterus was less in F1 dams exposed to dex prenatally at D11-15 than in the other two groups. However, F2 litter size and fetal and placental weights were unaffected by the treatment of their maternal grandmother (Table 2). Clearance of MeAIB across the F2 placenta was 75% greater in F1 dams exposed prenatally to dex at D14-18, compared to the other two groups (Figure 2C). In contrast, expression of *Slc38a4*, but not *Slc38a1* or *Slc38a2*, was significantly lower in the F2 placentae of the F1 mothers dex exposed at D14-18 , compared to the control group (Fig 2D). At D19 of pregnancy, plasma corticosterone concentrations were elevated above control values (644 ± 48 ng ml^{-1}, n = 5) in F1 dams exposed prenatally to dex at D14-18 (1119 ± 131 ng ml^{1}, n = 6, P<0.05) but not at D11-15 dams (600 ± 38 ng ml^{-1}, n = 5, P>0.05). The volumes of the Lz and Jz in the F2 placenta were similar in the three groups of F1 dams (Table 2).

DISCUSSION

This study is the first to demonstrate altered function of the F2 mouse placenta as a consequence of treating F0 pregnant dams with the synthetic glucocorticoid, dexamethasone. F1 neonates of F0 dams treated with dex close to term were growth restricted at birth but subsequently caught up in weight with controls whereas those exposed to dex earlier in gestation had normal birth weights but grew poorly in the period immediately after birth. Irrespective of the timing of F0 dex treatment, male F1 offspring had abnormal glucose tolerance as adults. However, prenatal dex exposure of the F1 males had no effect on F2 fetal weight or placental phenotype. In contrast, prenatal dex exposure of F1 females led to an increase in F2 placental MeAIB transport that was specific to the grandprogeny of F0 dams treated with dex near to term.

Previously, dex treatment either from D11 to D15 or from D14 to D18 of mouse pregnancy has been shown to reduce fetal and placental weight at D19 (Vaughan *et al.* 2013). However, in the current study growth restriction was only seen at birth in the F1 neonates prenatally exposed to dex over the later of these two periods of F0 treatment. This indicates that compensatory increases in fetal growth can occur during late gestation in mice but only when there are more than 2 days between the end of the insult and birth. The growth restricted F1 neonates exposed to dex from D14-18 did attain normal weight by 3 weeks after birth, consistent with the catch-up growth reported in human infants of low birth weight (Morrison *et al.* 2010). In contrast, growth of the F1 neonates exposed to dex from D11-15 was slow after birth so that they were lighter than the other two groups at weaning and remained so for the rest of the experiment, despite a normal birth weight. This may be a consequence of reduced absorption of nutrients from the neonatal gut, or changes in the composition or volume of milk produced by the dams during lactation (Drozdowski *et al.* 2009). Placental production of lactogenic hormones may have been impaired by dex administration as the dex exposed placenta is known to weigh less at the end of treatment and remain small at D19 after treatment from D11-15 (Vaughan *et al.* 2013). Indeed, the higher growth rate seen in both groups of dex exposed F1 offspring relative to the controls after weaning suggests that there is a degree of growth constraint during the period of suckling after dex treatment of their mothers (Vaughan *et al.* 2013). With group-housed animals, food intake of the F1 offspring could not be determined in the current study so nothing is known about the potential contribution of glucocorticoid programmed changes in appetite to postnatal growth (Moisiadis and Matthews 2014; Ross and Desai 2014).

