Diabetologia (2006) 49: 1138–1141
DOI 10.1007/s00125-006-0256-x

COMMENTARY

C. E. McCurdy · J. E. Friedman

# Early foetal programming of hepatic gluconeogenesis: glucocorticoids strike back

Published online: 22 April 2006


**Abbreviations** HNF4A: hepatocyte 54 nuclear factor 4, alpha · PCK: phosphoenolpyruvate carboxykinase · PPARA: peroxisome proliferative activated receptor, alpha · PPARGC1A: peroxisome proliferative activated receptor, gamma, coactivator 1 alpha

The foetus, though protected by the placental barrier, is highly susceptible to changes in both maternal diet and the hormonal milieu. In particular, a chronically poor maternal diet resulting from either protein/calorie restriction or excess maternal nutrients, as well as elevated hormones, such as insulin and glucocorticoids, have major consequences for foetal development and metabolic disease in adult life. Maternal under-nutrition, for example, can lead to intrauterine growth retardation, which is linked to an increased risk of diabetes, hypertension and cardiovascular disease in the adult offspring [1–3]. Emerging evidence also suggests that maternal over-nutrition may have similar long-term metabolic consequences in the offspring [4–6], but the mechanisms underlying the foetal origins of these diseases have only begun to be investigated.

The concept of foetal 'programming' describes a change in gene expression due to an environmental exposure in utero, resulting in a persistent altered metabolic phenotype in the adult offspring [7]. During development, glucocorticoids are essential for organ maturation, particularly the foetal lung. Administration of synthetic glucocorticoids is currently recommended for mothers at risk of preterm and delivery between 24–36 weeks, in order to promote proper foetal lung maturation, and is successful in reducing neonatal mortality and chronic lung disease [8]. Numerous laboratories have, however, provided compelling evidence that foetal exposure to inappropriate amounts of glucocorticoids has profound effects on foetal growth, placental function, and foetal and post-natal brain development, and can result in persistent hyperglycaemia throughout life [9–11]. Suboptimal maternal nutrition [12] and maternal stress [13, 14] are also thought to expose the foetus to excess glucocorticoids. Regardless of the source, elevated glucocorticoid levels during pregnancy predispose to in utero growth retardation and low birthweight [15, 16].

In this issue of *Diabetologia*, Nyirenda et al. [17] report an exciting new potential mechanism for glucocorticoid-induced foetal programming of hyperglycaemia. The authors' previous work demonstrated that exposure to prenatal dexamethasone resulted in persistent upregulation of the mRNA and activity of the cytosolic form of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PCK) in adult offspring [18]. Furthermore, these changes in PCK expression in adult offspring could not be attributed to altered postnatal maternal behaviour, suggesting that excess foetal glucocorticoid exposure has a permanent effect, programming increased gluconeogenesis [19]. Nyirenda et al. [17] have now followed up this work by investigating the effects of supraphysiological levels of glucocorticoids, administered to female rats during the last week of pregnancy, on key hepatic transcription factors known to regulate PCK expression in the rat foetus and adult offspring. Prenatal dexamethasone resulted in an early increase in the transcription of the gene encoding foetal hepatic nuclear factor 4 (*Hnf4a*), which remained elevated into adulthood, and paralleled the rise in *Pck1* expression and hyperglycaemia. Interestingly, dexamethasone treatment not only increased *Hnf4a* mRNA expression, but altered the expression of the of *Hnf4a* isoforms, such that the adult isoforms (*Hnfa1/2*) were favoured over the foetal isoforms (*Hnf4a7/8*). Expression of the different isoforms results from alternative promoter usage and differential splicing. An early promoter switch to produce

C. E. McCurdy (✉) · J. E. Friedman
Department of Pediatrics,
University of Colorado School of Medicine,
Mail Stop 8106,
P.O. Box 6511, Aurora, CO 80045, USA
e-mail: carrie.mccurdy@uchsc.edu

the adult HNF4A isoform thus represents a novel mechanism by which early exposure to glucocorticoids may potentially induce *Pck1* mRNA and result in a premature increase in gluconeogenesis.

