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## Short communication

## Altered placental methyl donor transport in the dexamethasone programmed rat

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## ABSTRACT

There is increasing evidence for a role for epigenetic modifications in early life 'programming' effects. Altered placental methyl donor transport may impact on the establishment of epigenetic marks in the fetus. This study investigated the effects of prenatal glucocorticoid overexposure on placental methyl donor transport. Glucocorticoids increased folate but decreased choline transport and reduced fetal plasma methionine levels. There was no change in global DNA methylation in fetal liver. These data suggest prenatal glucocorticoid overexposure causes complex alterations in the placental transport of key methyl donors which may have important implications for maternal diet and nutrient supplementation in pregnancy.

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## 1. Introduction

The association between exposure to an adverse early life environment and increased cardiometabolic disease risk has led to the development of the early life origins hypothesis [1]. Potential mechanisms include altered maternal/fetal nutrition [2,3] and prenatal glucocorticoid overexposure [4]. We have developed a rat model of 'programming' by fetal glucocorticoid overexposure in which prenatal exposure to a synthetic glucocorticoid, dexamethasone (Dex) reduces birthweight and leads to insulin resistance and hypertension in adulthood [4]. Maternal stress or inhibition of 11 $\beta$ -hydroxysteroid dehydrogenase type 2 (11 $\beta$ -HSD2, the placental 'barrier' to maternal physiological glucocorticoids) results in similar effects on offspring phenotype [5]. Increased fetal glucocorticoid overexposure may also be important in humans. The efficiency of placental 11 $\beta$ -HSD2 near term varies considerably in humans [6] and the lowest placental 11 $\beta$ -HSD2 activity is seen in babies with the smallest birth weights, suggesting increased fetal exposure to maternal glucocorticoids [6]. Additionally, exogenous glucocorticoids which readily cross the placenta are used in obstetric practice to accelerate lung maturation in cases of threatened preterm labour [7] and in women at risk of bearing fetuses at risk of congenital adrenal hyperplasia. Finally, placental 11 $\beta$ -HSD2 is not a complete barrier to glucocorticoids, so that increased circulating levels in the mother may result in increased fetal exposure; indeed maternal antenatal stress/anxiety has been associated with programming effects in the offspring [8,9].

Recent evidence suggests early life programming effects may be mediated by epigenetic modifications including DNA methylation

and histone marks [10,11]. The availability of methyl donors such as choline, methionine and folic acid during fetal development can influence the establishment of epigenetic modifications in the fetus [11–14]. Alterations in placental nutrient transport have been described in animal models of programming including prenatal glucocorticoid overexposure [15,16]. The purpose of this study was to explore the effects of prenatal glucocorticoid overexposure on the placental transport of methyl donors. This was achieved via characterization of placental methyl donor transport and gene expression; plasma methionine levels and DNA methylation levels in fetal liver.

