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Folic acid supplementation in the juvenile-pubertal period in rats leads to persistent tissue-specific changes in the expression and methylation of the tumour suppressor gene BRCA1

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Micronutrient intake has been shown to influence cancer risk at least in part by modifying the epigenome⁽¹⁾. However, the effects of folic acid intake during critical phases of development on future cancer risk are not known. We investigated whether variations in folic acid intake in the juvenile-pubertal (JP) period in rats induced lasting changes in the expression of the tumour suppressor gene BRCA1, which plays a key role in maintaining genome integrity⁽²⁾.

The study was carried out in accordance with the Home Office Animals (Scientific Procedures) Act (1986). Female Wistar rats were fed a modified AIN93M semi-purified diet containing either 1 mg/kg feed folic acid (folate adequate (AF)) or 5 mg/kg feed folic acid (folic acid supplemented, FS) for 28 d from PN28 to PN56. After this period all rats were given AIN93M supplemented with 1mg/kg folic acid. Tissues were collected on PN84. mRNA expression measured by real time RT PCR⁽³⁾ and BRCA1 promoter methylation was measured by sodium bisulphite pyrosequencing⁽⁴⁾.

JP folic acid supplementation induced tissue specific changes in BRCA1 mRNA expression that persisted beyond the period of supplementation. BRCA1 mRNA expression was significantly increased in adipose tissue, but decreased in muscle. Altered BRCA1 expression was accompanied by tissue-specific changes in the methylation of specific CpGs in its promoter. In adipose tissue, folic acid supplementation increased the methylation of CpGs -15, +14, +33 and +47, while in muscle there was an increase in methylation at CpG+33.

	Control AF AF		Control FS		<i>t</i> -test (<i>p</i>)
	Mean	SD	Mean	SD	
BRCA1 mRNA expression ($\Delta\Delta Ct$)					
Adipose	0.86	0.21	1.42	0.62	0.02
Muscle	1.15	0.26	0.45	0.27	0.0001
BRCA1 DNA methylation (% methylation)					
Adipose CpG -15	3.4	0.51	5.83	0.75	0.0001
Adipose CpG +14	2.8	0.42	3.83	0.96	0.01
Adipose CpG +33	8.4	1.43	11.0	3.54	0.05
Adipose CpG +47	3.4	0.84	8.00	2.65	0.01
Muscle CpG -15	4.00	1.85	3.40	2.25	NS
Muscle CpG +14	2.57	1.27	1.57	0.1.51	NS
Muscle CpG +33	8.76	0.88	11.1	2.79	0.03
Muscle CpG +47	4.12	0.64	5.00	1.59	NS

mRNA and methylation levels were compared by Student's unpaired *t*-test. *n* 8 per treatment. CpG locations (bp) are relative to the transcription start site.

These data show that FA supplementation induced tissue-specific changes in the expression and methylation of BRCA1 which persist beyond the period of feeding the modified diet. This suggests that altered epigenetic regulation of BRCA1 is one possible mechanism by which folic supplementation, at least during the JP period, may modify cancer risk. Differences in the effect of folic acid between tissues may have implications for understanding variations in the effectiveness of folic acid supplementation in cancer prevention between disease types and clinical trials⁽⁵⁾.

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