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Effects of folate depletion *in utero* and a high fat diet post-weaning on DNA methylation in the adult mouse small intestine

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Increasing evidence from animal studies shows that nutritional insults during development can lead to adverse health in later life. Altered patterns of DNA methylation is a potential mechanism for this programming effect because when DNA is methylated, gene expression is usually repressed. Folate is a major methyl donor so folate depletion in early life may affect DNA methylation and gene expression, leading to increased risk of disease throughout life [(1,2)]. We have reported that maternal folate depletion influences methylation in the fetal mouse gut [(3)]. Here we investigated the effects on adult offspring of maternal folate depletion and/or high dietary fat intake post-weaning on gene-specific methylation in the mouse proximal small intestine (SI).

Female C57BL/6J mice were randomly assigned to folate-adequate (FA, 2 mg/kg) or folate-depleted (FD, 0.4 mg/kg) diets 4 weeks prior to mating and assigned diets were maintained during pregnancy and lactation. At weaning, offspring were randomised to a low fat (LF, 5%) or a high fat (HF, 20%) diet. Allocated diets were continued for 6 months when proximal SI samples were collected and snap frozen. DNA was extracted and gene-specific DNA methylation was quantified at ten loci within 6 genes (*Esr1*, *Igf2*-DMR1, *Slc39a4*-CGI1 & -CGI2, *p16*, *Obfc2a*-amp1, -amp2 & -amp3, and *Ppm1k*-amp1 & -amp2) by Pyrosequencing.

There were no significant effects of maternal folate supply on methylation at any of the loci investigated ($n = 24$ for FA, $n = 24$ for FD diet, ANOVA, $p > 0.05$). However, as summarised in the table, methylation at all 9 CpGs and overall mean methylation across all nine CpGs in *Slc39a4*-CGI1 was significantly lower in DNA from mice fed the HF diet ($n = 24$ for LF, $n = 24$ for HF diet, ANOVA, $p < 0.05$). Conversely, methylation at CpGs 3, 4, 5 and mean methylation across all nine CpGs at *Obfc2a*-amp1, CpGs 1, 4, 6, and overall mean across all nine CpGs at *Obfc2a*-amp2 were higher in the HF group. Similarly, higher methylation was found at CpGs 2, 4 and mean methylation across all five CpGs in *p16*; CpGs 1, 2, 4 and overall mean across all four CpGs in *Ppm1k*-amp1, and CpGs 2, 5, 7 and mean methylation across all seven CpGs in *Ppm1k*-amp2 in the HF group (ANOVA, $p < 0.05$).

Locus	*Esr1*	*Igf2*-DMR1	*Slc39a4*-CGI1	-CGI2	*p16*	*Obfc2a*-amp1	-amp2	-amp3	*Ppm1k*-amp1	-amp2
FD diet*	No	No	No	No	No	No	No	No	No	No
HF diet*	No	No	Yes↓	No	Yes↑	Yes↑	Yes↑	No	Yes↑	Yes↑

*Yes = significant ($p < 0.05$) effects observed, No = no significant effects observed on methylation in response to FD or HF diet within each locus, ↑ = higher % methylation & ↓ = lower % methylation observed in the HF diet group.

In conclusion, feeding a high fat diet from weaning influenced methylation at *Slc39a4*-CGI1, *Obfc2a*-amp1, -amp2, *p16*, *Ppm1k*-amp1 and -amp2 in adult mouse proximal SI. This effect was locus and CpG specific.

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