In common with the adult offspring of rats treated with dexamethasone in late pregnancy (Lindsay *et al.* 1996a; Lindsay *et al.* 1996b; Nyirenda *et al.* 1998; Woods and Weeks 2005; Franko *et al.* 2010) adult F1 male mice of F0 dex treated dams had abnormal glucose tolerance in the current study with a glucose increment that was smaller initially but more prolonged than seen in the control animals. Reduced maximum blood glucose increment may suggest improved glucose tolerance and is consistent with the faster post-weaning weight gain of the dex exposed F1 animals. However, increased glucose uptake in the juvenile animals may lead to greater fat deposition and eventually insulin resistance in old age (Nyirenda *et al.* 1998). Alternatively, the smaller rise in blood glucose level in dexamethasone exposed F1 male mice may be due to impaired absorption of the glucose injected intraperitoneally. The increases in heart weight in these F1 males also suggests that there are changes in adult cardiovascular function as a result of prenatal dex exposure, as seen in other species (Woods and Weeks 2005). Maternal dex treatment during pregnancy has also been shown to alter the response of the hypothalamic-pituitary-adrenal (HPA) axis to stress in the adult offspring in rats and other species (Shoener *et al.* 2006; Hauser *et al.* 2009; Iqbal *et al.* 2012; Long *et al.* 2013a). However, there was little evidence of programmed changes in adrenocortical secretion in the F1 dex exposed adult males studied here, as plasma corticosterone concentration did not differ with F0 treatment. Other than body weight, no phenotypic data was collected on non-pregnant *F1* female mice in the present study as all were mated in order to create the F2 generation. Metabolic phenotyping of these females, for example by measuring glucose tolerance, either before or during pregnancy may also have confounded the F2 outcomes by introducing additional stresses.

There were no differences in feto-placental weight, placental MeAIB clearance or placental structure between F2 litters of control and F1 dex-exposed male mice. Previous studies in rats have shown inheritance of metabolic traits to F2 adults via the male offspring of F0 dams treated with a similar dose of dex in late pregnancy (Drake *et al.* 2005). These changes associated with a small decrease in F2 placental weight at day 20 of pregnancy (Drake *et al.* 2011). However, the present data do not support a major role for the placenta in transmitting a dex programmed F1 phenotype to the F2 generation patrilinearly in mice. Alternative mechanisms, for example epigenetic changes in imprinted or other genes more influential in post-natal growth and glucose tolerance than placental phenotype, may result in germ-line

dependent paternal intergenerational inheritance, as occurs following undernutrition of F0 pregnant mice (Radford *et al.* 2014).

When F1 female offspring were bred with control males, there was a reduction in the weight of the entire gravid uterus specifically in those dams exposed to dex between D11 to D15 of their intrauterine development. As F1 females from this group were lighter than controls at weaning, and tended to remain so at mating, there may have been a greater degree of maternal constraint on expansion of the uterus and its contents during pregnancy, despite no significant differences in weights of the individual *F2* fetuses and placentae. F1 dams given dex prenatally from D14 to D18 of gestation had raised plasma corticosterone and greater materno-fetal MeAIB clearance, a measure of F2 placental System A amino acid transport capacity, on D19 of their own pregnancy. In common with our previous findings (Vaughan *et al.* 2012; Vaughan *et al.* 2013), increased System A activity was not explained by changes in gene expression of *Slc38a* transporters. Indeed, F2 placental expression of the imprinted *Slc38a4* gene was reduced significantly in D14-18 dams that had the highest F2 placental MeAIB clearance. In contrast, *Slc38a4* expression is increased in the F2 rat placenta after exposure of the F1 mother to dex prenatally in late gestation (Drake *et al.* 2011) but its expression is unaltered in the F2 placenta following F1 prenatal undernutrition in mice (Radford *et al.* 2012). Species differences in the effects of F0 manipulations on F2 placental *Slc38a4* abundance may reflect yet unidentified morphological changes in the placenta or promoter-specific regulation of the various *Slc38a4* transcripts in the present and previous studies (Constancia *et al.* 2005).

In the present study, the F1 pregnant dams with the greatest placental capacity for System A amino acid transport had the highest maternal corticosterone concentrations. In previous studies, placental MeAIB clearance was *reduced* when corticosterone was given directly to pregnant mice for several days in late gestation (Vaughan *et al.*, 2012). The high corticosterone concentration observed in the pregnant F1 dam exposed prenatally to dex from D14-18 may, therefore, be due to an elevated but transient HPA response to the acute stress of handling and terminal anaesthesia in line with the enhanced HPA responsiveness seen in adult offspring of other species after prenatal exposure to dex (Liu *et al.* 2001; Shoener *et al.* 2006). Alternatively, since transplacental transfer of fetal corticosterone can contribute to maternal levels during mouse pregnancy (Barlow *et al.* 1974; Montano *et al.* 1993; Cottrell *et al.* 2012), the high maternal corticosterone levels may reflect enhanced HPA activity of the

