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The effect of low doses of betaine on plasma homocysteine in healthy volunteers

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Homocysteine is a risk factor for vascular diseases, and lowering of plasma total homocysteine (tHcy) may be beneficial for health. Homocysteine can be remethylated to methionine by betaine–homocysteine methyltransferase using betaine (2(*N*,*N*,*N*-trimethyl)glycine) as methyl donor. A dose of 6 g betaine/d has been used in the treatment of homocystinuria, but data on the dose–response are scarce. Thirty-four healthy men and women were supplied with doses of 1, 3 and 6 g betaine and then with 6 g betaine + 1 mg folic acid for four consecutive 1-week periods. The mean plasma tHcy concentration decreased by 1·1 (NS), 10·0 and 14·0 % ($P<0·001$) after supplementation with 1, 3 and 6 g betaine respectively. A further decrease in plasma tHcy by 5 % ($P<0·01$) was achieved by combining 1 mg folic acid with the 6 g betaine dose. Plasma betaine increased from 31 (SD 13) to 255 (SD 136) μmol/l in a dose-dependent manner (R^2 0·97). We conclude that plasma tHcy is lowered rapidly and significantly by 3 or 6 g betaine/d in healthy men and women.

Betaine: Homocysteine: Dose–response: Folic acid

Homocystinuria is a rare inherited disease in which homocysteine accumulates in high concentrations in the body (Mudd *et al.* 1995). The much more common state, mild hyperhomocysteinemia, is considered to be a risk factor for vascular diseases (Eikelboom *et al.* 1999); lowering plasma total Hcy concentration may thus be beneficial for health. Plasma total homocysteine (tHcy) is determined by intake of B-group vitamins, and by lifestyle and genetic factors. It has been shown convincingly in clinical trials that a moderate dose of folic acid lowers plasma tHcy to normal levels in healthy and in mildly hyperhomocysteinemic subjects (Homocysteine Lowering Trialists' Collaboration, 1998; Brouwer *et al.* 1999). Homocysteine may undergo transsulfuration to cysteine in a reaction requiring pyridoxine as cofactor and transmethylation to methionine by two different reactions: one requiring both 5-methyltetrahydrofolate and methylcobalamin, the other requiring betaine (2(*N*,*N*,*N*-trimethyl)glycine) from catalysis by betaine–homocysteine methyltransferase (Mudd *et al.* 1995). The common polymorphism C677T, which reduces the activity of the enzyme methylenetetrahydrofolate reductase, is associated with approximately 40 % higher plasma tHcy in carriers of the TT genotype compared with those with the wildtype (CC) (Gudnason *et al.* 1998).

Oral betaine has been used in addition to B-group vitamins in the treatment of homocystinuria since the early 1950s (Wilcken *et al.* 1983). The doses have varied between treated patients and the effects have not been well documented. Studies on the pharmacokinetics of betaine in patients with homocystinuria and in healthy adults have only recently been reported (Matthews *et al.* 2002; Schwahn *et al.* 2003). However, data on the dose–response of betaine on plasma tHcy has not been available. The aim of the present study was to find the effect of graded doses of betaine on plasma tHcy concentration.

Materials and methods

Subjects

Adult volunteers (eleven men and twenty-four women) were enrolled from the staff of a brewery (Oy Sinebrychoff Ab, Kerava, Finland). They were apparently healthy and had normal serum lipids, alanine aminotransferase, γ-glutamyltransferase and creatinine levels. One female subject discontinued the study after 2 weeks due to an intervening illness unrelated to the study. Her data were omitted from the calculations. The subjects gave their written consent and the study plan was approved by the local Ethics Committee.

Study design

During the first week the subjects received 1 g anhydrous betaine (Finnfeeds, Kantvik, Finland)/d dissolved in

Abbreviation: tHcy, total homocysteine.

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200 ml mineral water. The drink was consumed during working hours. After 1 week the dose of betaine was increased to 3 g/d and in the beginning of the third week to 6 g/d. During the fourth week the subjects continued to receive 6 g betaine/d and received in addition 1 mg folic acid (Folvite®; Wyeth Lederle, Nordiska AB, Solna, Sweden)/d. The 1-week periods on each dose of betaine were chosen on basis of a pilot study performed on six healthy subjects consuming 6 g betaine/d for 8 d. Their plasma tHcy concentrations reached a plateau in 5 d with no further reduction thereafter.

