British Journal of Nutrition (2007), **97**, 435–439
DOI: 10.1017/S0007114507352392

Dietary protein restriction of pregnant rats in the F_0 generation induces altered methylation of hepatic gene promoters in the adult male offspring in the F_1 and F_2 generations

Graham C. Burdge[1]*, Jo Slater-Jefferies[1], Christopher Torrens[1], Emma S. Phillips[2], Mark A. Hanson[1] and Karen A. Lillycrop[2]

[1]*Developmental Origins of Health and Disease Division, University of Southampton, Southampton, UK*
[2]*Development and Cell Biology, University of Southampton, Southampton, UK*

(Received 12 June 2006 – Revised 18 September 2006 – Accepted 9 October 2006)

Epidemiological studies and experimental models show that maternal nutritional constraint during pregnancy alters the metabolic phenotype of the offspring and that this can be passed to subsequent generations. In the rat, induction of an altered metabolic phenotype in the liver of the F_1 generation by feeding a protein-restricted diet (PRD) during pregnancy involves the altered methylation of specific gene promoters. We therefore investigated whether the altered methylation of PPARα and glucocorticoid receptor (GR) promoters was passed to the F_2 generation. Females rats (F_0) were fed a reference diet (180 g/kg protein) or PRD (90 g/kg protein) throughout gestation, and AIN-76A during lactation. The F_1 offspring were weaned onto AIN-76A. F_1 females were mated and fed AIN-76A throughout pregnancy and lactation. F_1 and F_2 males were killed on postnatal day 80. Hepatic PPARα and GR promoter methylation was significantly ($P<0{\cdot}05$) lower in the PRD group in the F_1 (PPARα 8 %, GR 10 %) and F_2 (PPARα 11 %, GR 8 %) generations. There were trends ($P<0{\cdot}1$) towards a higher expression of PPARα, GR, acyl-CoA oxidase and phosphoenolpyruvate carboxykinase (PEPCK) in the F_1 and F_2 males, although this was significant only for PEPCK. These data show for the first time that the altered methylation of gene promoters induced in the F_1 generation by maternal protein restriction during pregnancy is transmitted to the F_2 generation. This may represent a mechanism for the transmission of induced phenotypes between generations.

Fetal programming: Transgeneration: Epigenetic: Liver

Developmental plasticity allows the generation of a number of phenotypes from a single genotype (Gluckman & Hanson, 2004). Epidemiological (Godfrey & Barker, 2001) and experimental (Bertram & Hanson, 2002) studies show that aspects of the prenatal environment such as maternal nutrition and stress levels provide cues that modify the phenotype of the offspring without overt reductions in fetal growth. Such nutritional cues operate within the normal range for the human population and contribute to the early-life origins of risk of chronic diseases such as the metabolic syndrome (Godfrey & Barker, 2001).

There is evidence in humans and in experimental models for non-genomic transmission between generations of induced phenotypic traits associated with an impaired capacity to maintain energy balance. The mortality from diabetes was increased in men if the paternal grandfather had been exposed to abundant nutrition during puberty (Pembrey *et al.* 2006). The daughters of women exposed to nutrient restriction and environmental stress during pregnancy as a result of the Dutch Hunger Winter showed a decreased birth weight and an increased risk of insulin resistance, and their daughters also were born with a lower birth weight (Stein & Lumey, 2000; Painter *et al.* 2005). In rats, feeding a protein-restricted diet (PRD) to the F_0 generation during pregnancy resulted in elevated blood pressure and endothelial dysfunction (Torrens *et al.* 2002) and insulin resistance (Martin *et al.* 2000; Zambrano *et al.* 2005) in the F_1 and F_2 generations, despite normal nutrition during pregnancy in the F_1 generation. The adverse effects on glucose homeostasis of feeding a PRD during pregnancy in the F_0 generation have been found in the offspring up to F_3 generation (Benyshek *et al.* 2006). The administration of dexamethasone to dams in late pregnancy induced an increased expression of the glucocorticoid receptor (GR) and its target gene phosphoenolpyruvate carboxykinase (PEPCK) in the liver of the F_1 and F_2 offspring (Drake *et al.* 2005). This effect was transmitted through both the male and female F_1 lines. These changes in gene expression were not, however, present in the F_3 generation.