## 2. Methods

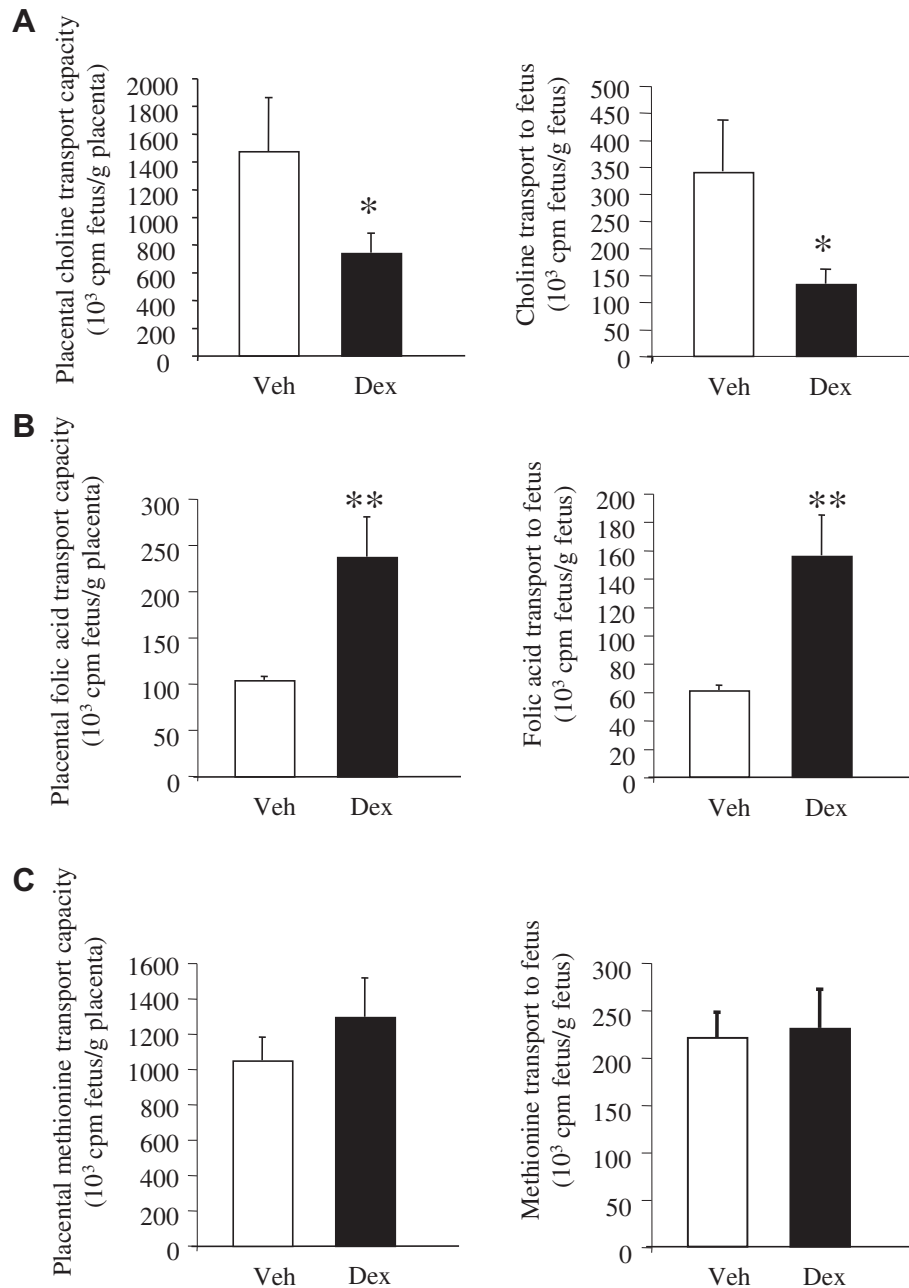
## 2.1. Animals

Virgin female Wistar rats (200–250 g; Harlan UK) maintained under conditions of controlled lighting and temperature (22 °C) were timed-mated and injected subcutaneously with 100  $\mu$ g/kg Dex or vehicle (Veh) from embryonic day (E) 15–19 as described [4]. Eight females per group were culled at E20. All studies were conducted under licensed approval by the UK Home Office, under the Animals (Scientific Procedures) Act, 1986, and with local ethical committee approval. Maternal and fetal plasma (pooled for offspring from one litter) was stored at –20 °C. Placental labyrinth was stored at –80 °C.

## 2.2. Placental transport of methyl donors at E20

Placental transport of choline, folic acid or methionine was measured using modified methods [17]. 8–10 pregnant rats were anesthetized and 100  $\mu$ l PBS containing 3.5  $\mu$ Ci of <sup>14</sup>C-choline chloride, <sup>14</sup>C-methionine or <sup>3</sup>H-folic acid (American Radiolabelled Chemicals (UK) Ltd.) injected intravenously. Animals were killed and fetuses and placentas weighed after 7 min (a timepoint found in preliminary experiments to be on the linear scale of placental transfer). Fetuses were lysed overnight at 55 °C in Biosol (National Diagnostics, UK). Radioactive counts (Tri-Carb 2100TR; Packard, UK) in each fetus were used to calculate the amount of radioisotope transferred/g placenta (a measure of placental transfer), or per gram of fetus (a

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**Fig. 1.** Changes in placental transport of <sup>14</sup>C-choline chloride (A), <sup>3</sup>H-folic acid (B) and <sup>14</sup>C-methionine (C) in vehicle and dexamethasone-treated rats at E20 expressed per gram of placenta or per gram of fetus.  $N = 8$  females per group. Values are mean  $\pm$  SEM; \* $P < 0.05$ ; \*\* $P < 0.01$ .

measure of the amount of solute received by the fetus). Average values for fetuses within a litter were used to calculate a mean for all litters.

### 2.3. Quantification of mRNA by real-time PCR

Total RNA was extracted from placental labyrinth, reverse transcribed and real-time PCR performed as previously described [18] to analyse the expression of genes involved in folate and choline transport using predesigned assays from Applied Biosystems, UK (Folate receptor (FR): Rn00591759\_m1; Reduced folate carrier (RFC): Rn00446220\_m1; Organic cation transporter 1 (OCT1): Rn00562250\_m1; Organic cation transporter 3 (OCT3): Rn00580082\_m1). Results were corrected for the expression of cyclophilin A (Rn00690933\_m1).

### 2.4. Plasma methionine levels

Plasma methionine levels were measured by the Biochemistry Department, Royal Hospital for Sick Children, Edinburgh, UK using a Biochrom 30 amino acid analyser (Biochrom Ltd, Cambridge, UK).

### 2.5. Genome-wide DNA methylation

Our previous studies have shown altered expression and DNA methylation of specific genes in fetal liver [19]. In order to determine whether these changes reflected global alterations in DNA methylation, DNA was prepared from fetal liver by phenol-chloroform extraction and global cytosine methylation measured as previously described [20].

### 2.6. Statistical analysis

Data were analysed by independent Student *t* testing and are expressed as mean  $\pm$  SEM, with each litter representing  $n = 1$ .