The gluconeogenic genes encoding PCK and glucose-6-phosphatase, which, respectively, catalyse the first and final steps in hepatic gluconeogenesis, together with the transcription factors that regulate their expression, are likely targets for foetal programming of hyperglycaemia. These genes are usually not expressed before birth, since the maternal glucose supply is sufficient to meet the needs of the foetus during development. In the immediate postnatal period, however, neonatal glucose falls and there is a rapid increase in stress hormones (cAMP and glucocorticoids), triggering increased transcription of the *Pck1* gene, which codes for the first enzyme in the gluconeogenic pathway [20]. Glucocorticoids stimulate hepatic *Pck1* gene expression by altering the protein levels of specific transcription factors and co-activators and promoting a complex series of interactions between these factors and the extended glucocorticoid regulatory unit (GRU) in the *Pck1* promoter [21, 22] (Fig. 1). Glucocorticoids have been shown to enhance the binding of specific transcription factors, including FOXO1, peroxisome proliferative activated receptor alpha (PPARA), and HNF4A to the extended glucocorticoid regulatory unit of the *Pck1* promoter [22]. Additionally, both *Pck1* and the gene for glucose-6-phosphatase contain glucocorticoid receptor binding elements, allowing glucocorticoids to enhance gene expression [23, 24]. The co-activator peroxisome proliferative activated receptor, gamma, coactivator 1 alpha (PPARGC1A) also activates *Pck1* expression through specific interaction with HNF4A, without binding directly to the *Pck1* promoter [25]. HNF4A has been associated with recruiting coactivator protein (CREB binding protein/p300) and the ability to mediate chromatin remodelling [26–28]. Whether these other regulators are also involved in stimulating PCK gene transcription directly in response to early glucocorticoid exposure was not explored in the report by Nyirenda et al. [17]. A more thorough and direct analysis of the DNA binding factors at the protein level could be explored using foetal rat liver nuclear extracts and DNA binding assays. In addition, research into the potential mediators that regulate alternative splicing may also further our understanding of the mechanisms whereby glucocorticoids alter HNF4A and its transcriptional activation pattern.

Animal models of excess glucocorticoid during late pregnancy are often used as a means of understanding how maternal stress can induce changes in the foetus. The foetus is normally protected from excess maternal glucocorticoid, as 11-beta-hydroxysteroid dehydrogenase type 2 (11β-