The mechanism for the transgenerational transmission of induced phenotypes is not known. Stable changes to gene expression that underlie individual phenotypes are the result of the epigenetic regulation of transcription, which includes DNA methylation and covalent modifications to histones. The methylation of cytosines in CpG dinucleotides in the promoter region of genes permanently suppresses transcription (Bird, 2002). Soon after fertilization, the genome undergoes demethylation. This is followed by methylation of the

Abbreviations: AOX, acyl-CoA oxidase; GR, glucocorticoid receptor; PEPCK, phosphoenolpyruvate carboxykinase; PRD, protein-restricted diet; RD, reference diet.

* **Corresponding author:** Dr G. C. Burdge, fax +44 (0)2380 594379, email g.c.burdge@soton.ac.uk

promoters of specific genes in the early embryo (Bird, 2002). Such epigenetic gene-silencing is critical for cellular differentiation and is maintained throughout the lifespan. Allele-specific silencing of imprinted genes by DNA methylation is well established (Arnaud & Feil, 2005; Lander-Diner & Cedar, 2005), and methylation patterns are resistant to demethylation during the early development of the embryo, although the underlying mechanism is unclear (Lane *et al.* 2003).

We have shown recently that feeding a PRD to pregnant rats resulted in hypomethylation and increased expression of the PPAR-α and $GR1_{10}$ promoters in the liver of the offspring on postnatal day 34 (Lillycrop *et al.* 2005). This shows that the induction of different metabolic phenotypes in the offspring by maternal nutrition during pregnancy in non-imprinted genes also involves an altered epigenetic regulation of gene expression. Moreover, in our previous study, this epigenetic change in the PPARα and GR promoters was prevented by supplementing the PRD with folic acid during pregnancy (Lillycrop *et al.* 2005), which suggests that altered 1-carbon metabolism is involved in the process of inducing altered DNA methylation.

In the present study, we have tested the hypothesis that the transmission of phenotypes between the F_1 and F_2 generations involves an altered epigenetic regulation of specific genes. We report the effect of feeding a PRD during pregnancy in the F_0 generation on the methylation status and expression of the GR and PPARα promoters, and on the expression of their respective target genes PEPCK and acyl-CoA oxidase (AOX) in the liver of the F_1 and F_2 offspring.

Materials and methods

Animal procedures

Female Wistar rats (F_0) were mated and then fed throughout pregnancy either a reference diet (RD) containing 180 g/kg (w/w) casein or an isocaloric PRD containing 90 g/kg (w/w) casein as described (Langley & Jackson, 1994; Table 1). At delivery, litters were reduced to eight pups. Dams were fed purified AIN-76A diet throughout lactation (Table 1). The offspring were weaned onto AIN-76A 28 d after birth. Male offspring (F_1) were killed on postnatal day 80. The livers were removed immediately, frozen in liquid N and stored at −80°C. Two female F_1 offspring were selected from each litter by random removal from the cage and were mated on postnatal day 125 with males that had received adequate nutrition throughout life. F_1 females were fed AIN-76A (Table 1) throughout pregnancy and lactation. Litters were reduced to eight at birth, and the F_2 offspring were weaned at postnatal day 28. Male offspring were killed on postnatal day 80. The livers were immediately removed, frozen in liquid N and stored at −80°C. Livers were selected at random for studies of gene methylation and expression by removal from collections of stored specimens without knowledge of any aspect of the phenotype of the offspring. Liver from one offspring from each litter was studied.

