Programming hyperglycaemia in the rat through prenatal exposure to glucocorticoids – fetal effect or maternal influence?

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Abstract

In a previous study, we showed that exposure of rats to dexamethasone (Dex) selectively in late pregnancy produces permanent induction of hepatic phosphoenolpyruvate carboxykinase (PEPCK) expression and hyperglycaemia in the adult offspring. The mechanisms by which glucocorticoids cause this programming are unclear but may involve direct actions on the fetus/neonate, or glucocorticoids may act indirectly by affecting maternal postnatal nursing behaviour. Using a cross-fostering paradigm, the present data demonstrate that switching the offspring at birth from Dex-treated dams to control dams does not prevent induction of PEPCK or hyperglycaemia. Similarly, offspring born to control dams but reared by Dex-treated dams from birth maintain normal glycaemic control. During the neonatal period, injection of saline *per se* was sufficient to cause exaggeration in adult offspring

responses to an oral glucose load, with no additional effect from Dex. However, postnatal treatment with either saline or Dex did not alter hepatic PEPCK activity. Prenatal Dex permanently raised basal plasma corticosterone levels, but under stress conditions there were no differences in circulating corticosterone levels. Likewise, Dex-exposed rats had similar plasma catecholamine concentrations to control animals. These findings show that glucocorticoids programme hyperglycaemia through mechanisms that operate on the fetus or directly on the neonate, rather than via effects that alter maternal postnatal behaviour during the suckling period. The hyperglycaemic response does not appear to result from abnormal sympathoadrenal activity or hypothalamic–pituitary–adrenal response during stress.

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Introduction

Increasing epidemiological evidence in humans suggests that early life events are important determinants of common cardiovascular and metabolic diseases in adulthood (Barker *et al.* 1989, Hales *et al.* 1991, Curhan *et al.* 1996). These observations have been proposed to exemplify the phenomenon of ‘programming’, whereby a factor or manipulation acting in early life can alter the development of organs, causing permanent dysfunction and ultimately resulting in disease (Barker *et al.* 1993, Edwards *et al.* 1993). The mechanisms involved in this programming are unclear but the timing of exposure to an insult is thought to be particularly critical, perhaps because organs develop at different stages (and rates) and the susceptibility of a system to a programming agent will be highest during its critical developmental window(s).

Recently, we have shown that prenatal exposure to glucocorticoids in rats induces permanent hyperglycaemia in the adult offspring (Lindsay *et al.* 1996a, Nyirenda *et al.* 1998). This is associated with hyperinsulinaemia and a lifelong elevation in hepatic mRNA expression and activity of phosphoenolpyruvate carboxykinase (PEPCK)

(Nyirenda *et al.* 1998), a key enzyme of gluconeogenesis (Pilkis & Granner 1992) – suggesting that an increase in hepatic glucose production contributes to the hyperglycaemia. Importantly, this programming occurred only when glucocorticoids were given during the last week of pregnancy and not with earlier treatment (Nyirenda *et al.* 1998). These findings indicate that the window of sensitivity to glucocorticoid programming of hyperglycaemia might lie late in gestation. In this model, a synthetic glucocorticoid, dexamethasone (Dex), is administered to the mother to avoid fetal compromise by repeated direct fetal injections. Thus, both the fetus and its mother received the glucocorticoid load, making it difficult to determine whether programming of hyperglycaemia is through direct action of glucocorticoids upon the fetus or indirectly through effects on the mother. For example, glucocorticoids are known to have profound effects on the central nervous system; they alter many aspects of cognition and behaviour (McLay *et al.* 1998, de Quervain *et al.* 1998) and are implicated in the pathogenesis of depression (Rubinow *et al.* 1984, Thakore & Dinan 1995). Glucocorticoid exposure in late gestation might, therefore, influence maternal behaviour and postnatal care, thus