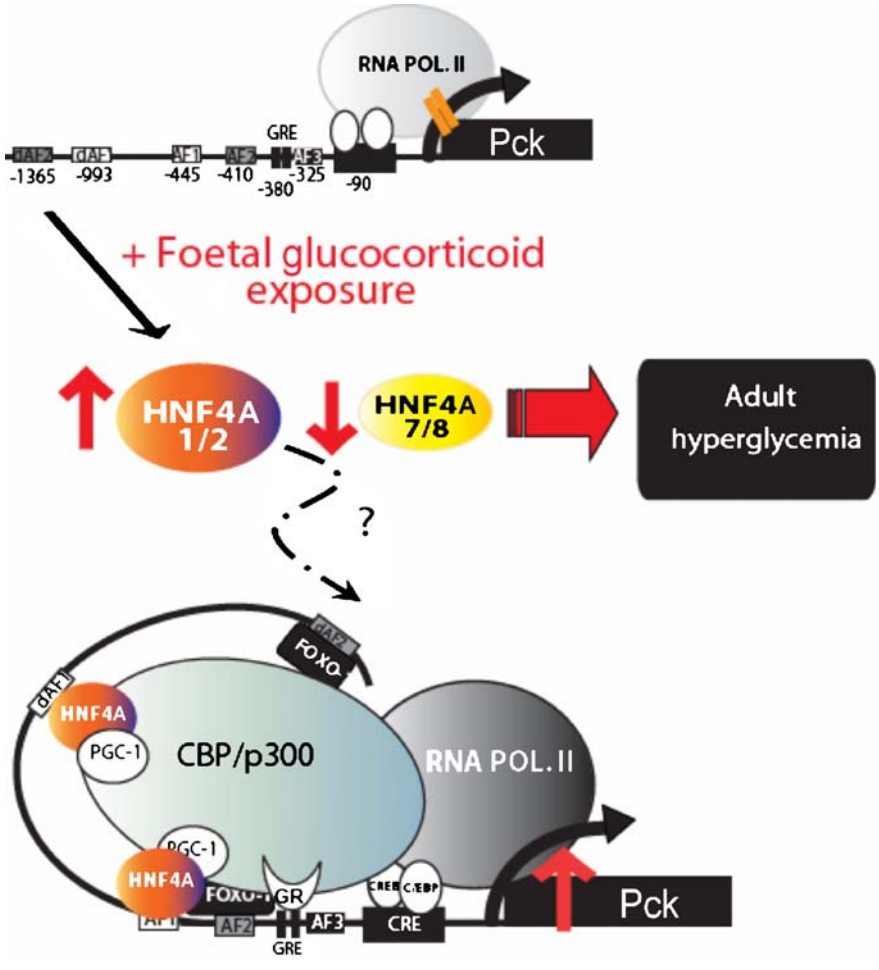


**Fig. 1** Extended model for the effect of excess glucocorticoid exposure on PCK gene transcription and persistent hyperglycaemia in adult offspring. Prenatal exposure to excess glucocorticoids leads to a specific increase in the expression of *Hnf4a* but not the gene for glucocorticoid receptor (*Nr3c1*), *Hnf1a* or *Ppargcla* in foetal rat liver. Isoform analysis of *Hnf4a* mRNA revealed that the glucocorticoid exposure induces promoter switching that prematurely upregulates the adult isoforms (HNF4A1/2) and downregulates the foetal isoforms (HNF4A7/8). These changes in *Hnf4a* are persistent and parallel the increases in *Pck1* expression and hyperglycaemia in the exposed adult offspring. These new findings suggest that changes in *Hnf4a* expression in utero may be a driving force for foetal programming of glucocorticoid-induced insulin resistance, possibly through premature assembly of transcription factors on the *Pck1* promoter. *AF* Accessory factor, *GRE* glucocorticoid regulatory element

HSD2) acts as a foeto-placental barrier, inactivating circulating maternal cortisol to inert cortisone, and thereby preventing foetal exposure to elevated glucocorticoid. In rats, inhibition of 11β-HSD2 causes postnatal development of hyperglycaemia, increased blood pressure, and increased hypothalamic–pituitary–adrenal (HPA) axis activity in adult offspring [29–31]. Additionally, prenatal betamethasone exposure resulted in a persistent increase in glucose-6-phosphatase activity in sheep adult offspring [32] and PCK in rat adult offspring [18], supporting the concept that changes in gluconeogenic enzyme expression may lead to the chronic hyperglycaemia in adult offspring associated with prenatal glucocorticoid exposure.

Although the paper from Nyirenda et al. does not provide direct evidence that the change in *Hnf4a* expression is the primary effect of dexamethasone, it does provide insight into what may be a common underlying mechanism for foetal programming. Different foetal insults (e.g. over- and under-maternal nutrition, placental insufficiency, maternal stress, and excess glucocorticoid exposure) can all result in a similar outcome: offspring predisposed to adult hyperglycaemia and insulin resistance. In the liver, HNF4A is thought to regulate the transcription of over half of all hepatic genes [33]. How HNF4A is targeted to genes is under active investigation, and is probably related to its interactions with other regulatory transcriptional complexes. However, it is important to note that its activation and expression are altered in response to both dietary and hormonal factors, thus making HNF4A an important intersection for nutritional and/or hormonal regulation of gene expression and cell function [34–36]. We have recently found, in a non-human primate model, that a high-fat, high-calorie maternal diet also results in a premature upregulation of gluconeogenic gene expression and increased HNF4A protein expression in the foetal liver (*Unpublished results*). A more focused study may elucidate whether HNF4A acts as the primary mediator or a corollary of foetal programming.

It is also important to note that prenatal glucocorticoid exposure in rats has intergenerational effects, resulting in adult offspring with increased PCK and hyperglycaemia in the subsequent $F_2$ generation, despite normal pregnancies [37]. Transcriptional control of gene expression depends on DNA accessibility, which is epigenetically regulated by histone modification, DNA methylation and chromatin remodelling. The notion that maternal nutrition and glucocorticoids may lead to epigenetic changes that underlie the foetal programming of adult disease, is increasingly gaining acceptance [38, 39]. The mechanisms involved in generating such responses are not well characterised, however, opening up new study areas that promise new insights into foetal programming of adult disease.

Diabetes is a polygenic disorder, and its onset is influenced as much by our inherited genes as by our exposure to environmental factors, starting at our earliest developmental stages in utero. While studies of families with disease clusters may be informative for identifying highly penetrant gene variants, other approaches are needed to identify factors that determine environmental susceptibility to diabetes, including prenatal and postnatal nutrition, stress, and physical activity. The study by Nyirenda et al. [17] highlights a new window of opportunity for investigation of the origins and mechanisms of adult hyperglycaemia.

