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ORIGINAL ARTICLE

Folic acid supplementation during pregnancy may protect against depression 21 months after pregnancy, an effect modified by MTHFR C677T genotype

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Background/Objectives: As low folate status has been implicated in depression, high folate intake, in the form of supplements, during pregnancy might offer protection against depression during pregnancy and postpartum.
Subjects/Methods: We examined the association between change in self-reported depressive symptoms (Edinburgh Postnatal Depression Scale) at different timepoints during and following pregnancy and self-reported folic acid supplementation during pregnancy in a prospective cohort of 6809 pregnant women. We also tested whether there was a main effect of methylenetetrahydrofolate reductase (*MTHFR)* C677T genotype (which influences folate metabolism and intracellular levels of folate metabolites and homocysteine) on change in depression scores, and carried out our analysis of folic acid supplementation and depression stratifying by genotype.
Results: We found no strong evidence that folic acid supplementation reduced the risk of depression during pregnancy and up to 8 months after pregnancy. However, we did find evidence to suggest that folic acid supplements during pregnancy protected against depression 21 months postpartum, and that this effect was more pronounced in those with the MTHFR C677T TT genotype (change in depression score from 8 months to 21 months postpartum among TT individuals was 0.66 (95% CI = 0.31–1.01) among those not taking supplements, compared with −1.02 (95% CI = −2.22–0.18) among those taking supplements at 18 weeks pregnancy, $P^{\text{difference}} = 0.01$).
Conclusions: Low folate is unlikely to be an important risk factor for depression during pregnancy and for postpartum depression, but may be a risk factor for depression outside of pregnancy, especially among women with the MTHFR C677T TT genotype.
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Introduction

Several studies have shown that low folate levels are associated with depression in the general population (Bjelland *et al*., 2003; Paul *et al*., 2004; Lewis *et al*., 2006; Morris *et al*., 2006) and some clinical trials also show that folate may have a therapeutic effect on depression, either alone or in combination with antidepressants (Taylor *et al*., 2004). A number of biological mechanisms have been postulated to explain how folate may affect central nervous system pathways that regulate mood. These include folate participation in serotonin metabolic pathways, decreasing homocysteine levels, which seems to have an excitotoxic effect on brain receptors, and DNA methylation—a process essential for gene regulation (Paul *et al*., 2004).

During pregnancy, the demand for folate is increased. Without adequate supplementation, concentrations of maternal serum folate decrease gradually from the fifth month of pregnancy onwards, and remain low for several months after childbirth, something which is further accentuated with shorter inter-pregnancy intervals (Smits and

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Essed, 2001). A short inter-pregnancy interval, which allows less time for restoration of folate levels between pregnancies, has been shown to be strongly associated with postpartum depression in a small observational study (Gurel and Gurel, 2000). Thus, if low folate levels contribute to causing depression, the increased likelihood of lower folate levels during the maternal perinatal period could explain the common occurrence of depression at this period. However, there is a paucity of studies of this association at this critical period. We found only three studies examining the association between folate levels and postpartum depression with contrasting results. Two of these studies were extremely small; Rouillon *et al*. (1992) ($n=25$) found no relationship between measured folate levels on the third day after delivery and postpartum depression, whereas, Abou-Saleh *et al*. (1999) ($n=5$) found strong evidence ($P<0.01$) of an association between low folate levels and depression 7 days after delivery. To our knowledge, there has been only one study investigating the relationship between dietary folate intake during pregnancy and postnatal depression in a population-based sample. This prospective cohort study of 865 pregnant women found no evidence of an association; however, the number of women classified as depressed was relatively small (14%) considering that a threshold of 9 or more on the Edinburgh Postnatal Depression Scale (EPDS) was used, and folic acid supplementation was not included in the analysis (Miyake *et al*., 2006). Cho *et al*. (2008) investigated whether intake of prenatal multivitamins containing folic acid was associated with decreased rates of depression among 1277 pregnant women. The overall prevalence of depression was 9.4% among those taking multivitamins and 6.9% among those not taking multivitamins, but the study did not show strong evidence of an effect ($P=0.11$).

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 homocysteine levels, has been identified. TT homozygotes have higher homocysteine compared with CC homozygotes (Frosst *et al*., 1995), although these differences in levels by genotype are diminished with folic acid supplementation (Yang *et al*., 2008). If low folate levels are an important factor in postpartum depression, the MTHFR C677T genotype should be associated with increased rates of depression, and the protective effect of supplementation should be greatest among those with MTHFR C677T TT genotype.