indirectly affecting neonatal biology. This notion is supported by well established evidence demonstrating that changes in maternal postnatal behaviour can permanently influence offspring neuroendocrine function and behaviour (Meaney *et al.* 1989, Maccari *et al.* 1995). Thus, enrichment of the maternal postnatal environment programmes a favourable offspring hypothalamic–pituitary–adrenal (HPA) response, while maternal stress is associated with permanent HPA hyperactivity in the offspring (Meaney *et al.* 1989, Maccari *et al.* 1995). Prenatal Dex treatment mimics the effect of maternal postnatal stress, in that offspring of dams treated with Dex in late gestation have elevated basal corticosterone levels (Levitt *et al.* 1996). In humans, high adult plasma cortisol levels are found in individuals with low birth weight, and may contribute to the increased cardiovascular risk observed in these individuals (Levitt *et al.* 2000, Phillips *et al.* 2000). Low birth weight is also associated with high resting pulse rate in adult life (Phillips & Barker 1997, Levitt *et al.* 2000), suggesting that the increased cardiovascular risk may, in part, be mediated by activation of the sympatho-adrenal system. Indeed, patients with type 2 diabetes mellitus show increased sensitivity of blood pressure, glucose tolerance and vascular responsiveness to catecholamines (Bruce *et al.* 1992, Hogikyan *et al.* 1999). These data suggest that altered development of mechanisms of the stress response may be important in mediating the effects of early environmental insult on later cardiovascular and metabolic control.

The aims of this study were (1) to determine whether hyperglycaemia programmed by prenatal glucocorticoid exposure is a result of fetal programming or is mediated indirectly by altering subsequent maternal postnatal behaviour; (2) to examine the effect of prenatal glucocorticoid treatment on the offspring stress response (HPA and sympathetic activity); (3) additionally, we examined whether a brief exposure to Dex just before birth or treatment in the postnatal period can reproduce the hyperglycaemic phenotype induced by treatment throughout the last week of pregnancy.

Materials and Methods

Animals

Wistar rats (200–250 g, Harlan UK Ltd, Bicester, Oxon, UK) were maintained under conditions of controlled lighting (lights on 0700–1900 h) and temperature (22 °C) and allowed free access to food (standard rat chow; 56.3% carbohydrate, 18.3% protein, NaCl 0.7%; B.S.&S Scotland Ltd, Edinburgh, UK) and tap water.

Prenatal glucocorticoid exposure

Time-mated female rats (10 per group) were given subcutaneous injections of either Dex (100 µg/kg/day,

dissolved in 4% ethanol–0.9% saline, 200 µg/ml) or vehicle during the last week of pregnancy (embryonic days (E) 15–21). Daily maternal weights were measured. In a separate cohort of rats, fasting plasma glucose and basal corticosterone levels were measured at day 20 of pregnancy. A cross-fostering experiment was undertaken at birth, with half the number of litters born to Dex-treated dams switched to and nursed by control dams, and vice versa. The offspring were weighed and litters were culled to eight; only litters born on the same day were crossed. There were no problems with pup acceptance by substitute mothers of either group. In further experiments to refine the window of Dex sensitivity, treatment with Dex or vehicle was restricted to the last two days of gestation (E20–21). To determine the effect of postnatal Dex exposure, pups born to untreated dams received subcutaneous injections of either Dex (0.01 µg/g) or saline on postnatal days 1, 3 and 5. Body weight was measured during and after treatment. Weaning was carried out at postnatal day 21 in all experiments, and adult male offspring, selected at random from each litter, were used in all subsequent experiments.

Oral glucose tolerance test

The offspring underwent an oral glucose tolerance test at the age of 6 months. Rats were handled regularly during the week before the experiment and habituated to being held in a folded tea towel. Animals were fasted overnight and tests were started between 0830 and 0900 h the following morning. Blood sampling was carried out by tail tipping from rats loosely restrained in the tea towel. A fasting sample was collected in heparinised eppendorf tubes at time 0 and further sampling was carried out 30 and 120 min after an oral glucose load of 2 g/kg was given by gavage. Plasma was stored at –20 °C or –80 °C for glucose or catecholamine assay respectively. Glucose was determined by the enzymatic (hexokinase) method using a glucose assay kit supplied by Sigma Chemical Co. (Poole, Dorset, UK). The intra-assay and interassay coefficients of variation were <2%. Plasma catecholamine levels were estimated commercially at St Helier Hospital (Carshalton, Surrey, UK) using the high-pressure liquid chromatography (HPLC) method. The intra-assay coefficients of variation were 5% and 7% for noradrenaline and adrenaline respectively.

Induction of stress by restraint procedure

Basal corticosterone levels (6-month-old rats) were determined in tail tip blood samples collected at 0830 h. To assess corticosterone response to stress, rats were placed in a Perspex cylinder (7.5 cm diameter) for 20 min before returning them to their cages. Blood samples were

Table 1 Maternal weight gain during the last week of pregnancy (3rd week), gestation length, litter size, male:female ratio and offspring birth weight in the three treatment groups. Results are means $\pm$ S.E.M.

