Short communication

Low-dose folic acid supplementation does not influence plasma methionine concentrations in young non-pregnant women

Ingeborg A. Brouwer[1,2]*, Marijke van Dusseldorp[1], Marinus Duran[3], Chris M. G. Thomas[2,4], Joseph G. A. J. Hautvast[1], Tom K. A. B. Eskes[2] and Régine P. M. Steegers-Theunissen[2,5]

[1]*Division of Human Nutrition and Epidemiology, Wageningen Agricultural University, Wageningen, The Netherlands*
[2]*Departments of Obstetrics & Gynaecology,* [4]*Chemical Endocrinology and* [5]*Epidemiology, University Hospital Nijmegen St Radboud, Nijmegen, The Netherlands*
[3]*Laboratory of Metabolic Diseases, Wilhelmina Children's Hospital, Utrecht, The Netherlands*

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An elevated plasma total homocysteine (tHcy) concentration is a risk factor for cardiovascular disease and for having offspring with a neural-tube defect. Folate is a methyl donor in the remethylation of homocysteine into methionine. Although folic acid supplementation decreases tHcy concentrations, effects of folic acid supplementation on plasma methionine concentrations are unclear. There is also concern that folic acid supplementation negatively affects vitamin B_{12} status. We studied effects of low-dose folic acid supplementation on methionine and vitamin B_{12} concentrations in plasma. We also investigated whether baseline plasma methionine and tHcy concentrations correlated with the baseline folate and vitamin B_{12} status. For a period of 4 weeks, 144 young women received either 500 μg folic acid each day, or 500 μg folic acid and placebo tablets on alternate days, or a placebo tablet each day. Plasma methionine, tHcy and plasma vitamin B_{12} concentrations were measured at start and end of the intervention period. Folic acid supplementation had no effect on plasma methionine or plasma vitamin B_{12} concentrations although it significantly decreased tHcy concentrations. Plasma methionine concentrations showed no correlation with either tHcy concentrations (Spearman $r_s - 0{\cdot}01$, $P = 0{\cdot}89$), or any of the blood vitamin variables at baseline. Baseline tHcy concentrations showed a slight inverse correlation with baseline concentrations of plasma vitamin B_{12} ($r_s - 0{\cdot}25$, $P < 0{\cdot}001$), plasma folate ($r_s - 0{\cdot}24$, $P < 0{\cdot}01$) and erythrocyte folate ($r_s - 0{\cdot}19$, $P < 0{\cdot}05$). In conclusion, low-dose folic acid supplementation did not influence plasma methionine or plasma vitamin B_{12} concentrations. Furthermore, no correlation between plasma methionine concentrations and the blood folate and vitamin B_{12} status was shown.

Folic acid: Methionine: Homocysteine: Vitamin B_{12}

Homocysteine has received a lot of attention because hyperhomocysteinaemia has been shown to be a risk factor for cardiovascular disease and for having offspring with a neural-tube defect (Steegers-Theunissen *et al*. 1994; Boushey *et al*. 1995; Mills *et al*. 1995). Homocysteine is the transmethylation product of the essential amino acid methionine. Methionine can be transmethylated to homocysteine, which in turn can be remethylated to methionine. Thus, in theory, an increase in plasma methionine is expected after a decrease in plasma homocysteine. *N*-5-methyltetrahydrofolate, the predominant form of the B-vitamin folic acid in the blood, serves as a methyl donor for this remethylation reaction and supplementation with folic acid decreases plasma total homocysteine (tHcy) concentrations (Ward *et al*. 1997; Brouwer *et al*. 1999). Vitamin B_{12} (methylcobalamin) is a cofactor in this remethylation reaction. Supplementation with folic acid or vitamin B_{12} increased plasma methionine concentrations in folate- or vitamin B_{12}-deplete subjects (Guttormsen *et al*. 1996).

Since 1947 there has been concern that folic acid supplementation may have deleterious effects on cobalamin deficiency (Heinle & Welch, 1947). We therefore checked in

Abbreviation: tHcy, total homocysteine.

* **Corresponding author:** Dr Ingeborg A. Brouwer, fax +31 317 483342, email ingeborg.brouwer@staff.NutEpi.wau.nl

this study whether low-dose folic acid supplementation influenced plasma vitamin B_{12} concentrations.