F2 fetuses. Certainly, intergenerational transmission of an altered HPA phenotype is seen in other species after F0 dex treatment (Long *et al.* 2013a; Moisiadis and Matthews 2014). The high corticosterone concentration observed in the pregnant F1 dam exposed prenatally to dex from D14-18 may, therefore, be the consequence rather than the cause of the changes in F2 placental function. Programmed adaptations in F1 maternal glucose metabolism may also contribute to the changes in F2 placental function, since prenatal dex exposure is known to alter adult glucose tolerance in F1 females in large animals (Long *et al.* 2012) and in F1 males in the present study.

The mechanism by which F0 dex treatment during mouse pregnancy leads to enhanced amino acid transport of the F2 placenta via the maternal lineage, therefore, remains unclear. It may involve changes in the chromatin, cytoplasm or mitochondria of the oocyte induced during either oogenesis in the F0 dam or folliculogenesis in the F1 mother as seen after periconceptional dietary manipulations of F0 mouse dams (Watkins *et al.* 2008; Igosheva *et al.* 2010; Lager *et al.* 2014). Alternatively, increased F1 maternal constraint associated with her poor postnatal growth or programmed changes in the F2 fetus may lead to an increased fetal demand for nutrients, which then results in an increased placental nutrient supply. Enhanced placental amino acid transport has been observed previously in response to fetal demand signals when mismatches between the fetal drive for growth and the placental nutrient supply are induced either nutritionally or genetically (Vaughan *et al.*, 2012). In turn, an altered placental phenotype may influence the maternal metabolic profile by changing the maternal endocrine environment and resource allocation between the mother and her offspring with consequences for subsequent pregnancies and the reproductive performance of the next generations (Fowden and Moore 2012).

In summary, the effects of F0 dex treatment on F2 placental phenotype depend on the period of F0 treatment and whether the F1 mother or father was overexposed *in utero*. Changes in the F2 placental capacity for amino acid transport were only seen with F0 treatment close to term transmitted via the maternal lineage, even though there were changes in growth in both sexes and metabolic alterations in the F1 males. Taken together, these observations suggest that the placenta can contribute to intergenerational inheritance of phenotypic traits via the female but not the male lineage in mice. However, the epigenetic mechanisms involved in determining the F2 placental phenotype still remain to be established.

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TABLES

Table 1 F1 offspring postnatal growth

	F0 treatment		
F1 weight (g)	**Control**	**Dex D11-15**	**Dex D14-18**
Neonates	n=33	n=34	n=36
Birth *	1.30 ± 0.03^{a}	1.27 ± 0.01^{ab}	1.19 ± 0.03^{b}
Males	n=7	n=9	n=14
3 weeks*	9.46 ± 0.24^{a}	7.97 ± 0.30^{b}	9.02 ± 0.22^{a}
6 weeks*	21.8 ± 0.4^{ab}	20.2 ± 0.7^{a}	22.2 ± 0.5^{b}
FGR 0-3 weeks ($g\ g^{-1}week^{-1}$)*	2.09 ± 0.08^{ab}	1.79 ± 0.07^{a}	2.19 ± 0.07^{b}
FGR 3-6 weeks ($g\ g^{-1}week^{-1}$)*	0.43 ± 0.02	0.50 ± 0.02	0.50 ± 0.02
Females	n=7	n=5	n=5
3 weeks*	9.33 ± 0.32^{a}	7.79 ± 0.47^{b}	9.02 ± 0.22^{ab}
6 weeks*	18.8 ± 0.5	17.5 ± 0.2	17.4 ± 0.3
FGR 0-3 weeks ($g\ g^{-1}week^{-1}$)	2.08 ± 0.14	1.75 ± 0.12	2.05 ± 0.12
FGR 3-6 weeks ($g\ g^{-1}week^{-1}$)	0.33 ± 0.02	0.41 ± 0.02	0.35 ± 0.02

Mean (±SEM) body weight of F1 offspring of F0 dams either untreated (control) or treated with dexamethasone (dex) from days (D) 11-15 or D14-18 of pregnancy. FGR, Fractional growth rate = (final weight - initial weight)/(initial weight × 3 weeks). * indicates significant effect of F0 treatment by one-way ANOVA ($P<0.05$). Values within rows with different superscripts are significantly different from each other by Bonferroni *post-hoc* test.