Measurement of DNA methylation

The methylation status of the $GR1_{10}$ and PPARα promoters was determined by methylation-sensitive real-time PCR (Lillycrop *et al.* 2005). Briefly, genomic DNA (5 μg) was isolated from liver using standard methods and treated with the methylation-sensitive restriction enzymes *Aci* I and *Hpa* II as instructed by the manufacturer (New England Biolabs (UK), Hitchin, Hertfordshire, UK). Purified DNA was then amplified by real-time PCR using the primers listed in Table 2. The reaction was carried out in a total volume of 25 μl with SYBR Green Jumpstart ready mix as described by the manufacturer (Sigma, Poole, Dorset, UK). The promoter region of the rat PPARγ2 gene, which contains no CpG islands and no *Aci* I or *Hpa* II recognition sites, was used as an internal control. There was no effect of maternal diet or generation on the methylation status of the hepatic PPARγ2 promoter. All cycle threshold (Ct) values were normalized to the internal control.

Measurement of mRNA expression

mRNA expression was determined by real-time RTPCR amplification (Harris *et al.* 2002). Briefly, total RNA was isolated from cells using TRIZOL reagent (Invitrogen, Paisley, Renfrewshire, UK), and 0·1 μg was used as a template to prepare cDNA using 100 U Moloney murine leukaemia virus reverse transcriptase. The primer sequences are listed in Table 2. The PCR reaction

Table 1. Composition of the diets fed

	F_0 pregnancy diets		Diet fed to F_0 dams during lactation and to F_1 and F_2 offspring
	Reference diet	Protein-restricted diet	AIN-76A
Casein (g/kg)	180	90	200
Folic acid (mg/kg)	1	1	2
Corn starch (g/kg)	425	482	150
Sucrose (g/kg)	213	243	500
Choline chloride (g/kg)	2	2	2
DL-Methionine (g/kg)	5	5	3
Vitamin mix† (g/kg)	5	5	5
Mineral mix‡ (g/kg)	20	20	20
Cellulose (g/kg)	50	50	50
Corn oil (g/kg)	100	100	50
Total metabolizable energy (MJ/kg)	20·2	19·9	15·5

† Vitamin mix: thiamine hydrochloride 2·4 mg/kg, riboflavin 2·4 mg/kg, pyridoxine hydrochloride 2·8 mg/kg, nicotinic acid 12·0 mg/kg, D-calcium pantothenate 6·4 mg/kg, biotin 0·01 mg/kg, cyanocobalbumin 0·003 mg/kg, retinyl palmitate 6·4 mg/kg, DL-tocopherol acetate 79·9 mg/kg, cholecalciferol 1·0 g/kg, menaquinone 0·02 mg/kg.

‡ Mineral mix: calcium phosphate dibasic 11·3 g/kg, NaCl 1·7 g/kg, potassium citrate monohydrate 5·0 g/kg, K_2SO_4 1·2 g/kg, $MgSO_4$ 0·5 g/kg, $MgCO_3$ 0·1 g/kg, ferric citrate 0·1 g/kg, $ZnCO_3$ 36·2 mg/kg, $CuCO_3$ 6·8 mg/kg, KIO_3 0·2 mg/kg, sodium selenite 0·2 mg/kg, chromium potassium sulphate 12·5 mg/kg.

Table 2. PCR primers for analysis of promoter methylation and mRNA expression

Gene	Forward primer	Reverse primer
Methylation-sensitive PCR		
$GR1_{10}$	TCCTCCATTTTTGCGAGCTC	CCACCGCAGCCAGATAAAC
PPAR-γ_2	GTCTCTGCTCTGGTAATTC	AAGGCTTGTGGTCATTGAG
PPARα	CGACTGTGAGGAGCAAGG	CCCAGGTCTCTTCTTCAG
mRNA expression		
$GR1_{10}$	TGACTTCCTTCTCCGTGACA	GGAGAATCCTCTGCTGCTTG
PPARα	CTGGTCAAGCTCAGGACACA	AAACGGATTGCATTGTGTGA
AOX	CCAATCACGCAATAGTTCTGG	CGCTGTATCGTATGGCGAT
PEPCK	AGCTGCATAATGGTCTGG	GAACCTGGCGTTGAATGC
Ribosomal 18S	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGTAGCG

Primers designed by QIAGEN Ltd UK, Crawley, UK.
AOX, acyl-CoA oxidase; $GR1_{10}$, glucocorticoid receptor; PEPCK, phosphoenolpyruvate carboxykinase.

was carried out in a total volume of 25 μl with SYBR Green Jumpstart ready mix as described by the manufacturer (Sigma). mRNA expression was normalized using the housekeeping gene ribosomal 18S RNA using the change in ΔCt method (Bustin, 2000). There was no effect of maternal diet or generation on the mRNA expression of 18S ribosomal RNA.