The present study aimed to determine the effects of low-dose folic acid supplementation on plasma methionine and plasma vitamin B_{12} concentrations in healthy volunteers. We compared these effects with the decreasing effect on plasma tHcy concentrations (Brouwer *et al*. 1999). We also examined whether plasma methionine and tHcy were correlated with the blood folate and vitamin B_{12} status.

Subjects and methods

The methods of this experiment have previously been described in more detail (Brouwer *et al*. 1999). In short, these are as follows.

Subjects

We recruited healthy, non-pregnant women (18–40 years of age). Exclusion criteria were: smoking, gastrointestinal disorder, use of vitamins, minerals, yeast or seaweed, malaria prophylaxes and anti-convulsants. The final study group comprised 144 women.

The Medical Ethical Committee of the Wageningen Agricultural University approved the study design. All women gave written informed consent.

Methods

After stratification for the use of oral contraceptives, the women were randomized over three intervention groups and received either 500 μg folic acid/d, or 500 μg folic acid and a placebo tablet every second day (on average 250 μg folic acid/d), or a placebo tablet daily (Pharmachemie B.V., Haarlem, The Netherlands) for 4 weeks. The group sizes were chosen to detect an 11 % decrease in plasma tHcy concentrations after 4 weeks of intervention (Brouwer *et al*. 1999). The subjects and the laboratory staff were blind to the group assignment. Subjects followed their regular diet except for refraining from the consumption of liver and Marmite, a yeast extract. A 24 h recall was obtained from each subject once during the intervention period to check their intake of macronutrients, vitamin B_6, B_{12} and folate (Stichting NEVO, 1993; Brants & Hulshof, 1995).

Venous blood samples were collected after overnight fasting at the start (week 0) and at the end of the 4-week intervention period (week 4). We determined plasma methionine concentrations and plasma tHcy, plasma folate, erythrocyte folate and vitamin B_{12} concentrations in all samples.

Measurements

Samples for determinations in plasma were immediately placed on ice and centrifuged within 60 min at 3000 ***g*** for 10 min. Plasma was separated and stored at −20° for methionine, at −35° for folate and vitamin B_{12} and at −80° for tHcy. For determination of folate concentrations in erythrocytes, whole blood was diluted 5-fold with aqueous sodium ascorbate (10 g/l) and stored at −35°. The haemolysates were further diluted with IMx Folate RBC Lysis Reagent (Abbott Laboratories, North Chicago, IL, USA). To enable expression of the folate concentration in erythrocytes, packed cell volumes were also measured.

For methionine analysis a quantitative amino acid analysis in plasma was performed on a Biotronik 7000 amino acid analyser (Biotronik, Maintal, Federal Republic of Germany) using *S*-(aminoethyl)cysteine as internal standard. The separation was achieved by a stepwise gradient of lithium citrate buffers; the detection and quantification was performed with the classical ninhydrin reaction and spectrophotometry at 440 and 570 nm. The method had inter- and intra-assay CV < 5 %.

Plasma tHcy was measured by an HPLC technique and fluorimetric detection (intra- and inter-assay CV < 8 %) (Araki & Sako, 1987). Folate concentrations in plasma and erythrocytes and vitamin B_{12} in plasma were determined with the Abbott IMx Vitamin B_{12} and Folic Acid assays (Abbott Laboratories). The intra-assay CV of the folate assay was < 6 %, while the inter-assay CV was $<$ 10 %. For vitamin B_{12}, both the intra- and inter-assay CV were lower than 5 %.

Statistics

The response to treatment was calculated for each subject as the change in plasma methionine, tHcy and plasma vitamin B_{12} concentrations between the start (week 0) and the end of the intervention period (week 4). These responses were normally distributed as checked by visual inspection of the normal probability plots (univariate procedure; Statistical Analysis Systems Inc., Cary, NC, USA). To analyse differences in response to intervention Student's *t* tests were used with a significance level of $P < 0{\cdot}05/3 = 0{\cdot}017$. Spearman rank correlation coefficients were calculated for the blood variables, because the concentrations (with the exception of plasma methionine) were not normally distributed.

