Short Communication

Body composition at age 9 years, maternal folate intake during pregnancy and methyltetrahydrofolate reductase (MTHFR) C677T genotype

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Nutrition during pregnancy and in early life may influence developmental plasticity and alter susceptibility to obesity and adult disease. One mechanism by which this could occur is through epigenetic changes, such as changes in methylation levels, which modify gene expression patterns. Folate intake during pregnancy, as well as maternal methyltetrahydrofolate reductase (MTHFR) C677T genotype, influences the availability of methyl donors for methylation during gestation and therefore may be associated with offspring body composition in childhood. We looked at associations between maternal folic acid supplementation at 18 and 32 weeks of pregnancy, folate intake in the diet (from self-reported FFQ) at 32 weeks of pregnancy and offspring body composition at age 9 years among 5783 children from a population-based birth cohort study in the UK. We also looked at maternal and offspring’s MTHFR C677T genotype in relation to offspring body composition. We found no evidence to support the hypothesis that intra-uterine exposure to folate influences childhood body composition.

Folate: Maternal nutrition: Body composition: Methyltetrahydrofolate reductase C677T genotype

It has been postulated that nutrition during pregnancy and in early life influences developmental plasticity and alters susceptibility to obesity and adult disease11. Methylation of cytosines in cytosine-guanine (CpG) dinucleotides is an important epigenetic modification which affects gene expression and thus cellular function. Environmental factors which modify DNA methylation levels at critical time points, such as maternal folate intake during pregnancy, may therefore influence adult phenotype50. In support of the hypothesis that epigenetic changes are linked to adult obesity is the observation that in humans several genes have been shown to exhibit changes in expression which correlate closely with BMI and/or waist:hip ratio52.

Methyl groups required for methylation are synthesised de novo or are supplied in the diet primarily from methionine and choline, but also from folate. In animal models dietary methyl supplementation of mothers during pregnancy increased DNA methylation and resulted in healthier longer-lived mice42. However, in humans, observational studies of folate intake during pregnancy and offspring body composition are subject to confounding, and randomised controlled trials carried out to date have not reported on offspring body composition. Mendelian randomisation, the use of genetic variants as a surrogate for measuring exposures in epidemiological studies, is a promising alternative to traditional observation epidemiology, because associations between genotypes and disease are not usually subject to confounding by lifestyle factors5. So, for example, an association between body composition and a gene that affects the biological availability of folate is not subject to confounding by other dietary intakes.

Methylation is catalysed by methyltransferases that use the universal methyl donor S-adenosyl-l-methionine. The enzyme methyltetrahydrofolate reductase (MTHFR) metabolises folate and in doing so produces a methyl donor for the synthesis of methionine from homocysteine, and the precursor of S-adenosyl-l-methionine. A common polymorphism (C677T) in the MTHFR gene, which is associated with MTHFR activity and circulating folate and homocysteine levels, has been identified10. MTHFR C677T TT homozygotes have been shown to exhibit a diminished level of DNA methylation compared with CC homozygotes7. Since MTHFR C677T genotype mediates the effect of dietary folate on methylation levels, it is possible to use MTHFR genotype as a surrogate for measuring folate intake, in order to study the effect of folate on body composition. We looked

Abbreviations: ALSPAC, Avon Longitudinal Study of Parents And Children; MTHFR, methyltetrahydrofolate reductase.

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at the association between folate intake during pregnancy, maternal and offspring’s MTHFR C677T genotype and body composition of the children at age 9 years in the Avon Longitudinal Study of Parents And Children (ALSPAC).

Methods

Study population

ALSPAC is a population-based prospective study investigating factors that affect the health and development of children and their parents. The study methods are described elsewhere(8). Pregnant women living in Bristol, England who had an expected date of delivery between April 1991 and December 1992 were eligible. A total of 14,663 women enrolled in the study of which 14,273 (97.3%) were singleton pregnancies. Extensive data have been collected from the children and parents from pregnancy onwards by questionnaire, abstraction from medical notes, record linkage and by attendance at research clinics.