Treatment	Maternal weight gain (g)	Gestation length (days)	Litter size	Male:female ratio	Birth weight (g)
Saline	81.2 $\pm$ 3.3 (n=10)	21.1 $\pm$ 0.3	10.5 $\pm$ 0.6	1.3 $\pm$ 0.3	6.2 $\pm$ 0.1 (n=27)
Dex E20–21	75.8 $\pm$ 4.3 (n=10)	21.1 $\pm$ 0.5	10.6 $\pm$ 0.9	0.9 $\pm$ 0.3	6.1 $\pm$ 0.2 (n=31)
Dex E15–21	58.3 $\pm$ 6.7* (n=10)	21.2 $\pm$ 0.4	11.4 $\pm$ 1.1	1.4 $\pm$ 0.4	5.5 $\pm$ 0.1* (n=33)

* $P < 0.05$ compared with saline treatment group.

collected 15 min into the restraint procedure and at further time points after the restraint. Plasma corticosterone levels were estimated as described below.

Corticosterone assay

Plasma samples were diluted in buffer (135 mmol/l sodium borate, 0.5% bovine serum albumin, 1% methanol, 0.1% ethylene glycol, pH 7.4) and heated at 70 °C to inactivate corticosterone-binding globulin. Antibody specific to corticosterone (final titre 1:10 000) was added along with [3 H]corticosterone (Amersham International, Amersham, Bucks, UK), and samples were incubated at 4 °C overnight. Unbound activity was estimated in a beta counter (Minaxi Tricab 4000, Canberra Packard, Pangbourne, Bucks, UK) after addition of scintillant (Picofluoro 40, Canberra Packard). Corticosterone was estimated by comparison with unlabelled corticosterone standards (Sigma Chemical Co.). The intra-assay coefficient of variation was 4%.

PEPCK assay

Fed animals were killed by decapitation between 0900 and 1000 h and the livers removed immediately. Fresh liver homogenates (10% (w/v)) were prepared at 0 °C in medium containing 250 mM sucrose and 5 mM Hepes, pH 7.4. Homogenates were centrifuged at 3600 g for 10 min to sediment intact cells. The supernatant fractions were further centrifuged at 130 000 g for 30 min. The resulting supernatant (cytosol) was collected and stored at -70 °C for PEPCK assay. Protein content of the preparations was determined by the method of Peterson (1977). PEPCK activity was assayed at 30 °C by coupling the formation of oxalacetate with NADH oxidation in the presence of excess malate dehydrogenase (Boehringer Mannheim UK, Lewes, East Sussex, UK), as described by Petrescu *et al.* (1979). The reaction mixture (1 ml final volume) contained 50 mM Hepes (pH 6.5), 50 mM sodium bicarbonate, 1 mM MnCl_2 , 0.25 mM NADH, 1 mM phosphoenolpyruvate, 1.5 U malate dehydrogenase and 10 μ l of liver cytosolic preparation. The reaction was

initiated with 0.15 mM deoxy-GDP and a decrease in absorbance at 340 nm was followed for 5 min. A reaction mixture lacking bicarbonate was used as control.

Statistics

All data are expressed as means $\pm$ S.E.M. Data were compared using unpaired Student's *t*-tests or one-way ANOVA followed by Newman-Keuls post-hoc multiple comparisons test, where appropriate. Values were considered significant when $P < 0.05$.

Results

Gestational weight gain and offspring birth weight

Dex administration throughout the third week of gestation caused a significant reduction in maternal weight gain (Table 1). As can be predicted from the inhibitory effect on the HPA axis, Dex-treated dams had lower plasma corticosterone levels than controls (3.7 ± 0.7 vs 16.5 ± 2.5 μ g/dl). However, there were no significant differences in maternal plasma glucose concentrations between the groups (6.1 ± 0.4 vs 5.5 ± 0.3 mmol/l). Likewise, the length of gestation, litter size or viability were not affected by Dex treatment (Table 1). As we have reported previously, administration of Dex throughout the third week of pregnancy caused a significant reduction in the birth weight of offspring (Nyirenda *et al.* 1998, and data included in Table 1). When treatment was restricted to the last two days of pregnancy, Dex neither altered maternal weight gain nor affected offspring birth weight (Table 1).