Results

Low-dose folic acid supplementation for 4 weeks had no effect on plasma methionine concentrations, whereas folate concentrations in plasma and erythrocytes increased and plasma tHcy concentrations decreased (Table 1; Brouwer *et al*. 1999). Intervention with folic acid had no effect on plasma vitamin B_{12} concentrations (Table 1).

The mean change in plasma methionine in the 500 μg group corrected for the change in the placebo group was 0·37 (95 % CI −1·6, 2·3) μmol/l; the mean change in plasma methionine in the 250 μg group corrected for the change in the placebo group was −1·2 (95 % CI −3·2, 0·7) μmol/l. Fig. 1 shows the individual values of plasma methionine and plasma tHcy before and after intervention.

Baseline plasma tHcy and methionine concentrations of the subjects were not correlated (Spearman r_s −0·01, P = 0·89). Baseline plasma tHcy concentrations showed a slight, but significant correlation with baseline concentrations of plasma vitamin B_{12} (r_s −0·25, $P = 0{\cdot}003$), plasma folate (r_s −0·24, $P = 0{\cdot}004$) and erythrocyte folate (r_s −0·19,

Table 1. Plasma folate, erythrocyte folate, plasma vitamin B_{12}, total plasma homocysteine and plasma methionine concentrations before and after 4 weeks of folic acid supplementation

(Values are means and standard deviations)

	500 μg folic acid/d group (*n* 45)		250 μg folic acid/d group (*n* 50)		Placebo group (*n* 49)	
	Mean	SD	Mean	SD	Mean	SD
Initial plasma folate (nmol/l)	11	4·0	11	3·4	12	4·2
Final plasma folate (nmol/l)	27	5·1	23	5·3	17	6·1
Initial erythrocyte folate (nmol/l)	400	107	386	85	422	181
Final erythrocyte folate (nmol/l)	508	176	432	126	401	182
Initial plasma vitamin B_{12} (nmol/l)	265	117	290	88	286	110
Final plasma vitamin B_{12} (pmol/l)	265	110	300	98	287	121
Initial total plasma homocysteine (μmol/l)	11·1	2·6	9·7	2·2	10·4	2·5
Final total plasma homocysteine (μmol/l)	8·5	1·7	8·5	1·7	10·3	2·5
Initial plasma methionine (μmol/l)	22·6	4·8	23·4	4·3	23·4	4·1
Final plasma methionine (μmol/l)	23·7	5·7	22·9	4·3	24·1	4·7

$P = 0{\cdot}03$). However, none of these blood vitamin concentrations was correlated with plasma methionine concentrations.

Users of oral contraceptives (*n* 97) had significantly lower baseline plasma vitamin B_{12} concentrations (252 (SD 84) pmol/l than non-users (346 (SD 118) pmol/l). However, no differences were observed for plasma tHcy and folate concentrations between users and non-users of oral contraceptives.

Discussion

This present study shows that 4 weeks of low-dose folic acid supplementation did not influence plasma methionine or plasma vitamin B_{12} concentrations although it decreased plasma tHcy concentrations and increased plasma and erythrocyte folate concentrations (Brouwer *et al*. 1999). Plasma methionine concentrations were also not correlated with tHcy or plasma or erythrocyte folate concentrations, while tHcy concentrations were inversely correlated with these blood vitamin variables.

In contrast to our study, Guttormsen *et al*. (1996) showed significantly increased fasting plasma methionine concentrations after intervention with folic acid (5 mg/d for 10 d) and/or intramuscular injections with cobalamin (3–5 mg in 2–3 weeks). However, they supplied much higher doses of folic acid and all subjects in that study had a deficient or marginal folate and vitamin B_{12} status and elevated plasma tHcy concentrations (Guttormsen *et al*. 1996). Our results are supported by those of others who also showed no effects of increased folate intake on plasma methionine concentrations in healthy young men, although plasma tHcy concentrations increased after depletion and decreased after repletion (Jacob *et al*. 1994, 1995; Ubbink *et al*. 1995). All our subjects had plasma methionine concentrations in the normal range (Scriver *et al*. 1985), but they were not in optimal folate status because folic acid supplementation decreased their plasma tHcy concentrations (Brouwer *et al*. 1999). Effects of folic acid supplementation might be different in subjects with either high or low plasma methionine and tHcy concentrations. Repetition of the analysis