Table 2 Biometry of pregnant F1 offspring and F2 conceptuses

	F0 treatment		
Weight	**Control**	**Dex D11-15**	**Dex D14-18**
F1 male x untreated female	n=9 litters	n=13 litters	n=18 litters
Maternal weight (g)	38.6 ± 0.6	36.1 ± 0.8	35.4 ± 1.2
Carcass	25.6 ± 0.3	24.2 ± 0.5	23.5 ± 0.6
Gravid Uterus	13.1 ± 0.7	11.9 ± 0.5	11.8 ± 0.7
F2 fetus (mg)	1207 ± 17	1204 ± 20	1220 ± 15
F2 placenta (mg)	87 ± 6	86 ± 2	84 ± 1
Junctional zone	22 ± 3	21 ± 2	23 ± 3
Labyrinthine zone	47 ± 4	49 ± 1	43 ± 4
Litter size (pups)	7.7 ± 0.5	7.1 ± 0.5	7.0 ± 0.3
F1 female x untreated male	n=5 litters	n=5 litters	n=6 litters
Maternal weight (g)*	37.9 ± 1.0[a]	33.6 ± 0.9[b]	35.5 ± 1.0 [ab]
Carcass	24.2 ± 0.9	23.4 ± 0.4	22.8 ± 1.4
Gravid Uterus*	13.7 ± 0.5[a]	10.2 ± 0.9[b]	12.6 ± 0.6[a]
F2 fetus (mg)	1125 ± 21	1097 ± 14	1135 ± 38
F2 placenta (mg)	88 ± 5	84 ± 2	85 ± 3
Junctional zone	24 ± 3	26 ± 2	25 ± 4
Labyrinthine zone	54 ± 5	46 ± 3	48 ± 2
Litter size (pups)	7.8 ± 0.6	6.4 ± 0.8	7.5 ± 0.2

Mean (±SEM) weights of pregnant mothers, fetuses and placentae on D19 of pregnancy in untreated females mated to F1 male offspring or F1 female offspring mated to untreated males where F1 offspring was either untreated (control) or treated with dexamethasone (DEX) from days (D) 11-15 or D14-18 of the F0 pregnancy * indicates significant effect of F0 treatment by one-way ANOVA ($P<0.05$). Values within rows with different superscripts are significantly different from each other by Bonferroni *post-hoc* test.

Figure 1

Main figure, mean (±SEM) increments in blood glucose concentrations from baseline (0min) values in response to glucose administration at 0 minutes in adult F1 males of F0 dams that were untreated (Controls, open symbols) or treated with dexamethasone (Dex) at days (D) 11-15 (black symbols) or D14-18 (grey symbols). The effects of time and maternal treatment were determined by two-way ANOVA (treatment $P=0.0436$, time $P<0.0001$, interaction $P<0.0001$), different letters a and b indicate different treament groups at each time point by Bonferroni post test. *Inset*, mean (±SEM) area under the curve of blood glucose concentration increment with time from glucose administration in adult F1 males as above. There was no significant effect of maternal treatment by one-way ANOVA. F1 n values: Control n=7, dex D11-15 n=9, dex D14-18 n=14

Figure 2

Mean (±SEM) values of materno-fetal MeAIB clearance across F2 placentae (A and C) and of placental *Slc38a* gene expression (B and D) in offspring of F1 fathers (A and B) and F1 mothers (C and D) that were either untreated (controls) or exposed prenatally to dexamethasone (Dex) at days (D) 11-14 or D14-18 of the F0 pregnancy. The effect of F1 prenatal treatment was determined by one-way ANOVA, different letters a and b indicate significantly different values by Bonferroni post-hoc test. (A), Control n=9, dex D11-15 n=13, dex D14-18 n=17. (C), Control n=4, dex D11-15 n=4, dex D14-18, n=6. (B and D) n=5 per treatment group.