Prenatal Dex, cross-fostering at birth and offspring glucose homeostasis

Confirming our previous observations, the offspring of rats treated with Dex during the whole of the last week of pregnancy had permanent fasting and reactive hyperglycaemia, with elevated hepatic PEPCK activity (Nyirenda

Figure 1 Cross-fostering and offspring glucose homeostasis. Pregnant rats received vehicle alone (SALINE) or Dex (DEX E15–21) during the third trimester. At birth, half the litters born to Dex-treated dams were cross-fostered and reared by saline-treated dams (DEX E15–21/CROSSED), while half the litters born to saline-treated dams were transferred to Dex-treated dams (SALINE/CROSSED). After an overnight fast, adult offspring underwent an oral glucose tolerance test (A) and hepatic PEPCK activity was measured from cytosolic preparations from fed rats (B). $n=9$ per group; $*P<0.05$ compared with SALINE and SALINE/CROSSED groups.

et al. 1998, and Fig. 1). We used this phenotype in a cross-fostering paradigm to establish whether the effect of Dex was on the fetus or was mediated postnatally through altered maternal nursing behaviour. Cross-fostering pups between control and Dex-treated dams did not change the pattern of glycaemic control in the adult offspring. Thus, offspring of Dex-treated dams had significantly higher levels of fasting glucose and showed exaggerated plasma glucose levels following an oral glucose challenge, irrespective of the treatment group of the rearing mother (Fig. 1A). Likewise, induction of hepatic PEPCK activity by

Figure 2 Length of prenatal Dex exposure and offspring glucose control. Pregnant rats were given Dex either throughout the third week of pregnancy (DEX E15–21, $n=8$) or during the last two days of gestation (DEX E20–21, $n=12$). A control group (SALINE, $n=8$) received vehicle throughout the third week of pregnancy. Following an overnight fast, adult offspring from each group underwent an oral glucose tolerance test (panel A). Panel B shows hepatic PEPCK activity in fed adult rats. $*P<0.05$ compared with SALINE group.

prenatal Dex administration was unaltered by cross-fostering pups to control dams at birth (Fig. 1B). Offspring born to control dams exhibited normal glycaemic control and hepatic PEPCK activity, and these indices were not affected by cross-fostering of these offspring to Dex-treated dams (Fig. 1).

Defining the window of Dex sensitivity

Exposure to Dex on E20–21 and later glycaemic control When Dex was given to rats only during the last two days (instead of the entire last week) of pregnancy,

Table 2 Effect of early postnatal Dex administration on subsequent body weight. Results are means $\pm$ S.E.M.

Treatment	Body weight at 4 weeks (g)	Body weight at 6 months (g)
Postnatal saline	96.4 $\pm$ 10.6 (n=16)	492 $\pm$ 24 (n=16)
Postnatal Dex	58.9 $\pm$ 8.3* (n=14)	478 $\pm$ 18 (n=14)

* $P < 0.01$ compared with saline treatment group.

there was no significant effect on glycaemic control in the adult offspring (Fig. 2A). Likewise, hepatic PEPCK activity of offspring exposed to Dex during E20–21 was similar to the activity in control animals (Fig. 2B).

Postnatal Dex and glycaemic control in adulthood

In order to test whether the window of programming of hyperglycaemia extended into the neonatal period, pups from untreated dams were given Dex or vehicle on postnatal days 1, 3 and 5. All pups survived after this treatment. Animals treated with Dex showed a slower rate of weight gain initially, so that at 4 weeks of age Dex-treated animals were 40% lighter compared with those that received saline injections (Table 2). However, by the age of six months the difference in body weight did not reach statistical significance (Table 2). There were no differences in fasting plasma glucose level, plasma glucose response to oral glucose load or hepatic PEPCK activity between animals that received saline or Dex postnatally (Fig. 3). Intriguingly, when compared with controls that were not manipulated postnatally, neonatal injection *per se* (either saline or Dex) induced an exaggerated glycaemic response to glucose challenge in the adult animals (uninjected 8.0 $\pm$ 0.3; postnatal saline 9.4 $\pm$ 0.4 mmol/l, $P < 0.05$, 30 min post loading). However, neonatal injection did not alter adult hepatic PEPCK activity (uninjected 7.4 $\pm$ 0.5; postnatal saline 7.7 $\pm$ 0.6 nmol/min/mg protein).