## References


1. Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS (1993) Fetal nutrition and cardiovascular disease in adult life. Lancet 341:938–941
2. Valdez R, Athens MA, Thompson GH, Bradshaw BS, Stern MP (1994) Birthweight and adult health outcomes in a biethnic population in the USA. Diabetologia 37:624–631
3. Barker DJ, Hales CN, Fall CH, Osmond C, Phipps K, Clark PM (1993) Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. Diabetologia 36:62–67
4. Armitage JA, Taylor PD, Poston L (2005) Experimental models of developmental programming: consequences of exposure to an energy rich diet during development. J Physiol (Lond) 565:3–8
5. Boney CM, Verma A, Tucker R, Vohr BR (2005) Metabolic syndrome in childhood: association with birth weight, maternal obesity, and gestational diabetes mellitus. Pediatrics 115: e290–e296
6. Fall CH, Stein CE, Kumaran K et al (1998) Size at birth, maternal weight, and type 2 diabetes in South India. Diabet Med 15:220–227
7. West-Eberhard MJ (2005) Developmental plasticity and the origin of species differences. Proc Natl Acad Sci USA 102:6543–6549
8. Friedman S, Shinwell ES (2004) Prenatal and postnatal steroid therapy and child neurodevelopment. Clin Perinatol 31: 529–544
9. Sloboda DM, Newnham JP, Challis JR (2000) Effects of repeated maternal betamethasone administration on growth and hypothalamic–pituitary–adrenal function of the ovine fetus at term. J Endocrinol 165:79–91
10. Sloboda DM, Challis JR, Moss TJ, Newnham JP (2005) Synthetic glucocorticoids: antenatal administration and long-term implications. Curr Pharm Des 11:1459–1472
11. Seckl JR (2001) Glucocorticoid programming of the fetus; adult phenotypes and molecular mechanisms. Mol Cell Endocrinol 185:61–71
12. Edwards LJ, Coulter CL, Symonds ME, McMillen IC (2001) Prenatal undernutrition, glucocorticoids and the programming of adult hypertension. Clin Exp Pharmacol Physiol 28:938–941
13. Kapoor A, Matthews SG (2005) Short periods of prenatal stress affect growth, behaviour and hypothalamo–pituitary–adrenal axis activity in male guinea pig offspring. J Physiol 566: 967–977
14. Jones A, Godfrey KM, Wood P, Osmond C, Goulden P, Phillips DIW (2006) Fetal growth and the adrenocortical response to psychological stress. J Clin Endocrinol Metab (in press) DOI 10.1210/jc2005-2077
15. Bayol SA, Simbi BH, Stickland NC (2005) A maternal cafeteria diet during gestation and lactation promotes adiposity and impairs skeletal muscle development and metabolism in rat offspring at weaning. J Physiol 567:951–961
16. Reinisch JM, Simon NG, Karow WG, Gandelman R (1978) Prenatal exposure to prednisone in humans and animals retards intrauterine growth. Science 202:436–438
17. Nyirenda MJ, Dean S, Lyons V, Chapman E, Seckl JR (2006) Prenatal programming of hepatocyte nuclear factor 4α in the rat: a key mechanism in the 'foetal origins of hyperglycaemia'? Diabetologia DOI 10.1007/s00125-006-0188-5