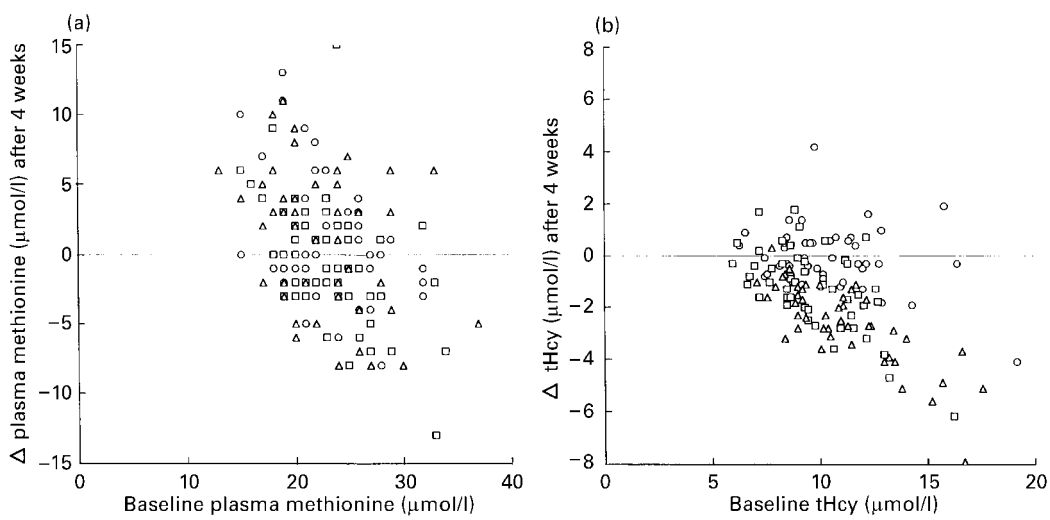

Fig. 1. Changes in (a) plasma methionine and (b) plasma total homocysteine (tHcy) in healthy young women (*n* 144) after four weeks of placebo (○), 250 μg folic acid/d (□) or 500 μg folic acid/d (△).

with exclusion of those subjects with plasma tHcy concentrations above 15 μmol/l did not change the results of the current study.

Potgieter *et al*. (1997) noticed that methionine is very sensitive to oxidation and may even be converted to methionine sulfoxide when samples are stored at −20° until analysis. Thus, in our study spontaneous oxidation of methionine might have occurred and it can not be excluded that this contributed to the lack of effect on plasma methionine.

Differences in methionine intake between the groups is not a very likely explanation for the lack of effect in the present study. Although we did not estimate methionine intake in the subjects, we know from a 24 h recall from all participants during the trial that the total dietary protein intake and the intake of animal protein reflected by the vitamin B_{12} intake were similar among the groups (Brouwer *et al*. 1999). This suggests comparable methionine intakes between the groups. Besides, it is unlikely that a slight difference in methionine intake would have influenced plasma methionine concentrations: Jacob *et al*. (1995) showed that changes in the intake of dietary methyl groups did not influence plasma methionine concentrations. Moreover, Andersson *et al*. (1990) found that a 3-fold increase in the daily intake of methionine for 13 d had no effect on fasting plasma methionine concentrations on day 14.

On the basis of the actual group sizes (placebo group, *n* 49; 500 μg/d group, *n* 45) and the standard deviations for the change in plasma methionine (placebo group, 4·8; 500 μg/d group, 5·1) and a power of 90 %, we calculated that a difference of 3·3 μmol/l in plasma methionine between the placebo and the 500 μg/d group would have been statistically significant. Therefore, we can exclude effects of low-dose folic acid supplementation on methionine above this level.

We found no effect of folic acid supplementation on plasma vitamin B_{12} concentrations. However, users of oral contraceptives had a lower plasma vitamin B_{12} concentration than non-users at baseline. Plasma tHcy concentrations were not affected by use of oral contraceptives. Green *et al*. (1998) also found lower (33 %) plasma vitamin B_{12} concentrations, but similar plasma tHcy concentrations in adolescent females who used oral contraceptives than those who did not use oral contraceptives. Our study suggests that this finding is not just confined to adolescent females.

In conclusion, fasting plasma methionine levels in healthy women were not related to plasma folate and vitamin B_{12} concentrations and were not influenced by low-dose folic acid supplementation.

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