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Short Communication

Maternal vitamin D deficiency during rat gestation elicits a milder phenotype compared to the mouse model: Implications for the placental glucocorticoid barrier

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ABSTRACT

Maternal vitamin D deficiency disturbs fetal development and programmes neurodevelopmental complications in offspring, possibly through increased fetal glucocorticoid exposure. We aimed to determine whether prenatal exposure to excess glucocorticoids underlies our rat model of early-life vitamin D deficiency, leading to altered adult behaviours. Vitamin D deficiency reduced the expression of the glucocorticoid-inactivating enzyme *Hsd11b2* in the female placenta, but did not alter maternal glucocorticoid levels, feto-placental weights, or placental expression of other glucocorticoid-related genes at mid-gestation. This differs to the phenotype previously observed in vitamin D deficient mice, and highlights important modelling considerations.

1. Introduction

Early-life vitamin D deficiency is associated with disturbed fetal development and numerous postnatal health complications in offspring, including neuropsychiatric disorders [1]. The specific mechanisms underlying these adverse outcomes are still relatively unclear, but emerging evidence suggests they may be partially mediated via altered fetal glucocorticoid exposure during vitamin D deficient pregnancies [2].

Glucocorticoids are required for fetal brain and tissue maturation, however excessive glucocorticoid exposure impairs fetal growth and increases the risk for adverse adult health outcomes [3]. In healthy pregnancy, fetal glucocorticoid exposure is regulated in part by placental *Hsd11b2*, which inactivates glucocorticoids [4]. For much of rat gestation, *Hsd11b2* expression remains high within the placental labyrinth zone (LZ), but declines before the end of gestation to facilitate fetal glucocorticoid exposure and thus organ maturation in preparation for birth [4].

Our group has previously demonstrated that vitamin D deficiency increases maternal corticosterone levels and diminishes the placental glucocorticoid barrier in a BALB-C mouse model, resulting in elevated fetal glucocorticoid exposure [5]. This occurred with reductions in placental vascular development [5] and changes to fetal brain morphology [6]. Studies in rats have confirmed that the offspring of vitamin D deficient mothers display altered brain morphology and behavioural outcomes in adulthood, however maternal corticosterone levels and offspring HPA axis activity remain largely unaffected in these models [7,8]. This suggests that rats and mice may exhibit differences in HPA axis sensitivity to developmental vitamin D deficiency, however it is unclear whether this also occurs in relation to the placental glucocorticoid barrier. Consequently, we aimed to determine if the reduced placental glucocorticoid transfer previously reported in mice [5] also occurs in our rat model of maternal vitamin D deficiency, and whether this may underlie the altered behavioural outcomes we have recently described in the male offspring [7]. We measured the expression of glucocorticoid-related genes in the LZ at E16, before down-regulation of *Hsd11b2* commences [4].

Abbreviations: 11-DHC, 11-dehydrocorticosterone; ***3-epi-25(OH)D3***, C-3 epimer of 25-hydroxyvitamin D3; ***5(OH)D3***, 25-hydroxyvitamin D3; ***Con***, control; ***Def***, vitamin D deficient; **E**, embryonic day; ***Hsd11b1***, hydroxysteroid 11-beta dehydrogenase 1; ***Hsd11b2***, hydroxysteroid 11-beta dehydrogenase 2; ***JZ***, junctional zone; ***LZ***, labyrinth zone; ***Nr3c2***, nuclear receptor subfamily 3 group C member 2; ***Tsc22d3***, TSC22 domain family member 3; ***Vegfa***, vascular endothelial growth factor alpha

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Table 1
Maternal, fetal and placental characteristics at E16 of gestation in control ($n = 7$) and vitamin D deficient ($n = 8$) animals.

	Control	Vitamin D deficient
Pregnancy characteristics		
Litter size	14.1 ± 1.8	14.4 ± 1.0
Weight (g)		
Maternal body E1	259 ± 4.2	253 ± 3.4
Maternal body	335.8 ± 3.8	353.3 ± 7.1*
Maternal carcass	329.4 ± 3.8	346.3 ± 7.2
Maternal brain	1.96 ± 0.01	1.91 ± 0.03
Fetal	0.25 ± 0.01	0.28 ± 0.02
Whole placental	0.21 ± 0.01	0.22 ± 0.01
LZ	0.09 ± 0.01	0.10 ± 0.01
JZ	0.10 ± 0.01	0.10 ± 0.01
Placental area (%)		
Chorionic plate	5.00 ± 0.84	2.92 ± 0.50
LZ	32.29 ± 4.74	35.83 ± 2.75
JZ	51.83 ± 5.07	47.81 ± 1.58
Decidua	11.08 ± 1.95	13.54 ± 1.80
Maternal plasma concentration (nmol/L)		
25(OH)D3	47.5 ± 5.4	4.4 ± 0.6**
3-Epi-25(OH)D3	73.1 ± 23.5	7.4 ± 2.8**
Corticosterone	600 ± 146	510 ± 147
11-DHC	10.3 ± 2.0	10.0 ± 2.3

