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Hepatic phosphoenolpyruvate carboxykinase promoter methylation is contingent on pre- and post-natal diet in female rats

S. P. Hoile[1], E. S. Garratt[1], K. A. Lillycrop[2], N. A. Thomas[1], M. A. Hanson[1] and G. C. Burdge[1]
[1]*University of Southampton, School of Medicine, Southampton SO16 6YD, UK and* [2]*Faculty of Natural Sciences, University of Southampton, Southampton SO16 6YD, UK*

The quality of nutrition during development acting via developmental plasticity and altered methylation of gene promoters is an important determinant of future disease risk(1). Little is known about the relative impact of pre- and post-natal nutrition on such epigenetic effects. Induced changes to the homeostatic capacity of females may affect their ability to provide an optimal developmental environment. We investigated the effect of variation in maternal dietary protein and post-weaning folic acid (FA) intake on the methylation of individual CpG dinucleotides in the hepatic phosphoenolpyruvate carboxykinase (PEPCK) promoter in adult female rats.

The study was carried out in accordance with the Home Office Animals (Scientific Procedures) Act (1986). Virgin female Wistar rats (*n* 9 per dietary group) were fed either protein-sufficient (S, 18% (w/w) casein) or protein-restricted (R, 9% (w/w) casein) diets during pregnancy and AIN93G during lactation(2). Offspring were weaned on PN day 28 onto folic acid adequate (FA, 1 mg/kg feed) or FA-supplemented (FS, 5 mg/kg) diets for 28 d, and then an FA diet containing 18%(w/w) fat until day 84(1). Offspring (*n* 8 females per group) were fasted for 12 h prior to sample collection. Hepatic PEPCK mRNA expression was measured by real-time RTPCR(2). PEPCK promoter methylation was determined by pyrosequencing(3). Statistical analysis was by a general linear model (GLM) with maternal (M) and post-weaning (P) diets as fixed factors. The numerical designation of CpGs refers to their location (bp) from the transcription start site.

	Expression (log ΔΔct)		CpG methylation (%)											
			-606		-508		-440		-129		-90		-81	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
S/FA	0.8[a]	0.2	37.7[a]	3.2	22.5[a]	2.0	25.3[a]	2.0	26.4[a]	4.8	20.1[a]	1.5	6.0[a]	0.8
R/FA	1.3[c]	0.1	27.7[b]	3.4	26.8[b]	2.2	34.1[b]	5.0	18.7[b]	3.9	14.1[b]	1.7	14.3[b]	1.1
S/FS	0.8[ab]	0.2	29.2[b]	1.8	28.1[b]	1.8	38.9[b]	3.2	18.1[b]	2.5	15.9[b]	2.1	16.2[b]	1.9
R/FS	1.0[b]	0.1	30.6[b]	2.4	25.4[ab]	3.0	31.7[ab]	6.6	17.6[b]	2.2	14.1[b]	1.9	14.9[b]	2.0
							GLM (*P*)							
M	<0.0001		0.001		0.473		0.594		0.003		<0.0001		<0.0001	
P	NS		0.03		0.01		0.001		0.001		0.004		<0.0001	
M*P	<0.0001		<0.0001		<0.0001		<0.0001		0.008		0.003		<0.0001	

Different superscripts indicate significantly different values ($P<0.05$) by Bonferroni's *post-hoc* test; NS, not significant.

Post-weaning FA supplementation induced differential changes in PEPCK mRNA expression and on the methylation of individual CpGs contingent on maternal protein intake. The FS diet altered the methylation of CpGs in offspring of S dams, but not R dams. The changes induced in DNA methylation by FS diets were directionally opposite for CpGs-606, -129 and -90 compared to CpGs-508, -440 and -81. CpGs-606 ($r = -0.509$, $P = 0.003$), located proximal to a heat shock factor response element and -90 ($r = -0.524$, $P = 0.002$), located within a cAMP response element, were correlated negatively with PEPCK expression. These results show that maternal protein intake and post-weaning FA intake interact to induce persistent changes in hepatic PEPCK mRNA expression, which reflect altered methylation of specific CpG dinucleotides. These findings suggest that nutrition throughout early life may have long-term effects on the metabolism of females, which may affect their ability to nourish their offspring.

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