Measurement of folate intake

Dietary folate intake was estimated from a self-administered FFQ completed by mothers at 32 weeks’ gestation, and includes natural food folate and folic acid-fortified foods. The FFQ covered seventy-five types of foods and drinks commonly consumed in Britain. Nutrient intakes of the pregnant women in this survey compared very closely with the reported nutrient intakes for all women aged 16–64 years in the 1987 National Dietary and Nutritional Survey(9). British food tables were used to obtain folate contents of standard portions of each of these dietary items according to the UK Ministry for Agriculture, Fisheries and Food(10), and intakes of folate were estimated using their frequency of consumption. In addition, information on the use of folic acid supplements was collected from mothers at 18 weeks’ gestation based on whether these had ever been taken during pregnancy, and at 32 weeks’ gestation based on whether these had been taken within the previous 3 months. Some women who answered ‘no’ to the questions on folic acid supplementation stated that they were taking (unspecified) vitamins; in case these vitamins included folic acid we performed the analysis with and without inclusion of those women who reported taking unspecified supplements.

Measurement of body composition

Children were invited to attend a special clinic at about age 9 years to undergo a variety of psychometric and physical assessments including a dual-energy X-ray absorptiometry scan. Total body weight, fat mass and lean mass were measured (g).

Measurement of confounders

The following variables can influence folate levels and body composition and thus were included in the models as confounders; mother’s social class (manual or non-manual, using the UK Registrar General’s Classification to define manual as: IIM, skilled manual; IV, partly skilled manual; V, unskilled manual) was determined by questionnaire at baseline. Maternal energy intake (from FFQ) and educational level (less than age 18 years or education to age 18 years and above) were all determined by questionnaire at 32 weeks’ gestation. Child’s sex and age (in weeks) when the body composition measurements were taken were also included in the analysis.

Laboratory methods

DNA was extracted by a salting-out procedure(11). Genotyping was undertaken by KBioscience Ltd (Hoddesdon, Herts, UK; www.kbioscience.co.uk), who use their own form of competitive allelic-specific PCR system (KASPar) and Taqman™, for single nucleotide polymorphism analysis.

Statistical methods

Total body weight, fat mass and trunk fat mass were log-transformed to approximate to normal distributions. Separate linear regression models were used to assess associations between body composition and folate intake measured by FFQ, folate supplementation at 18 weeks’ and at 32 weeks’ gestation and MTHFR C677T genotype. Adjustments were made as described in Table 1. A test for transmission distortion of the MTHFR C677T genotype from mother to child was carried out by assuming the same distribution of genotype frequencies among fathers as mothers and then calculating the expected distribution of CC, CT and TT genotypes among children born to CT mothers. A χ² test was performed to test whether the observed genotypes of children born to CT mothers differed from expected. Assumptions of Hardy–Weinberg equilibrium were formally tested in mothers and children separately using a likelihood ratio test. All data were analysed using STATA 8.0 (Stata Corp. LP, College Station, TX, USA).

Results

We included only children of confirmed white ethnicity (n 11 233) whose mothers had provided data on folate intake at 18 weeks and 32 weeks of pregnancy (n 10 511) in the present study. A further 4629 (44%) of these children did not have complete data for the dual-energy X-ray absorptiometry whole-body scan at age 9 years, and ninety-nine children (1.7%) were excluded due to missing data on maternal social class or educational level, so this analysis is based on data from 5783 children. A total of 11·0 % of their mothers reported taking folic acid supplements at 18 weeks’ gestation; this figure had increased to 28·8 % by 32 weeks’ gestation. If we include women taking unspecified vitamins in these groups the proportions taking supplements increases to 24·8 % at 18 weeks and 36·1% at 32 weeks of pregnancy. Folate intake from the diet at 32 weeks of pregnancy ranged from 64·6 to 581·5 μg/d with a mean of 255·6 μg/d. Among the children the mean total body weight was 34·7 (sd 7·29) kg, mean fat mass was 8·51 (sd 5·06) kg, lean mass was 24·5 (sd 3·17) kg and trunk fat mass was 3·42 (sd 2·46) kg.