Blood samples and assessment of dietary intake

Fasting venous samples were drawn into EDTA-containing vacuum tubes between 08.00 and 09.00 hours 7 weeks before baseline, at baseline, at the end of each intervention week and 3 weeks post-supplementation. The plasma was separated within 1 h and stored at −70°C until analysed. One tube of whole blood was reserved for isolation of DNA.

During the study the subjects adhered to a low-folate diet in order to reduce fluctuations in folate intake. Two subjects regularly took B-group vitamin supplements: they were asked to discontinue their use 7 weeks before the intervention. On weekdays the subjects consumed their lunch at the same worksite cafeteria and during the weekends and the evenings they were instructed to avoid abundant consumption of fruits and vegetables. On each intervention week they kept food records during one weekday and one weekend day. The records were returned weekly and checked by a nutritionist. The nutrient intakes were calculated using the Fineli® food composition database of the National Public Health Institute (Ovaskainen, 2001).

Chemical methods

Plasma folate and cyanocobalamin were determined by ion-capture immunometric methods (IMX; Abbott Laboratories, IL, USA), betaine by HPLC (Laryea *et al.* 1998) and tHyc by 7-benzo-2-oxa-1,3-diazole-4-sulfonic acid (SBDF) reagent and HPLC separation according to Ubbink *et al.* (1991) with minor modifications (Knekt *et al.* 2001). The precision for tHcy between series (n 6) was 5·5 % and the mean bias was −4·0 % in a quality assurance programme (Möller *et al.* 1999). DNA was extracted by a standard method (Bell *et al.* 1981). The methylenetetrahydrofolate reductase genotypes were analysed as described previously (Alfthan *et al.* 2003).

Statistical methods

The mean values between doses were tested for difference by the Wilcoxon signed ranks test, and mean values between genders by t test, linear regression and Spearman correlations using SPSS (version 10.0; SPSS Inc., Chicago, IL, USA).

Results

The characteristics and baseline data of the subjects are shown in Table 1. Plasma HDL-cholesterol and tHcy differed significantly between men and women. There were two homozygotes for the methylenetetrahydrofolate reductase TT genotype, both male subjects.

Table 1. Characteristics of subjects at baseline†
(Mean values and standard deviations)

	Men		Women	
	Mean	SD	Mean	SD
Gender (n)	11		23	
Age (years)	46·9	10·7	41·7	8·8
BMI (kg/m^2)	25·2	4·3	25·0	3·2
Serum cholesterol (mmol/l)	5·57	0·89	5·35	0·86
HDL-cholesterol (mmol/l)	1·18	0·31	1·50*	0·38
Smoking (n)	2		2	
Plasma betaine (μmol/l)	38·5	16·6	31·4	13·9
Plasma tHcy (μmol/l)	13·2	4·6	10·4*	2·7
Plasma folate (nmol/l)	11·2	3·9	13·7	5·4
Plasma cyanocobalamin (pmol/l)	509	155	489	137

tHcy, total homocysteine.
Mean values were significantly different from those of men: *$P<0·05$.
† Methylenetetrahydrofolate reductase genotype for all subjects (%): CC 65·7, CT 28·6, TT 5·7.

The mean intakes (2 d) of nutrients during the baseline and each intervention week are shown for men and women separately in Table 2. The intake of folate from the diet did not change significantly in men or women during the four intervention weeks. The intake of energy was significantly lower ($P<0·05$) than the baseline value during weeks 1 and 2 for women; pyridoxine concentration during week 2 was significantly lower ($P<0·05$) than the baseline value for women; for men all nutrients remained unaffected.

The mean plasma betaine concentration (μmol/l) increased from the baseline value of 31·4 (SD 13·6) to 52·5 (SD 26·5) (67 %) after 1 g betaine/d, to 109 (SD 41) (247 %) after 3 g/d and to 255 (SD 136) (712 %) after 6 g betaine/d (Fig. 1). At no sampling point did the mean values differ between men and women. A second week on 6 g betaine/d did not further increase plasma betaine concentration. The increase in plasma betaine was dose-dependent (R^2 0·97). The mean betaine concentration had returned to baseline values 3 weeks post-supplementation.