Statistical comparisons

Normalized Ct values are presented as proportion of the RD group in the F_1 generation (mean (SEM); n 6 offspring per F_0 dietary group, one offspring per litter). Analysis of the covariate by independent variable interaction showed that the homogeneity of the regression slopes could be assumed for each of the genes studied. Therefore, ANOVA was used to assess the effects of diet and generation on promoter methylation status and mRNA expression. The extent of interactions between the diet of the F_0 dams and the generation of the offspring was determined by two-way ANOVA. Comparisons of DNA methylation and mRNA expression between F_0 dietary groups and generations of offspring were by one-way ANOVA with Dunnett's *post hoc* test (two-sided) using the RD F_1 group as the reference.

Results

DNA methylation

The results of measurements of the methylation status of the $GR1_{10}$ promoter, which is expressed in liver, and of the PPARα promoter are summarised in Fig. 1. Analysis by two-way ANOVA showed that there was no significant effect of generation or interaction between F_0 diet and generation on the methylation of the $GR1_{10}$ or PPARα promoters. There was, however, a significant effect of diet ($P<0{\cdot}001$) on the methylation status of both genes. Methylation of the $GR1_{10}$ promoter was significantly lower ($P<0{\cdot}05$) in the liver of the male offspring of the F_0 PRD group in the F_1 (10·2 %) and F_2 (7·9 %) generations compared with the F_0 RD group. Methylation of the PPARα promoter was significantly lower in the liver of the male offspring of the F_0 PRD group in the F_1 (8·2 %) and F_2 (10·5 %) generations compared with the F_0 RD group. There were no significant differences between the F_1 and F_2 generations within a F_0 maternal dietary group.

mRNA expression

The results of measurements of mRNA expression are summarised in Table 3. Analysis by two-way ANOVA showed

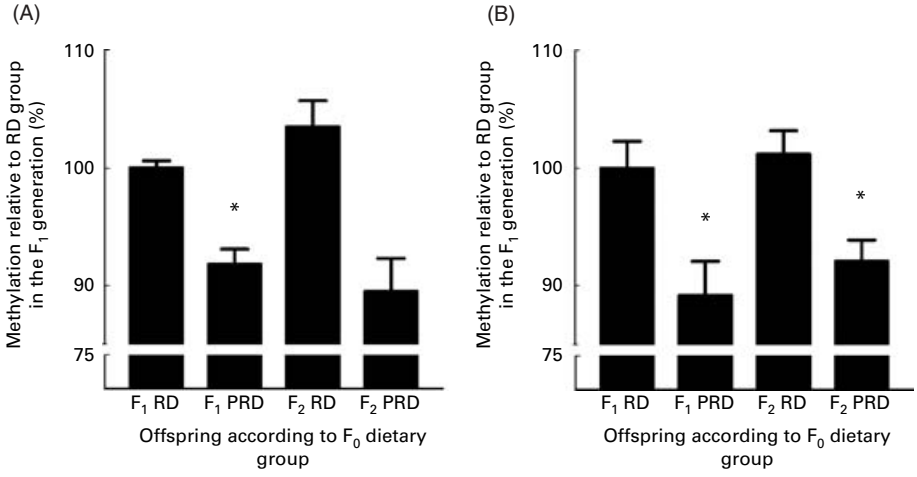

Fig. 1. Effect of maternal protein intake during pregnancy in the F_0 generation on hepatic (A) PPARα and (B) glucocorticoid receptor promoter methylation in the F_1 and F_2 male offspring. Values are means with their standard errors normalized to the offspring of the F_1 reference diet (RD) group (six per group). PRD, protein-restricted diet. * Mean values were significantly different between maternal diets within a generation by one-way ANOVA with Dunnett's *post hoc* test (two-sided) using the RD F_1 group as a reference: $P<0{\cdot}05$.