As increasing folate intake before conception and for the first 3 months of pregnancy reduces the risk of neural tube defects (MRC Vitamin Study Research Group, 1991), women planning or in the early stages of pregnancy are advised to take folic acid supplements until the end of the first trimester. Many women continue to take large amounts of folic acid throughout pregnancy, but the implications of this, including a potential beneficial effect on preventing pregnancy or postnatal depression, have not been adequately studied.

We examined the association between folic acid supplementation in pregnancy and MTHFR C677T genotype, and changes in depression score assessed by the EPDS between 18 and 32 weeks antenatally, between 32 pregnancy weeks and 8 weeks postpartum, between 8 weeks and 8 months postpartum, and between 8 and 21 months postpartum in a large prospective study, in order to test the hypothesis that high folate intake during pregnancy protects against depression.

Participants and methods

The Avon Longitudinal Study of Parents And Children (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 in detail elsewhere (http://www.alspac.bris.ac.uk and Golding *et al*., 2001). In brief, pregnant women living in Bristol, England who had an expected date of delivery between April 1991 and December 1992 were eligible. Of the 14 541 pregnancies enroled in the study, 14 273 were singleton pregnancies and 335 pregnancies were excluded because these were second or third pregnancies of mothers already in the study. Of the 13 928 remaining women, 13 299 had offspring surviving to 12 months. Extensive data have been collected from the mothers from pregnancy onwards by questionnaire, abstraction from medical notes, record linkage, and by attendance at research clinics. Ethical approval for the study was obtained from the ALSPAC Law and Ethics Committee and the Local Research Ethics Committees.

Measurement of folate intake

Information on use of folic acid supplements was collected from mothers (by self-report) 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 last 3 months. This questionnaire also included an open-ended question on supplement use; in some cases, women did not report taking folic acid when asked specifically but later recording either taking folic acid alone or folic acid with iron as free text. All these women were included in the group taking folic acid.

Measurement of depressive symptoms

Women completed the EPDS by questionnaire at 18 and 32 weeks of pregnancy and at 8 weeks, 8 months and 21 months postpartum. This questionnaire has 10 questions each with a maximum score of 3 points, with a higher score indicating a greater level of depression. Rather than using an arbitrary

cut-off point for cases of depression, something which is highly contentious, we used a continuous EPDS score, which also improved the statistical power to detect an association. Our interest was in the change in EPDS score between successive assessments because we wanted to capture the effects of folic acid supplementation on depression score rather than the effect of depressive symptoms on taking folic acid supplements. In addition, we wanted to study the effect of folic acid supplementation during specific periods; hence, using differences allows us to adjust for the starting scores in each period. Thus, we calculated the difference in EPDS scores between 18 and 32 weeks of pregnancy, between 32 weeks pregnancy and 8 weeks postpartum, between 8 weeks and 8 months postpartum, and between 8 and 21 months postpartum. Negative differences were equivalent to a decrease in depressive symptoms and positive differences in EPDS scores indicated greater levels of depressive symptoms.

Measurement of confounders

There are many potential confounders for the association between folic acid supplementation and depression score, and there is likely to be some bias and measurement error when assessing these confounders. However, potential confounders between folic acid intake and depression score are unlikely to be associated with MTHFR C677T genotype (we tested this assumption by looking at the association between genotype and several maternal characteristics, obtained by questionnaire completed by the mother during pregnancy). Thus, although we show results adjusted for a minimal set of confounders (mother's age, pre-pregnancy BMI, educational level and parity), rather than trying to adjust for all potential confounders in our analysis, and further reducing our dataset due to missingness, we used the association with genotype as a way of confirming that any associations with folate status were real. The MTHFR C677T genotype has been found to be associated with folate levels and to explain around 53% of the genetic variance in homocysteine levels (Bathum *et al*., 2007) with the difference in homocysteine levels between the CC and TT genotypes being approximately equal to the difference between those taking and not taking folic acid supplements. In addition, we determined whether any effect of folic acid supplementation was more pronounced among TT homozygotes, as a way of determining whether the effect was biological or simply due to confounding. The difference in serum folate and homocysteine concentrations by genotype is known to decrease as intake of folic acid increases; this is because the largest change in levels occurs in the MTHFR C677T TT genotype (Yang *et al*., 2008).