The mean plasma tHcy concentrations before, during and after supplementation are shown separately for men and women in Fig. 2. The mean values for men and women differed at all points ($P<0·05$) except at the post-supplementation period. The mean plasma tHcy concentration in men and women combined (11·3 (SD 3·6) μmol/l) decreased after 7 d of 1 g betaine/d (−1·1 %, 0·42 μmol/l; NS), 3 g betaine/d (−10·4 %, 1·3 μmol/l, $P<0·001$) and 6 g betaine/d (−14·2 %, 1·8 μmol/l; $P<0·001$ %) compared with baseline values (Fig. 3). The combined supplementation of 6 g betaine + 1 mg folic acid/d resulted in a further decrease of 5·1 % (−0·63 μmol/l; $P<0·01$) in tHcy concentration.

There were six subjects (three women and three men, two of whom were homozygotes for the T allele of methylenetetrahydrofolate reductase) who at baseline had a plasma tHcy value >14 μmol/l (mean 17·3 (SD 4·3) μmol/l; mean plasma folate 6·8 (SD 1·5) nmol/l). As expected, they benefited the most from betaine (Fig. 3). After the

Table 2. The intake of nutrients of men and women during baseline and the intervention weeks†
(Mean values with their standard deviations)

Week...	0		1		2		3		4	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Men (*n* 11)										
Energy (MJ)	10·5	2·66	9·48	4·29	8·73	3·59	9·95	4·47	11·2	3·6
Carbohydrate (% energy)	44·1	14·8	45·2	11·8	42·8	11	44·7	9	40·8	10·9
Fat (% energy)	35·1	11·9	34·3	9·3	34·8	8·6	35·7	7·28	33·7	8·77
Folate (μg)	259	90·8	246	128	247	114	286	156	280	88
Cyanocobalamin (μg)	6·13	5·22	5·37	3·68	7·85	7·81	6·94	5·24	8·05	5·32
Pyridoxine (mg)	2·08	1·12	1·99	1·56	2·02	1·06	2·36	1·41	2·43	1·11
Riboflavin (mg)	1·8	0·4	1·9	0·8	1·7	0·7	2·1	1·0	2·2	1·2
Women (*n* 23)										
Energy (MJ)	7·83	2·47	6·27*	1·92	6·05*	1·67	6·35	2·12	8·33	2·55
Carbohydrate (% energy)	43·7	11·8	45·7	9·23	46·5	8·08	44·7	10·6	41·0	9·35
Fat (% energy)	35·3	8·94	35·5	7·5	34·8	6·49	37·2	10·2	34·9	9·22
Folate (μg)	213	94·5	200	103	188	76·2	225	152	240	162
Cyanocobalamin (μg)	6·34	8·46	5·65	5·59	3·52	2·4	6·45	9·38	5·76	6·47
Pyridoxine (mg)	1·66	0·56	1·43	0·54	1·37*	0·51	1·53	0·7	1·69	0·59
Riboflavin (mg)	1·5	0·7	1·2	0·5	1·1	0·5	1·4	0·6	1·4	0·7

Mean values were significantly different from those at baseline (week 0): *$P<0·05$.
† For details of subjects and procedures, see Table 1 and p. 665.

doses of 1, 3 and 6 g betaine/d their mean plasma tHcy decreased by −11·8 % (2·2 μmol/l), −15·4 % (2·6 μmol/l, NS) and −21·9 % (3·9 μmol/l, $P<0·05$) respectively, with a further reduction of −6·7 % (0·9 μmol/l, $P<0·05$) after the additional folic acid.

The mean plasma folate concentration decreased ($P<0·01$) in both men and women after the first week on supplementation by 28 and 23 % respectively, but returned to baseline values for the next 2 weeks. After week 4, when the subjects were supplemented with betaine + folic acid, the mean plasma folate concentration increased by 59 % in men and 41 % in women ($P<0·001$) and returned to baseline 3 weeks later. Plasma cyanocobalamin remained unaffected during the whole intervention.

Correlation between plasma variables

Plasma tHcy was significantly ($P<0·05$) associated with plasma folate at all time points (r 0·35 to −0·66), but no correlations were found with plasma betaine or cyanocobalamin at any time point.

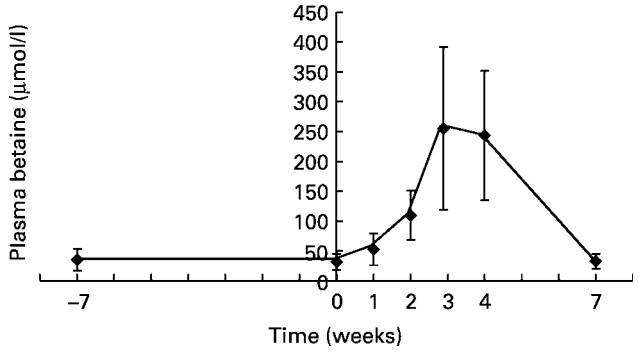

Fig. 1. Plasma betaine concentration of subjects (*n* 34) after consumption of 1, 3 and 6 g betaine/d and 6 g of betaine + 1 mg folic acid/d (each dose for 1 week). For details of subjects and procedures, see Table 1 and p. 665. Values are means with standard deviations shown by vertical bars.