Table 3. Measurements of hepatic mRNA expression

(Mean values and their standard errors for six male offspring in each F_0 dietary group in each generation)

	mRNA expression (%, compared with F_1 reference diet group)								
	F_1				F_2				
	Reference diet		Protein-restricted diet		Reference diet		Protein-restricted diet		
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	ANOVA
PPARα	100·0	15·0	129·4	15·8	106·2	5·1	143·8	33·2	0·093
$GR1_{10}$	100·0	24·0	115·0	18·2	105·6	14·4	130·9	24·4	0·080
AOX	100·0	14·0	164·8	7·6	101·2	19·8	204·6	37·3	0·039
PEPCK	100·0	16·0	158·7*	14·9	107·4	6·4	172·8*	13·2	0·023

AOX, acyl-CoA oxidase; $GR1_{10}$, glucocorticoid receptor; PEPCK, phosphoenolpyruvate carboxykinase.

*Mean values were significantly different between maternal diets within a generation by one-way ANOVA with Dunnett's *post hoc* test (two-sided) using the reference diet F_1 group as a reference: $P<0·05$.

that there was no significant interaction effect between generation and F_0 dietary group on the expression of any of the genes measured. Analysis by one-way ANOVA showed that there were no significant differences in the expression of any of these genes between the F_1 and F_2 generations with an F_0 maternal dietary group. There was no significant effect of F_0 maternal diet on the expression of PPARα or $GR1_{10}$ in the F_1 or F_2 generations, although there were trends ($P<0·1$) towards higher mRNA expression in the PRD group in the F_1 (29 % and 15 %, respectively) and F_2 (44 % and 31 %, respectively) compared with the F_1 RD group. Two-way ANOVA showed that there was a significant effect of the F_0 maternal diet on the expression of AOX ($P=0·016$) and PEPCK ($P=0·006$). For AOX, one-way ANOVA showed a significant difference between groups, although this did not reach statistical significance in pairwise comparisons. There were, however, trends (ANOVA $P<0·05$) towards higher AOX mRNA expression in the PRD group in the F_1 (65 %) and F_2 (105 %) generations, although these did not reach statistical significance in pairwise comparisons (Table 3). PEPCK expression was significantly ($P<0·05$) greater in the PRD group in the F_1 (59 %) and F_2 (73 %) generations (Table 3).

Discussion

The results of this study show for the first time that the altered methylation of gene promoters induced in the F_1 offspring by maternal protein restriction during pregnancy is transmitted to the F_2 offspring.

The majority of studies on the induction of an altered metabolic phenotype by maternal dietary restriction in humans and in experimental models have focused on the first-generation offspring. There is, however, evidence from epidemiological studies (Stein & Lumey, 2000; Painter *et al.* 2005), and in particular from animal models (Martin *et al.* 2000; Torrens *et al.* 2002; Zambrano *et al.* 2005; Benyshek *et al.* 2006), that the phenotype induced in the offspring of the F_1 generation can be transmitted to subsequent generations.

We have previously shown that an increased expression of $GR1_{10}$ and PPARα in the liver of the offspring on postnatal day 34 as a result of feeding a PRD in pregnancy is due to hypomethylation of the respective gene promoters (Lillycrop *et al.* 2005). Our current findings show that the methylation status of the $GR1_{10}$ and PPARα promoters was reduced in the F_1 and F_2 offspring of the F_0 PRD dams. One possible explanation is that the level of methylation of the $GR1_{10}$ and PPARα promoters was set during the development of the F_1 generation and that this is maintained through gamete production, through demethylation of the maternal and paternal genomes after fertilization and during gene specific remethylation in the early embryo. If so, this implies that the process which results in the hypomethylation of $GR1_{10}$ and PPARα in the liver also induces a stable reduction of methylation of these genes in the germ cells. As the F_1 females, but not the males with which they were mated, had been exposed to nutritional constraint during pregnancy, our findings suggest that the transmission of $GR1_{10}$ and PPARα hypomethylation must have occurred via the female genome and that this was sufficient to alter the methylation of the promoters of these genes in the livers of the F_2 males. Studies of the expression of intracisternal A-type particles show that the methylation of these repetitive sequences is resistant to demethylation during preimplantation development, and it has been suggested that such resistance may explain the inheritance of patterns of gene imprinting (Lane *et al.* 2003). If the level of methylation of non-imprinted genes was also 'protected' during post-fertilization demethylation, this might explain how patterns of $GR1_{10}$ and PPARα methylation induced in the F_1 generation may be transmitted to the F_2 generation.