## 3. Results and discussion

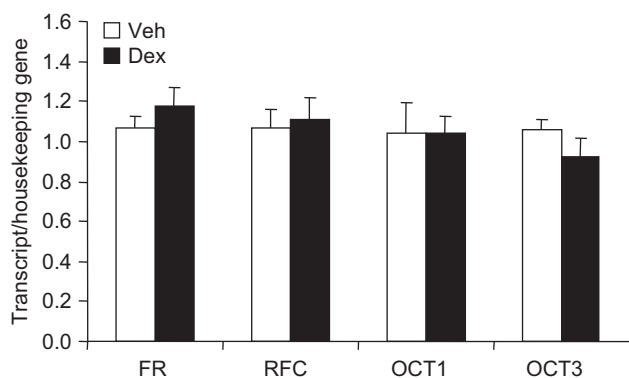
Prenatal Dex reduced fetal weight at E20 (Dex 2.16  $\pm$  0.03 vs Veh 2.34  $\pm$  0.02 g  $p < 0.0001$ ). We found opposite effects of

glucocorticoid exposure on placental choline and folate transport. The placental transport capacity of choline was reduced (39%;  $P < 0.001$ ) by Dex, such that the fetus received less choline per gram fetal weight (55% less than Veh fetuses;  $P < 0.001$ ; Fig. 1A). In contrast, Dex increased placental folate transport by 2.5 times ( $P < 0.05$ ) such that the Dex-exposed fetuses received 2.3 times more folate per gram fetal weight ( $P < 0.05$ ; Fig. 1B). The reason for these changes remain to be determined as we found no changes in mRNA levels of the folate transporters RFC and FR or the choline transporters OCT1 and OCT3 in the placental labyrinth (Fig. 2).

Placental methionine transport (Fig. 1C) and maternal plasma methionine concentrations were unaffected by Dex (Dex 37.4  $\pm$  1.9 vs Veh 41.5  $\pm$  1.8  $\mu\text{mol/l}$ ;  $p = 0.18$ ), however Dex exposure reduced fetal plasma methionine levels (Dex 69.8  $\pm$  7.1  $\mu\text{mol/l}$  vs Veh 99.8  $\pm$  2.6  $\mu\text{mol/l}$ ;  $p < 0.01$ ). There are complex interactions between choline, folate and methionine [21–24] with the folate and choline metabolic pathways meeting at the conversion of homocysteine to methionine and because of this, altered metabolism of one methyl donor can result in compensatory changes in another [25]. For instance, administration of a choline deficient diet is associated with reduced tissue concentrations of methionine in non-pregnant rats [26] and folate deficiency in pregnant rats increases choline availability in maternal liver [24]. Nevertheless, although the observed increase in placental folate transport may be a compensatory mechanism in the presence of reduced choline transport, this was not complete since Dex exposure was associated with reduced fetal methionine levels.

Fetal methyl donor availability may play a key role in the establishment of epigenetic marks in offspring [23]. Despite the Dex-induced alterations in methyl donor transport and the reduced fetal plasma methionine levels, we found no changes in global hepatic DNA methylation (Dex 3.52  $\pm$  0.25 vs Veh 3.31  $\pm$  0.41%;  $p = 0.67$ ), in agreement with studies in animal models of gestational dietary methyl donor deficiency [24]. Our results do not exclude the possibility that global DNA methylation is altered in other tissues such as brain, or at specific target genes. Indeed several studies suggest both global and gene-specific alterations in DNA methylation [12,27] including in this model [19]. The mechanisms underpinning the different effects reported in these studies are unclear but may reflect the nature and specific timing of the insult in relation to critical periods of organ development [28].

One-carbon donors have the potential to play a key role in developmental programming and the addition of folate to a maternal low protein diet appears to attenuate adverse programmed effects on vascular dysfunction [29] and prevents alterations in DNA methylation in offspring exposed to prenatal protein



**Fig. 2.** mRNA expression of placental transporters involved in the transport of folate: Folate receptor (FR), Reduced Folate Carrier (RFC) and choline: Oct1 and Oct3.  $N = 15$  Dex placentas from 10 litters and 12 vehicle placentas from 8 litters. Values are mean  $\pm$  SEM.

restriction [11] so that methyl donor supplementation has been proposed as one strategy to reduce the consequences associated with exposure to an adverse intrauterine environment. However, these data suggest that methyl donor supply is complex and that compensatory mechanisms may operate if deficiency occurs, highlighting the necessity for further studies to determine optimal interventions to reduce disease risk.

In conclusion, we show that glucocorticoid overexposure in pregnancy changes placental transport of folate and choline and reduces fetal plasma methionine levels. Changes in these key components of the methyl donor cycle may have implications for disease risk in the offspring. Given the intricate inter-relationships between the components of the methyl donor cycle, our findings illustrate the subtle complexities of the mechanisms which must be resolved before any appropriately targeted therapies can be devised.

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