Discussion

Oral dosing with betaine in healthy subjects resulted in a statistically significant lowering of plasma tHcy ($P<0·001$). The dose of 6 g betaine/d resulted in slightly lower efficacy in terms of percentage change per g betaine compared with 3 g/d (2·4 *v.* 3·4 % respectively). The duration of the study per dose was only 1 week, but according to our pilot study this was sufficient to reach a maximal response. However, the response was transient, as 3 weeks after supplementation plasma tHcy had returned to baseline values.

In a smaller study from the Netherlands also on healthy subjects, Brouwer *et al.* (2000) reported that 6 g betaine/d lowered plasma tHcy by 8 % in 2 weeks, a similar value (9 %) to that which was found in a study on obese subjects (*n* 42) who took 6 g betaine/d for 12 weeks (Schwab *et al.* 2002). In a recent study by Olthof *et al.* (2003), four groups

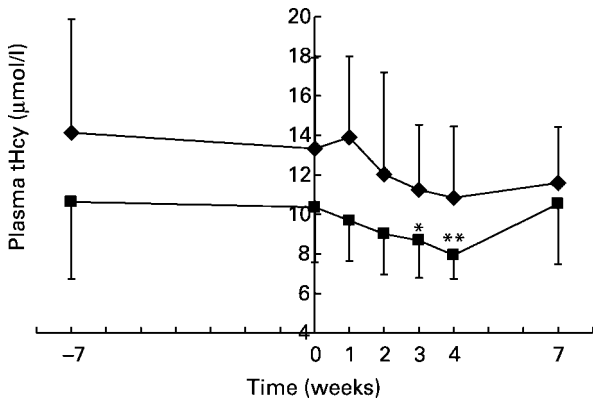

Fig. 2. Plasma total homocysteine (tHcy) concentration of men (*n* 11; ◆) and women (*n* 23; ■) after consumption of 1 (week 1), 3 (week 2) and 6 (week 3) g betaine/d and 6 g betaine + 1 mg folic acid/d (week 4) (each dose for 1 week). For details of subjects and procedures, see Table 1 and p. 665. Values are means with standard deviations shown by vertical bars. Mean values were significantly different from those at baseline: *$P<0·05$, **$P<0·01$.

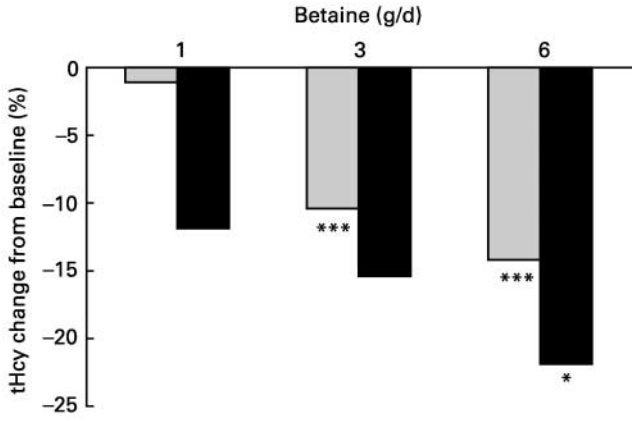

Fig. 3. Mean changes in plasma total homocysteine (tHcy) concentration after consumption of 1, 3 and 6 g betaine/d (each dose for 1 week) in all subjects (*n* 34; ▫) and in subjects with a tHcy concentration >14 μmol/l at baseline (*n* 6; ■). For details of subjects and procedures, see Table 1 and p. 665. Mean values were significantly different from those at baseline: **P*<0·05, ****P*<0·001.

(nineteen per group) of healthy adults were given 1·5, 3·0 or 6·0 g betaine or placebo twice per d for 6 weeks. Compared with the placebo group after 6 weeks, plasma tHcy concentration decreased 12, 15 and 20 % respectively. However, even after 2 weeks on betaine the plasma tHcy-lowering effect was maximal on all doses. This finding, together with that from our pilot study, suggests that betaine lowers plasma tHcy almost immediately (within 1–2 weeks).