Laboratory methods

DNA was extracted using the salting out procedure (Miller *et al*., 1988). Genotyping was undertaken by KBioscience Ltd (http://www.kbioscience.co.uk), who use their own form of competitive allele-specific PCR system (KASPar) and Taqman, for SNP analysis.

Statistical methods

We tested for associations between potential confounders during pregnancy and genotype using analysis of variance for continuous variables and χ^2 for categorical variables. Although the EPDS scores were negatively skewed, the differences in scores were symmetric and the distribution normal; hence, we used paired *t*-tests to determine whether differences in depression scores between the different time points were associated with folic acid supplementation and MTHFR C677T genotype. We used linear regression for multivariate analyses to adjust for confounders. We also carried out analyses between depression and folic acid supplementation with stratification by MTHFR C677T genotype. Finally, we looked for interactions between genotype and supplement intake using linear regression models with an interaction term; in this analysis, the TT genotype was compared with the CC and CT genotype combined. Assumptions of Hardy–Weinberg Equilibrium were formally tested using a likelihood ratio test. All data were analysed using Stata 8.0 (Stata Corporation, College Station, TX, USA).

Results

Information on folic acid supplementation at either 18 weeks or 32 weeks of pregnancy was available for 12 718 (95.6%) of eligible women. The MTHFR C677T was successfully genotyped in 7587 (59.7%) of these women (the genotyping call rate for this particular polymorphism was 96.8%). Of these 7587 women, 284 (3.7%) who were either of unknown or non-white ethnicity were excluded from analysis. A further 494 (6.8%) women did not complete the Edinburgh postnatal depression score at 18 weeks of pregnancy and so were excluded. This analysis is based on the remaining 6809 women.

At 18 weeks gestation, only 9.36% of women reported having taken folate supplements during their pregnancy, this figure had increased to 27.8% by 32 weeks gestation. The distribution of depression scores and changes in scores among mothers during and following pregnancy in ALSPAC has previously been reported (18), and are shown for this sample in Table 1 (defined using a EPDS cut-off >12). Despite being based on a smaller sample of the main cohort, our results correspond very well with those of Evans *et al.* (2001). Our mean scores based on the EPDS during and after pregnancy and mean changes in scores between time points are also shown in Table 1. We found that scores decreased after pregnancy, suggesting lower levels of depressive symptomatology and were at their lowest 8 months postpartum, but increased slightly again by 21 months postpartum.

In Table 2, we have shown the distribution of several potential confounding factors by genotype, which illustrates that the association between MTHFR genotypes is unlikely to be confounded by maternal age, parity, inter-pregnancy interval, alcohol intake during pregnancy, smoking, social class, educational level and folic acid supplementation.

The results of the analysis of folic acid intake and depression are shown in Table 3. Reported folic acid supplementation at 18 and 32 weeks of pregnancy and MTHFR C677T genotype were not associated with changes in depression score, as measured by the EPDS between 18 and 32 weeks of pregnancy, 32 weeks of pregnancy and 8 weeks postpartum, or between 8 weeks and 8 months postpartum. However, women taking folic acid supplementation at 32 weeks of pregnancy experienced on average a smaller increase in depression score between 8 months and 21 months postpartum than those not taking supplements. We analysed the above with adjustment for key confounders of mother's age, BMI, parity and educational level; this adjustment did not notably change our results.

In addition, the MTHFR C677T CT and TT genotypes were associated with greater increases in EPDS score between 8 month and 21 months post pregnancy than the CC genotype. In the analysis stratified by genotype, we found that the biggest effect of folic acid supplementation on change in depression score was seen among TT homozygotes (P-interaction = 0.02, Table 4). Among this genotype group, women who were taking folic acid supplements were less depressed (according to EPDS score) at 21 months postpartum than at 8 months postpartum, which is contrary to all other genotypes and to women who did not report taking folic acid at 32 weeks of pregnancy. We also stratified by genotype and looked at the association between folic acid supplementation and change in depression score at other time points, but found no association (data not shown).