One alternative explanation is that prenatal undernutrition induced changes in the F_1 females that constrained the intrauterine environment experienced by the F_2 male offspring, and that hypomethylation of the PPARα and $GR1_{10}$ promoters was thus induced *de novo* in the male offspring in each generation. This seems unlikely because of the similarity in the degree of hypomethylation induced in the F_1 and F_2 generations. It might be anticipated that if promoter hypomethylation were induced *de novo* in each generation, it would result in different levels of methylation because of differences in the degree of environmental constraint. However, a single environmental challenge in the F_0 generation might be expected to induce a similar level of promoter methylation in both generations if the effect on the F_1 generation were transmitted to the F_2 generation.

Feeding a PRD during pregnancy in the F_0 generation induced a trend towards an increased expression of PPARα and $GR1_{10}$, and their respective target genes AOX and PEPCK, in the liver of the F_1 and F_2 offspring at day 80, although only the increase in PEPCK expression reached statistical significance. The trend in the expression is consistent with reduced methylation of the $GR1_{10}$ and PPARα promoters. We have previously shown that feeding a PRD during pregnancy significantly increased the expression of PPARα, $GR1_{10}$ and AOX in the liver of the F_1 offspring on postnatal day 34 due to hypomethylation of the $GR1_{10}$ and PPARα promoters (Lillycrop *et al.* 2005), and others have shown increased gluconeogenesis in this model (Burns *et al.* 1997).

One possible explanation for the difference between our previous report (Lillycrop *et al.* 2005) and the present study in the extent to which hypomethylation of the GR and PPARα promoters altered the expression of $GR1_{10}$ and PPARα, and of their target genes AOX and PEPCK, is that the transcription of PPARα and GR is responsive to environmental stimuli such as dietary fat intake and stress, respectively. In the absence of a dietary or stress challenge, the elevated levels of transcription found in recently weaned animals (Lillycrop *et al.* 2005) may have diminished by day 80. For example, in the rat, hepatic PPARα expression decreases after weaning due to the reduction in fat intake (Panadero *et al.* 2000). In addition, PPARα expression is less sensitive to dietary fat intake in adult liver than neonates (Panadero *et al.* 2005). Nevertheless, feeding the PRD diet to F_0 dams induced in the F_1 and F_2 offspring the potential for an exaggerated response to stress or dietary fat.

These findings suggest that the transmission of an altered metabolic phenotype as a result of prenatal nutritional constraint to at least one subsequent generation is the result of an induction of altered epigenetic regulation of gene expression in both the F_1 and F_2 generations. This may also explain the transmission of induced phenotypes from the F_1 to F_2 generation in other experimental systems, such as increased hepatic GR expression and PEPCK activity as a result of exposure of the F_0 dams to dexamethasone in late gestation (Drake *et al.* 2005). If this occurs in humans, as indicated by epidemiological studies (Stein & Lumey, 2000; Painter *et al.* 2005), the findings would suggest that the nutrition of pregnant women has a critical impact not only on the health of their children, but also on subsequent generations.

Acknowledgements

G. C. B. and M. A. H. are supported by the British Heart Foundation, which also funded part of this study.

References

Arnaud P & Feil R (2005) Epigenetic deregulation of genomic imprinting in human disorders and following assisted reproduction. *Birth Defects Res (Part C)* **75**, 81–97.

Benyshek DC, Johnston CS & Martin JF (2006) Glucose metabolism is altered in the adequately-nourished grand-offspring (F(3) generation) of rats malnourished during gestation and perinatal life. *Diabetologia* **49**, 1117–1119.