All data are the mean ± SEM. *$P = 0.05$ and **$P < 0.01$ compared to control; *t*-test. Fetal and placental weights and placental area measures did not differ between sexes ($P > 0.05$; two-way ANOVA), so have been presented as combined sex averages.

2. Materials & methods

Female Sprague-Dawley rats were given free access to a vitamin D deficient (0 IU/kg) or control (2195 IU/kg) diet, as previously reported [7]. Deficient diets were supplemented with 25 g/kg calcium, and caloric content was controlled to ensure similar energy intake between groups (deficient: 15.3 MJ/kg; control: 15.8 MJ/kg). After five weeks of diet exposure, females were mated with control-fed males and maintained on the diets until tissue collection at E16. Maternal plasma was isolated for glucocorticoid and vitamin D measurement via LC-MS/MS [9,10]. Male and female placentas were fixed whole for histological measures, or separated into junctional (JZ) and labyrinth zones (LZ) and snap-frozen for molecular analyses. Zonal fractions (% overall placental sectional area) were calculated for the chorionic plate, LZ, JZ and decidua [11]. The relative mRNA expression of *Hsd11b1*, *Hsd11b2*, *Nr3c2* (the glucocorticoid receptor), *Tsc22d3* (an indicator of glucocorticoid receptor activation) and *Vegfα* was measured in LZ tissue [7,12].

3. Results

Mothers consuming the vitamin D deficient diet exhibited a marked reduction in plasma 25(OH)D3 and 3-epi-25(OH)D3 ($P < 0.01$), however corticosterone and 11-dehydrocorticosterone levels were unchanged (Table 1).

While vitamin D deficient and control dams did not differ in weight on the first day of pregnancy (Table 1), deficient mothers displayed a