Table 1 Mean Edinburgh postnatal depression scores and changes in scores at different time points during and after pregnancy

Women classified as depressed according to an EPDS score >12	*%*	N	*Total*
18 weeks pregnancy	12.7	865	6809
32 weeks pregnancy	14.0	908	6482
8 weeks postpartum	9.23	574	6218
8 months postpartum	8.01	481	6005
21 months postpartum	9.36	520	5557
Edinburgh postnatal depression score	*Mean*	*s.d.*	N
18 weeks pregnancy	6.84	4.78	6809
32 weeks pregnancy	6.83	4.95	6482
8 weeks postpartum	5.88	4.72	6218
8 months postpartum	5.23	4.58	6005
21 months postpartum	5.59	4.71	5557
Change in score between time points			
18–32 weeks pregnancy	0.06	4.15	6482
32 weeks pregnancy to 8 weeks postpartum	−0.89	4.49	6074
8 weeks postpartum to 8 months postpartum	−0.64	4.04	5842
8 months postpartum to 21 months postpartum	0.41	4.03	5360

Abbreviation: EPDS, Edinburgh postnatal depression scores.

Discussion

To our knowledge, this is the first longitudinal study of folic acid supplementation use and perinatal depression ever carried out. We had repeated measures of depression score covering pregnancy and up to 21 months postpartum, and this allowed us to test for changes in depression score, which may be associated with folic acid supplementation. In addition, we were able to look at the association between the MTHFR C677T polymorphism and postnatal depression, which, as far as we are aware, has so far not been reported in previous studies. We hypothesised that folic acid supplementation use during pregnancy may protect against increases in depressive symptoms. We found no strong

Table 2 Distribution of potential confounding factors by MTHFR C677T genotype

Maternal characteristics	*MTHFR C677T genotype*			P-*value for difference*
	CC (N = 3035)	*CT (N = 3017)*	*TT (N = 757)*	
Mean age	28.3 ± 4.67	28.4 ± 4.73	28.3 ± 4.72	0.87
Parity > 2	6.01%	5.53%	6.52 %	0.51
Short inter-pregnancy interval (<12 months)	24.7%	24.1%	26.2%	0.69
Mean alcohol intake	1.70 ± 3.40	1.79 ± 3.91	1.48 ± 3.07	0.27
Manual social class (n = total)	50.1%	49.3%	47.1%	0.35
Education < A-level	65.5%	63.4%	62.3%	0.14
Smoked pre-pregnancy	32.8%	31.1%	34.0%	0.20
Pre-pregnancy BMI	23.0 ± 3.94	23.0 ± 3.76	23.0 ± 3.97	0.93
Folate intake (FFQ)	249.1 ± 71.3	248.8 ± 71.4	250.6 ± 73.7	0.83
Folate supplements at 18 weeks of pregnancy	9.6%	9.6%	7.5%	0.18
Folate supplements at 32 weeks of pregnancy	28.4%	27.6%	25.9%	0.40
Folate supplements at both 18 and 32 weeks	7.3%	7.3%	5.5%	0.19

Abbreviations: BMI, body mass index; FFQ, food frequency questionnaire.

Table 3 Analysis of folic acid supplement use, MTHFR C677T genotype and change in Edinburgh postnatal depression

	18–32 weeks antenatal	*32 weeks antenatal to 8 weeks postnatal*	*8 weeks to 8 months postnatal*	*8 months to 21 months postnatal*
Supplements at 18 weeks				
Yes	0.08 (0.17) $n=611$	−0.80 (0.18) $n=577$	−0.52 (0.17) $n=565$	0.14 (0.18) $n=517$
No	0.06 (0.05) $n=5828$	−0.89 (0.06) $n=5467$	−0.65 (0.06) $n=5251$	0.43 (0.06) $n=4819$
	$P=0.91$, $P^{adjusted}=0.97$	$P=0.62$, $P^{adjusted}=0.73$	$P=0.47$, $P^{adjusted}=0.72$	$P=0.11$, $P^{adjusted}=0.38$
Supplements at 32 weeks				
Yes	0.13 (0.10) $n=1795$	−0.82 (0.11) $n=1724$	−0.54 (0.10) $n=1652$	0.24 (0.10) $n=1528$
No	0.04 (0.06) $n=4666$	−0.91 (0.07) $n=4332$	−0.66 (0.06) $n=4081$	0.47 (0.06) $n=3749$
	$P=0.44$, $P^{adjusted}=0.41$	$P=0.47$, $P^{adjusted}=0.93$	$P=0.32$, $P^{adjusted}=0.62$	$P=0.06$, $P^{adjusted}=0.08$
Supplements at both 18 and 32 weeks				
Yes	0.21 (0.19) $n=457$	−0.70 (0.21) $n=435$	−0.53 (0.20) $n=424$	0.19 (0.21) $n=389$
No	0.05 (0.05) $n=5961$	−0.90 (0.06) $n=5591$	−0.63 (0.05) $n=5283$	0.41 (0.06) $n=4864$
	$P=0.42$, $P^{adjusted}=0.41$	$P=0.39$, $P^{adjusted}=0.94$	$P=0.61$, $P^{adjusted}=0.97$	$P=0.29$, $P^{adjusted}=0.72$
MTHFR genotype[a]				
CC	0.10 (0.08) $n=2885$	−0.84 (0.09) $n=2710$	−0.57 (0.08) $n=2609$	0.24 (0.08) $n=2366$
CT	0.04 (0.08) $n=2869$,	−0.93 (0.09) $n=2691$,	−0.66 (0.08) $n=2588$,	0.54 (0.08) $n=2405$,
	$P=0.57$, $P^{adjusted}=0.67$	$P=0.48$, $P^{adjusted}=0.34$	$P=0.42$, $P^{adjusted}=0.37$	$P=0.009$, $P^{adjusted}=0.004$
TT	0.03 (0.15) $n=728$,	−0.90 (0.18) n = 673	−0.81 (0.16) n = 645	0.55 (0.17) n = 589
	$P=0.68$, $P^{adjusted}=0.72$	$P=0.78$, $P^{adjusted}=0.88$	$P=0.18$, $P^{adjusted}=0.26$	$P=0.09$, $P^{adjusted}=0.17$