Bertram CE & Hanson MA (2002) Prenatal programming of postnatal endocrine responses by glucocorticoids. *Reproduction* **124**, 459–467.

Bird A (2002) DNA methylation patterns and epigenetic memory. *Genes Dev* **16**, 6–21.

Burns SP, Desai M, Cohen RD, Hales CN, Iles RA, Germain JP, Going TC & Bailey RA (1997) Gluconeogenesis, glucose handling, and structural changes in livers of the adult offspring of rats partially deprived of protein during pregnancy and lactation. *J Clin Invest* **100**, 1768–1774.

Bustin SA (2000) Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. *J Mol Endocrinol* **25**, 169–193.

Drake AJ, Walker BR & Seckl JR (2005) Intergenerational consequences of fetal programming by in utero exposure to glucocorticoids in rats. *Am J Physiol* **288**, R34–R38.

Gluckman PD & Hanson MA (2004) The developmental origins of the metabolic syndrome. *Trends Endocrinol Metab* **15**, 183–187.

Godfrey KM & Barker DJ (2001) Fetal programming and adult health. *Public Health Nutr* **4**, 611–624.

Harris RG, White E, Phillips ES & Lillycrop KA (2002) The expression of the developmentally regulated proto-oncogene Pax-3 is modulated by N-Myc. *J Biol Chem* **277**, 34815–34825.

Lander-Diner L & Cedar H (2005) Silence of the genes - mechanisms of long-term repression. *Nat Rev Genet* **6**, 648–654.

Lane N, Dean W, Erhardt S, Hajkova P, Surani A, Walter J & Reik W (2003) Resistance of IAPs to methylation reprogramming may provide a mechanism for epigenetic inheritance in the mouse. *Genesis* **35**, 88–93.

Langley SC & Jackson AA (1994) Increased systolic blood pressure in adult rats induced by fetal exposure maternal low protein diets. *Clin Sci (Lond)* **86**, 217–222.

Lillycrop KA, Phillips ES, Jackson AA, Hanson MA & Burdge GC (2005) Dietary protein restriction of pregnant rats induces and folic acid supplementation prevents epigenetic modification of hepatic gene expression in the offspring. *J Nutr* **135**, 1382–1386.

Martin JF, Johnston CS, Han C-H & Benyshek DC (2000) Nutritional origins of insulin resistance: a rat model for diabetes-prone human populations. *J Nutr* **130**, 741–744.

Painter RC, Roseboom TJ & Bleker OP (2005) Prenatal exposure to the Dutch famine and disease in later life: an overview. *Repro Toxicol* **20**, 345–352.

Panadero M, Herrera E & Bocos C (2000) Peroxisome proliferator-activated receptor-alpha expression in rat liver during postnatal development. *Biochimie* **82**, 723–726.

Panadero M, Herrera E & Bocos C (2005) Different sensitivity of PPARalpha gene expression to nutritional changes in liver of suckling and adult rats. *Life Sci* **76**, 1061–1072.

Pembrey ME, Bygren LO, Kaati G, Edvinsson S, Northstone K, Sjostrom M & Golding JALSPAC Study Team (2006) Sex-specific, male-line transgenerational responses in humans. *Eur J Hum Genet* **14**, 159–166.

Stein AD & Lumey LH (2000) The relationship between maternal and offspring birth weights after maternal prenatal famine exposure: the Dutch Famine Birth Cohort Study. *Hum Biol* **72**, 641–654.

Torrens C, Brawley L, Dance CS, Itoh S, Poston L & Hanson MA (2002) First evidence for transgenerational vascular programming in the rat protein restriction model. *J Physiol* **543P**, 41P–42P.

Zambrano E, Martinez-Samayoa PM, Bautista CJ, Deas M, Guillen L, Rodriguez-Gonzalez GL, Guzman C, Larrea F & Nathanielsz PW (2005) Sex differences in transgenerational alterations of growth and metabolism in progeny (F2) of female offspring (F1) of rats fed a low protein diet during pregnancy and lactation. *J Physiol* **566**, 225–236.