Eight mother–child pairs were excluded from the analysis due to discordance between their genotypes. Genotype data were available on 3062 mother–offspring pairs.
Table 1. Linear regression models of folate intake and body composition adjusted for maternal education and mother’s social class
(β Coefficients and 95% confidence intervals)

<table>
<thead>
<tr>
<th>Supplement use</th>
<th>Log-weight (kg)*</th>
<th>Log-total fat (kg)*</th>
<th>Total lean (kg)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>95% CI</td>
<td>P</td>
</tr>
<tr>
<td>----------------</td>
<td>-----------------</td>
<td>---------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Mean</td>
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<td>7·29</td>
<td></td>
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<tr>
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<td></td>
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<td>18-week supplements</td>
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<td>0·003</td>
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<tr>
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<tr>
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<tr>
<td>18-week supplements</td>
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<td>4351</td>
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<td>0·002</td>
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<td>0·002</td>
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<tr>
<td>Supplements at both time points</td>
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<td>32-week dietary intake (μg/d)‡</td>
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<tr>
<td>Child’s MTHFR C677T genotype§</td>
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<td></td>
</tr>
<tr>
<td>TT (n 1365)</td>
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<tr>
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<tr>
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</tr>
<tr>
<td>TT (n 337)</td>
<td>Reference</td>
<td>−0·014</td>
<td>−0·006, 0·017</td>
</tr>
</tbody>
</table>

MTHFR, methyltetrahydrofolate reductase.
* Adjusted for age, sex, height, height squared, maternal education and mother’s social class.
† Including women who answered ‘yes’ to taking vitamins, but ‘no’ to a specific question on folic acid.
‡ Adjusted for 32-week daily energy intake plus variables outlined above. Mean dietary intake 255·6 (sd 69·7) μg/d.
§ Genetic analyses were not adjusted for maternal education and mother’s social class, but were adjusted for age, sex, height and height squared, plus total fat (for log-trunk fat analysis).
|| Analysis of maternal MTHFR genotype was further adjusted by child’s genotype.
Genotype frequencies in the mothers were as follows: CC, 43.3%; CT, 45.7%; TT, 11.0%. Genotype frequencies in the children: CC, 44.6%; CT, 44.8%; TT, 10.8%. The MTHFR C677T genotype was found to be in Hardy–Weinberg equilibrium in this cohort of mothers ($P=0.26$) and in the children ($P=0.44$). There was no evidence of transmission distortion of genotype from mother to child ($P=0.13$).

Table 1 shows the associations between folate variables, MTHFR genotype and child’s weight, total fat mass and lean mass at age 9 years. Folic acid supplementation at 18 weeks and 32 weeks of pregnancy were not found to be associated with body composition among the offspring at 9 years of age. There was some suggestion ($P=0.04$) that maternal dietary intake (estimated by FFQ) of folate at 32 weeks of pregnancy may be related to lean body mass. Neither maternal nor child’s MTHFR C677T genotype was associated with body composition of the child at age 9 years.

Discussion

We found no associations between the MTHFR C677T polymorphism and body composition in the present study. In addition, maternal folic acid supplementation during pregnancy did not influence body composition; the weak association between lean body mass and mothers’ self-reported dietary intake at 32 weeks of pregnancy is likely to have arisen from multiple testing, or confounding by other dietary or lifestyle factors. One further limitation is that in the present study we determined folate intake from foods by using a FFQ. It has been shown that FFQ are subject to substantial measurement error, which is likely to be nondifferential and therefore likely to lead to an underestimation of any effect if present. However, since the MTHFR C677T genotype provides an unconfounded measure of folate levels, our lack of an association between body composition and this genotype argues against an association between exposure to folate during pregnancy and childhood obesity. A recent family study(12) found associations between polymorphisms in the MTHFR gene and lean body mass among adults; unfortunately that study did not look at the C677T variant due to the fact that it was not in Hardy–Weinberg equilibrium among parents, and given the difference in age of the subjects, is not directly comparable with the present study. However, as the C677T variant has a functional effect on the resulting protein, we would expect to find an association with this polymorphism if this gene is involved in lean body mass from an early age. We therefore conclude that the MTHFR gene is not associated with lean body mass, fat mass and trunk fat among 9-year-old children, and as this gene influences the biological availability of folate, this lack of association argues against a role for folate in determining body composition at age 9 years. However, whether folate plays a role in body composition at different ages requires further clarification.

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The authors have no conflicts of interest in relation to this paper.

References