[a]*P*-values for genotype indicate comparison with CC genotype.
Numbers in parentheses indicate standard errors.
Adjusted *P*-values are adjusted by mothers' pre-pregnancy body mass index, age, parity and educational level.

Table 4 Change in depression score between 8 months and 21 months postpartum stratified by MTHFR C677T genotype

		CC	*CT*	*TT*	*All individuals*
Folic acid supplements at 18 weeks	No	0.25 (0.09) $n=2115$	0.56 (0.09) $n=2165$	0.66 (0.18) $n=539$	0.43 (0.06) $n=4819$
	Yes	0.11 (0.24) $n=240$	0.37 (0.27) $n=234$	−1.02 (0.61) $n=43$	0.14 (0.18) $n=517$
	$P=$	0.62	0.51	0.01	0.11
Folic acid supplements at 32 weeks	No	0.26 (0.10) $n=1632$	0.60 (0.10) $n=1690$	0.73 (0.20) $n=427$	0.47 (0.06) $n=3749$
	Yes	0.18 (0.15) $n=705$	0.37 (0.16) $n=669$	−0.10 (0.32) $n=154$	0.24 (0.10) $n=1528$
	$P=$	0.68	0.22	0.03	0.06

Numbers in parentheses indicate standard errors.
P-value for interaction between genotype (TT compared to CT + CC combined) and folic acid supplements $P=0.02$ (for supplements at 18 weeks) and $P=0.09$ (for supplements at 32 weeks).

evidence that folic acid supplementation in pregnancy, which could increase circulating folate levels and decrease homocysteine levels substantially, was associated with changes in depression score during pregnancy or up to 8 months postpartum. However, we did find evidence to suggest that folic acid supplementation in pregnancy may protect against an increase in depressive symptoms between 8 and 21 months postpartum. This association was most pronounced among those with the MTHFR C677T TT genotype. Folic acid supplementation has been shown to have the biggest impact on circulating folate and homocysteine levels in those with the MTHFR C677T TT genotype; in accordance with this, we found that the folic acid supplementation had the most beneficial impact on EPDS scores at 21 months postpartum among women with the TT genotype.

Observational studies are subject to confounding, measurement error and bias. Confounding is particularly likely to be a problem in observational studies of folate intake as supplement use and higher dietary intake are associated with a number of measures of social position, which have also been associated with risk of depression. However, associations between genetic variants and disease are not generally subject to the problem of confounding, as a genetic variant, which affects the metabolism of a nutrient, is unlikely to be associated with other dietary and lifestyle factors, which are typically associated with dietary folate intake (Davey Smith and Ebrahim, 2003, 2004; Lewis *et al*., 2006). Furthermore, unlike measurement of dietary intake (Lewis *et al*., 2006b), measurement of genotype has a very low error rate and is not generally subject to bias. Thus, associations between polymorphisms, which affect the biologically effective level of a

particular exposure, and disease can be used to make inferences about the given exposure and disease.