The plasma tHcy-lowering effect of betaine was quantitatively similar in both genders except in men after the first betaine dose, which resulted in an increase of 4·3 % in plasma tHcy compared with the decrease of 6·6 % in women (Fig. 2). Although the intake of folate from two dietary records during the first week indicated no significant change, plasma folate decreased in men in and women, possibly because they were asked to avoid foods particularly rich in folate.

Until recently it was believed that the intake of betaine from ordinary food is so low that plasma tHcy could not be affected by dietary means. Zeisel *et al.* (2003), however, analysed foods from the USA for betaine and estimated that significant amounts (0·5–2·0 g) of betaine may be attained from the diet, the main sources being wheat germ, spinach, beets and shrimps. Our preliminary estimate, based on the Finnish balance sheet supplemented with data from Zeisel *et al.* (2003), indicated a mean intake of 0·14 g/d; the intake calculated from the food records in the present study was 0·18 g/d (ML Ovaskainen, personal communication). The sources of betaine (Zeisel *et al.* 2003) and folate (Alfthan *et al.* 2003) are different and because the metabolic pathways through which they lower homocysteine are not related, they can provide additive plasma tHcy-lowering effects.

The six subjects with an initial plasma tHcy value >14 μmol/l benefited from betaine more than those with a normal tHcy at baseline. The other common feature of these six subjects was a low plasma folate (mean 6·8 nmol/l). Thus, it seems that mildly hyperhomocysteinemic subjects benefit the most from additional betaine, as has been shown for individuals with the homozygous methylenetetrahydrofolate reductase TT genotype from folic acid supplements (Homocysteine Lowering Trialists' Collaboration 1998; Pullin *et al.* 2001; Alfthan *et al.* 2003).

Our pilot study showed that by dosing with 6 g betaine/d a steady-state of plasma tHcy was reached after 5 d. Although respective data for folic acid are not available to our knowledge, we assumed in our present experiment that 7 d would suffice also for folic acid. A single dose of 400 μg folic acid has been shown to keep plasma folate elevated for 6 h (Prinz-Langenohl *et al.* 1999). The acute effect of betaine on plasma tHcy is rapid. After a single dose of 6 g betaine, plasma tHcy decreased by about 10 % after 1 h and stayed at this level for the remaining 24 h (U Schwab, personal communication). In the study by Olthof *et al.* (2003), the betaine dose was given two times per d, i.e. after breakfast and dinner. Our subjects were allowed to finish their drink during the day. Data simulated by Matthews *et al.* (2002) showed that multiple dosage seemed to improve the response only slightly compared with a single dose.

The sequential increasing dose study design was chosen because it allowed us to use a smaller number of subjects in a shorter time. As the dose increased, the carry-over effect was probably small.

In the present study we were not able to compare separately the effects of betaine and folic acid on plasma homocysteine. However, a relatively large dose of folic acid together with 6 g betaine contributed only slightly more to the lowering of plasma tHcy than betaine by itself. We chose to co-supplement betaine with only folic acid, as including pyridoxine and cyanocobalamin would probably have provided very little extra benefit (Ubbink *et al.* 1994). The intakes of pyridoxine, cyanocobalamin and riboflavin in our present subjects were adequate according to Finnish national recommendations (1998), but the intake of folate reached on average only 73 and 85 % of the energy-adjusted recommendation (36 μg/MJ) for men and women respectively. Steenge *et al.* (2003) compared the relative effects of 6 g betaine or 800 μg folic acid/d on plasma tHcy in mildly hyperhomocysteinemic subjects during a 6-week intervention. Betaine lowered plasma tHcy by 11 % and folic acid by 18 %, but the difference was not statistically significant between treatments. In another study in chronic haemodialysis patients, van Guldener *et al.* (1999) compared the relative effect of 5 mg folic acid supplementation with the combination 5 mg folic acid + 4 g betaine/d for 12 weeks. Compared with folic acid, betaine did not have additional homocysteine-lowering effects.

In subjects with a poor response to folic acid, a combined dose including betaine and folic acid may prove effective, since the two compounds are metabolized by different organs (betaine in the liver and kidney, folate remethylation in most cells). The product of the transmethylation of homocysteine is methionine. Long-term treatment of homocystinuric patients with betaine causes increased plasma methionine concentrations, which have remained elevated with no adverse effects (Wilcken & Wilcken, 1997).

We conclude that 3 or 6 g betaine/d lowers plasma tHcy significantly in healthy men and women.

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