The HPA and sympathoadrenal activity of the adult offspring

In utero exposure to Dex has been shown to cause a lifelong elevation in basal (diurnal nadir, unstressed) corticosterone levels (Levitt *et al.* 1996) and increased hepatic glucocorticoid receptor expression (Nyirenda *et al.* 1998). An exaggerated stress response during the glucose tolerance procedure would result in increased glycaemic responses. Therefore, we examined whether prenatal exposure to Dex (DEX E15–21) was associated with an increase in corticosterone response to stress. As shown in Fig. 4, no significant difference was observed in plasma corticosterone responses to stress between animals that were exposed to Dex *in utero* and control animals. There was a tendency for higher concentrations of catecholamines in Dex-treated

Figure 3 Effect of neonatal Dex on subsequent glucose homeostasis. Pups from untreated dams were given Dex (DEX, $n = 14$) or saline (SALINE, $n = 16$) in the first postnatal week. An oral glucose tolerance test was performed at 6 months of age following an overnight fast (A). Hepatic PEPCK activity was measured in cytosolic preparations from fed animals (B).

animals than in control rats, but this difference did not reach statistical significance (Table 3).

Discussion

Recent data have shown that exposure of pregnant rats and sheep to glucocorticoids, specifically Dex, produces permanent hyperglycaemia (Lindsay *et al.* 1996a, Nyirenda *et al.* 1998) and hypertension (Lindsay *et al.* 1996b, Dodic *et al.* 1998) in the adult offspring. Such programming of hyperglycaemia is associated with features of insulin resistance, as indicated by higher plasma insulin-to-glucose ratio, and a permanent increase in hepatic PEPCK expression (Nyirenda *et al.* 1998). In the rat, both PEPCK activity and adult hyperglycaemia are induced selectively when glucocorticoids are given during the last week of pregnancy (Nyirenda *et al.* 1998). The

Figure 4 Plasma corticosterone response to stress. Basal plasma corticosterone concentrations were determined (time 0), and rats were then subjected to 20 min of restraint. Further samples were collected 15 min into, and at several time points after the restraint. Animals had been exposed to either vehicle alone (SALINE) or Dex (DEX E15–21) during the last week of intrauterine life.

mechanisms through which glucocorticoids mediate these effects are unclear. In particular, it remains unknown whether this programming reflects direct glucocorticoid action on late fetal development or results from their effects on the mother. It is well established that mother–pup interactions, such as the level of maternal licking and grooming of pups, are crucial in programming essential biological systems such as responsiveness of the HPA axis (Meaney *et al.* 1989). Dex exposure in late gestation might, therefore, act on the mother to influence maternal behaviour and care during the suckling period and thus alter development of the offspring (Rubinow *et al.* 1984, Thakore & Dinan 1995). Our data do not indicate a role for Dex-induced altered maternal nursing behaviour in glucocorticoid programming of hyperglycaemia; both induction of hepatic PEPCK activity and glycaemic control were determined by prenatal treatment, and were not affected by cross-fostering offspring between Dex-treated and control dams at birth. These findings strongly suggest that glucocorticoids programme PEPCK expression and hyperglycaemia by acting on the fetus in late gestation.

Evidence from human studies and other animal models also suggest that late gestation is a critical period

in programming hyperglycaemia. In rats, diabetes (streptozotocin-induced) of the mother during pregnancy causes insulin resistance in the adult offspring, but normalisation of maternal hyperglycemia during the last week of gestation prevents insulin resistance in offspring (Aerts & Van Assche 1992). However, frank maternal diabetes does not appear to be the underlying mechanism in programming hyperglycaemia by Dex, as this treatment did not significantly affect maternal plasma glucose levels in the present study. Epidemiological data have shown that, of people born during the Dutch famine, exposure to famine during the third trimester (but not earlier exposures) was associated with an increased risk of glucose intolerance and type 2 diabetes in adult life (Ravelli *et al.* 1998). How maternal malnutrition in late pregnancy predisposes the offspring to later abnormal glucose control remains unclear, but glucocorticoids provide a potential mechanism. For example, maternal nutritional restriction is associated with an increase in plasma levels of stress hormones (Fowden 1995) as well as attenuation of the placental enzyme barrier to glucocorticoids (Langley-Evans *et al.* 1996), thus increasing fetal glucocorticoid load.