Some potential limitations need to be borne in mind. We did not conduct psychiatric interviews to assess depression but relied on the EPDS (Cox *et al*., 1987), a self-reported questionnaire that has shown excellent validity in measuring depressive symptoms during pregnancy and post-natal depression in the United Kingdom and elsewhere (Eberhard-Gran *et al*., 2001). We used a continuous measure of depression rather than using a cut-off for defining depressive disorder, this gave us more power to look at associations and also helps avoid the problem of setting an arbitrary and debatable cut-off point for caseness. We looked for changes in this score between time points in order to take account of prevalent depressive mood, this gave us an indicator of changes in the severity of depressive symptoms along a continuous measure. However, it is very difficult to quantify the clinical relevance of such changes at a population level. Once we excluded all women with missing data, we were left with a much smaller subset of the original ALSPAC study, however, our scores corresponded very well with those reported previously in a much large sample, (Evans *et al*., 2001) suggesting that the women included in this analysis were representative of the women who took part in the study. We did not have information on the dose of folic acid taken by women in the study, nor information on how long the folic acid was taken, similarly, many women reported taking multivitamins during pregnancy, but as we did not have any data on whether these contained folic acid (at the time of recruitment, it was not clear that folic acid was beneficial during pregnancy), we did not include women taking multivitamins (unless they specifically reported taking folic acid) in our exposed group. However, even if these multivitamins did contain folic acid this would usually be at a lower dose than a folic acid-only supplement, and excluding these women from the folic acid group would attenuate our results towards the null, and so makes our estimates of effect more conservative.

The association between folic acid supplementation at 32 weeks of pregnancy and change in depression score between 8 months and 21 months of pregnancy could be a chance finding given the large number of statistical tests performed in this analysis. However, our finding of an association between change in EPDS score and the MTHFR C677T genotype, and a greater effect of supplementation among those with the MTHFR C677T genotype suggests that this association may be true rather than due to multiple testing.

How could folic acid supplementation at 32 weeks of pregnancy affect changes in depression score between 8 and 21 months postpartum and not changes in depression score during pregnancy and up to 8 months after pregnancy? Twenty-one months postpartum is a time at which depression can no longer be classified as postnatal and is unlikely to be related to reproductive factors or changes in folate levels during or immediately after pregnancy. Postpartum depression does not seem to be different from other types of depression except for the timing at which it occurs. Many of the symptoms and risk factors that appear to be associated with depression during and following pregnancy are the same as those that are related to depression in general (Halbreich, 2005). However, low folate levels and high homocysteine levels have been suggested to increase infertility and miscarriages (Nelen *et al*., 2000; George *et al*., 2002). Therefore, perhaps the fact that all of the women in this study had a pregnancy, which went to term, meant that they also had a sufficiently high folate level so as not to influence depressive symptomatology. While nutrient intakes of the women in this study were similar to the reported nutrient intakes for all women aged 16–64 in the 1987 dietary and nutritional survey of British adults the intake of folate was much higher (Rogers and Emmett, 1998). The mean folate intake in this study was 250 μg/day while the mean intake among women in dietary and nutritional survey of British adults was just 213 μg/day (a difference of 27). In other studies, it has been observed that homocysteine concentrations are lower in pregnant women than in non-pregnant controls, and that this difference is independent of folic acid supplementation (Bonnette *et al*., 1998; Walker *et al*., 1999). This may be a physiological response to pregnancy, but may also protect against depression while pregnant and immediately afterwards. Of note is that depression scores among the women included in this study were lower postnatally than antenatally, and that the prevalence of depression (defined by an EPDS score $\geqslant$13) was lower than in women aged 26 in a population-based cohort study (Gale and Martyn, 2004). Depression during pregnancy may therefore be related to reasons other than a lack of folate, which may be a risk factor outside the perinatal period as suggested by previous studies (Bjelland *et al*., 2003; Morris *et al*., 2006).

It is not clear whether taking supplements at 32 weeks of pregnancy could lead to large increases in available folate at 21 months postpartum, as there is little data on folate levels several months after supplementation. One study based on a single administration of folic acid in just one subject suggested that the half-life of folate in body pools may be around 100 days (Krumdieck *et al*., 1998). However, it is possible that women who were taking folic acid supplements at 32 weeks of pregnancy continued to take these supplements after pregnancy. It is possible that these results have arisen by chance in this population and so further studies are required, preferably using a clinical diagnosis of depression to determine whether taking folic acid supplements can affect long-term depression risk and if this risk is moderated by the MTHFR genotype.

Conflict of interest

The authors declare no conflict of interest.

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