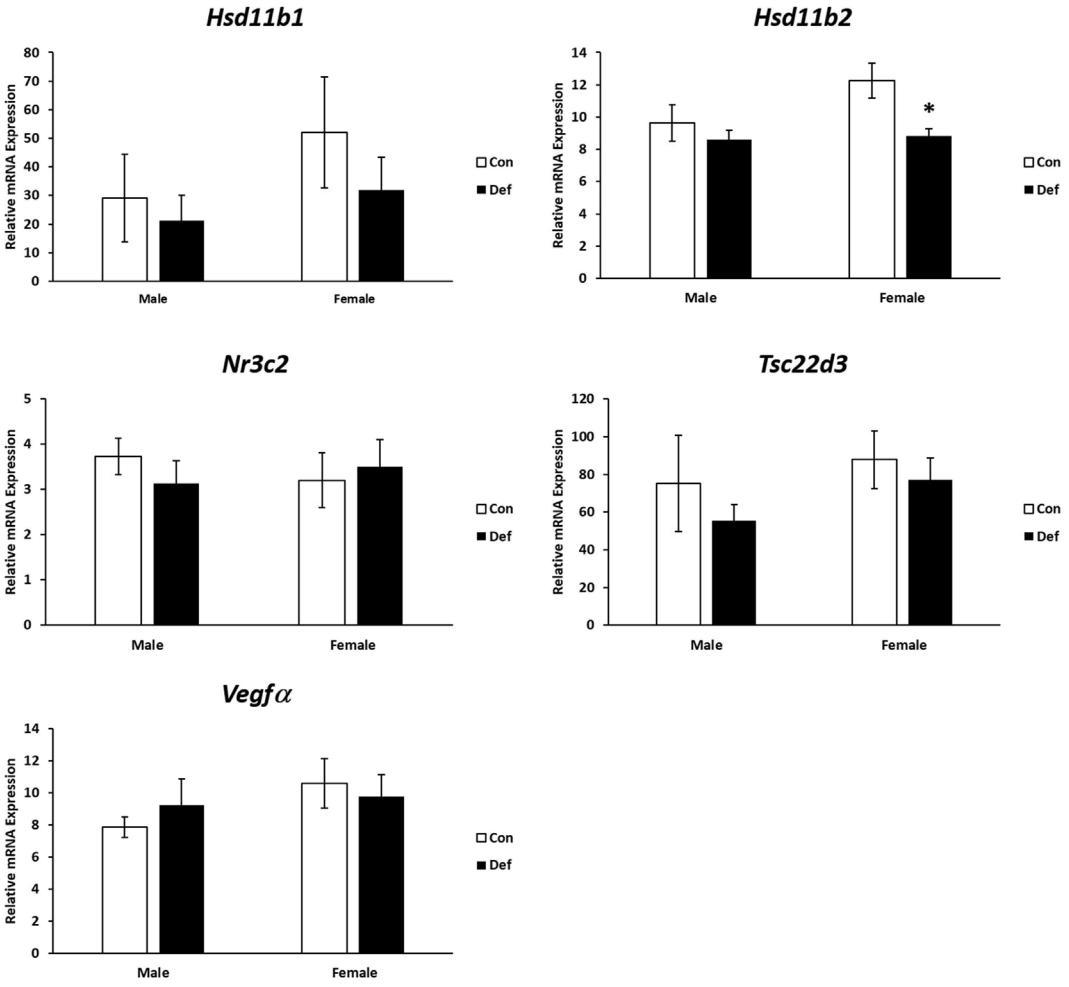

Fig. 1. Expression of glucocorticoid-related genes in the placental LZ of control (Con; $n = 7$) and vitamin D deficient (Def; $n = 8$) pregnancies at E16. Data are the mean ± SEM. *$P < 0.05$ compared to Con; LSD comparison following $P < 0.01$ diet effect in two-way ANOVA.

slight (5%) increase in body weight relative to controls by E16 ($P = 0.05$). This may be attributed to trends for increased fetal ($P = 0.06$) and placental ($P = 0.08$) weight that were noted in the deficient group, since vitamin D status did not significantly alter litter size or maternal brain or carcass weight. Fetal, placental and zonal weights were also not significantly altered by diet (Table 1). Similarly, while there was a trend for reduced chorionic plate fraction in deficient animals ($P = 0.07$), there were no significant differences in placental zonal fractions between diet groups (Table 1).

Vitamin D deficiency reduced *Hsd11b2* in the female LZ by 27% ($P < 0.05$), however expression of the remaining genes was unaffected by maternal vitamin D status (Fig. 1).

4. Discussion

These findings indicate that maternal vitamin D deficiency induces a milder phenotype in rats compared to the previously reported mouse model. In BALB-C mice, vitamin D deficiency elevated maternal corticosterone, reduced placental weights and vascular development, and altered placental *Hsd11b2* and *Tsc22d3* expression, indicative of increased placental glucocorticoid transfer [5]. Vitamin D deficiency did not alter maternal glucocorticoid levels in the current study, which corresponds to the maternal phenotype at weaning [7] and at E14 in another rat model [8]. Moreover, placental and fetal weights were unaffected by vitamin D deficiency, and the only clear glucocorticoid-related effect was the *Hsd11b2* reduction in the female LZ. Differences between rats and mice are likely to be important within the context of developmental programming research, and investigators should be wary of extrapolating glucocorticoid-related findings between these species. Indeed, recent studies on the effects of early-life vitamin D deficiency on adult social behaviour in different mouse strains showed no consistent phenotype [13], unlike the deficits observed in Sprague-Dawley rats [7]. Thus, there is likely a strong interaction between genetics and vitamin D deficiency in the determination of offspring phenotype.

The reduction in placental *Hsd11b2* suggests that female fetuses may be exposed to higher levels of glucocorticoids, and could therefore be susceptible to postnatal health complications [3,14]. Interestingly, vitamin D deficiency did not affect the placental glucocorticoid barrier in males, however we have recently reported that by postnatal day 1, cerebral expression of *Hsd11b2*, *Nr3c2* and *Tsc22d3* was altered in males born to vitamin D deficient mothers [7]. Since maternal corticosterone levels were unaffected at mid-gestation and weaning, this implies that vitamin D deficiency does not alter endogenous maternal or fetal HPA axis activity *per se*. However, changes to maternal care quality, fetal glucocorticoid sensitivity or placental glucocorticoid barrier disturbances after E16 could affect key aspects of fetal brain formation and contribute to the behavioural offspring outcomes. Thus, further studies are required to determine the exact mechanisms, both *in-utero* and during postnatal development, that result in altered offspring postnatal phenotypes.

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Declarations of interest

The authors declare no conflict of interest.

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