The molecular details of glucocorticoid programming of hyperglycaemia remain to be elucidated but may involve metabolic pathways that develop in late gestation. Gluconeogenic enzymes provide one such potential target. These enzymes, amongst which PEPCK is rate-limiting (Pilkis & Granner 1992), appear just before birth and are under tight glucocorticoid control (Ballard & Hanson 1967, Friedman *et al.* 1993). Prenatal glucocorticoid treatment was associated with a permanent induction of hepatic PEPCK, supporting the role of altered gluconeogenesis in the programming of hyperglycaemia. Although PEPCK is induced at birth, restricting glucocorticoid treatment to the last two days of pregnancy (rather than treatment for the whole of the last week of pregnancy) did not cause a permanent induction in hepatic PEPCK and the adult offspring showed normal glycaemic control – suggesting that the window of sensitivity to glucocorticoids during late gestation is discrete. Prenatal exposure to glucocorticoids may also alter later glucose metabolism by influencing development of other target organs such as skeletal muscle, adipose tissue and pancreas, as well as mechanisms that co-ordinate the function of these organs. In particular, there is evidence that the adult HPA axis and the sympathoadrenal activity may be increased by

Table 3 Plasma catecholamine concentration during oral glucose tolerance testing in the saline- or Dex-treated groups. Results are means ± S.E.M.

Treatment	Adrenaline (nmol/l)		Noradrenaline (nmol/l)	
	Fasting	30-min post-load	Fasting	30-min post-load
Saline	1.7 ± 1.3 (n=6)	0.16 ± 0.06 (n=6)	2.8 ± 1.5 (n=6)	0.32 ± 0.12 (n=6)
Dex E15–21	12.6 ± 6.0 (n=6)	1.64 ± 1.54 (n=6)	9.2 ± 4.4 (n=6)	0.92 ± 0.45 (n=6)

environmental stress in early life (Levitt *et al.* 2000, Phillips *et al.* 2000). It is thus intriguing that prenatal Dex exposure in rats caused lifelong elevation in basal corticosterone levels (Levitt *et al.* 1996) and hepatic glucocorticoid receptor expression (Nyirenda *et al.* 1998), although these animals had a similar plasma corticosterone response to stress as controls. Whatever the mechanism, our data do not suggest that increased glucose and PEPCK responses are a result of exaggerated corticosterone or catecholamine responses to stress, although increased basal glucocorticoid levels may be more pertinent.

In most mammalian species organ development continues well into the early postnatal period, making this another potential programming window. Indeed, there are several examples showing that manipulations during the neonatal period can induce permanent physiological and behavioural alterations (Arai & Gorski 1968, Gustafsson & Sternberg 1974, Csaba *et al.* 1979, Meaney *et al.* 1989). Therefore, we examined whether direct administration of Dex to the newborn would reproduce the phenotype of adult hyperglycaemia and elevated hepatic PEPCK expression. Intriguingly, pups that had saline injections showed higher adult glycaemic responses compared with animals that were untouched neonatally. This suggests that the stress of neonatal injection *per se* (perhaps through increased endogenous glucocorticoid levels) is enough to induce perturbation in later glycaemic control. Additional glucocorticoid loading with Dex postnatally had no further effect to injection alone, suggesting that either this mechanism is 'saturated' by the corticosterone stress response at this age (although this is usually slight during the immediate postnatal 'stress-hyporesponsive' period (Sapolsky & Meaney 1986)) or, perhaps more plausibly, that other components of the stress response are involved. In this light, the role of perinatal manipulations in determining the sensitivity of the pancreas and liver to catecholamines and their effects upon glucose–insulin homeostasis has received some attention (Huff *et al.* 1991). Unlike the effect of prenatal Dex, the hyperglycaemic responses that followed neonatal injections were not associated with an induction of adult hepatic PEPCK expression, again suggesting that different mechanisms may be involved.

Glucocorticoids are widely used in obstetric practice, as immunosuppressants for maternal disorders (Rayburn 1992) and for short term treatment of the fetus to accelerate lung maturation in preterm labour (Crowley *et al.* 1990). Additionally, long term use of glucocorticoids has been advocated for prenatal therapy of fetuses at risk of congenital adrenal hyperplasia (Forest *et al.* 1989, Carlson *et al.* 1999). Experimental data suggests that exposure to glucocorticoids during development is associated with a risk of later metabolic and cardiovascular disorders. Rigorous follow-up studies are required to evaluate whether prenatal glucocorticoid exposure in humans produces these adverse effects. Indeed, recent data have suggested that a short antenatal glucocorticoid exposure in preterm

babies is associated with increased blood pressure at 14 years (Doyle *et al.* 2000). Understanding of the action of glucocorticoids in development, including determination of the critical time window(s), may provide further insights into the pathogenesis of common cardiovascular and metabolic disorders, as well as allow a more rational therapeutic exploitation of glucocorticoids in early life.

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