18. Nyirenda MJ, Lindsay RS, Kenyon CJ, Burchell A, Seckl JR (1998) Glucocorticoid exposure in late gestation permanently programs rat hepatic phosphoenolpyruvate carboxykinase and glucocorticoid receptor expression and causes glucose intolerance in adult offspring. J Clin Invest 101:2174–2181
19. Nyirenda M, Welberg L, Seckl JR (2001) Programming hyperglycaemia in the rat through prenatal exposure to glucocorticoids-fetal effect or maternal influence? J Endocrinol 170:653–660
20. Girard J (1990) Metabolic adaptations to change of nutrition at birth. Biol Neonate 58(Suppl 1):3–15
21. Imai E, Stromstedt PE, Quinn PG, Carlstedt-Duke J, Gustafsson JA, Granner DK (1990) Characterization of a complex glucocorticoid response unit in the phosphoenolpyruvate carboxykinase gene. Mol Cell Biol 10:4712–4719
22. Cassuto H, Kochan K, Chakravarty K et al (2005) Glucocorticoids regulate transcription of the gene for phosphoenolpyruvate carboxykinase in the liver via an extended glucocorticoid regulatory unit. J Biol Chem 280:33873–33884
23. Vander Kooi BT, Onuma H, Oeser JK et al (2005) The glucose-6-phosphatase catalytic subunit gene promoter contains both positive and negative glucocorticoid response elements. Mol Endocrinol 19:3001–3022
24. Nakae J, Kitamura T, Silver DL, Accili D (2001) The forkhead transcription factor Foxo1 (Fkhr) confers insulin sensitivity onto glucose-6-phosphatase expression. J Clin Invest 108: 1359–1367
25. Yamamoto T, Shimano H, Nakagawa Y et al (2004) SREBP-1 Interacts with hepatocyte nuclear factor-4$\alpha$ and interferes with PGC-1 recruitment to suppress hepatic gluconeogenic genes. J Biol Chem 279:12027–12035
26. Dell H, Hadzopoulou-Cladaras M (1999) CREB-binding protein is a transcriptional coactivator for hepatocyte nuclear factor-4 and enhances apolipoprotein gene expression. J Biol Chem 274:9013–9021
27. Gautier-Stein A, Mithieux G, Rajas F (2005) A distal region involving hepatocyte nuclear factor 4$\alpha$ and CAAT/enhancer binding protein markedly potentiates the protein kinase a stimulation of the glucose-6-phosphatase promoter. Mol Endocrinol 19:163–174
28. Yoshida E, Aratani S, Itou H et al (1997) Functional association between CBP and HNF4 in trans-activation. Biochem Biophys Res Commun 241:664–669
29. Saegusa H, Nakagawa Y, Liu YJ, Ohzeki T (1999) Influence of placental 11beta-hydroxysteroid dehydrogenase (11beta-HSD) inhibition on glucose metabolism and 11beta-HSD regulation in adult offspring of rats. Metabolism 48:1584–1588
30. Lindsay RS, Lindsay RM, Waddell BJ, Seckl JR (1996) Prenatal glucocorticoid exposure leads to offspring hyperglycaemia in the rat: studies with the 11$\beta$-hydroxysteroid dehydrogenase inhibitor carbenoxolone. Diabetologia 39:1299–1305
31. Welberg LAM, Seckl JR, Holmes MC (2000) Inhibition of 11beta-hydroxysteroid dehydrogenase, the foeto-placental barrier to maternal glucocorticoids, permanently programs amygdala GR mRNA expression and anxiety-like behaviour in the offspring. Eur J Neurosci 12:1047–1054
32. Sloboda DM, Moss TJ, Li S et al (2005) Hepatic glucose regulation and metabolism in adult sheep: effects of prenatal betamethasone. Am J Physiol Endocrinol Metab 289: E721–E728
33. Odom DT, Zizlsperger N, Gordon DB et al (2004) Control of pancreas and liver gene expression by HNF transcription factors. Science 303:1378–1381
34. Rajas F, Gautier A, Bady I, Montano S, Mithieux G (2002) Polyunsaturated fatty acyl coenzyme A suppress the glucose-6-phosphatase promoter activity by modulating the DNA binding of hepatocyte nuclear factor 4alpha. J Biol Chem 277: 15736–15744
35. Jump DB, Clarke SD (1999) Regulation of gene expression by dietary fat. Annu Rev Nutr 19:63–90
36. Bailly A, Torres-Padilla ME, Tinel AP, Weiss MC (2001) An enhancer element 6 kb upstream of the mouse HNF4$\alpha$1 promoter is activated by glucocorticoids and liver-enriched transcription factors. Nucl Acids Res 29:3495–3505
37. Drake A, Walker B (2004) The intergenerational effects of fetal programming: non-genomic mechanisms for the inheritance of low birth weight and cardiovascular risk. J Endocrinol 180: 1–16
38. Reik W, Constancia M, Fowden A et al (2003) Regulation of supply and demand for maternal nutrients in mammals by imprinted genes. J Physiol 547:35–44
39. Gallou-Kabani C, Junien C (2005) Nutritional epigenomics of metabolic syndrome: new perspective against the epidemic. Diabetes 54:1899–1906