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ORIGINAL ARTICLE

Long-term effect of betaine on risk factors associated with the metabolic syndrome in healthy subjects

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Background/Objectives: To examine the effects of betaine on serum lipid profile, plasma homocysteine concentration and hemostatic factors in healthy subjects.
Subjects/Methods: Altogether, 63 volunteers (27 ± 8 years, body mass index $22.6 \pm 2.4\,kg/m^2$) participated in a placebo-controlled, randomized, parallel double-blinded study. The intervention lasted for 6 months during which the subjects consumed mineral water 500 ml/day with (betaine group, $n=32$) or without (control group, $n=31$) a 4-g betaine supplementation.
Results: There was a significant interaction of time and group (general linear model) in serum total and low-density lipoprotein (LDL)-cholesterol concentrations and total-to-high-density lipoprotein (HDL)-cholesterol ratio without a significant difference between or within the groups. Concentrations of serum HDL-cholesterol, triglycerides or oxidized LDL did not change during the study. Plasma homocysteine concentration did not change in either of the groups. Plasma plasminogen activator inhibitor 1 concentration increased in the betaine group ($P=0.028$) and decreased in the control group ($P=0.006$). There was a significant interaction of time and group (general linear model) in plasma fibrinogen and blood hemoglobin concentration without a significant difference between or within the groups. There were no changes in parameters regarding the function of the liver or kidney.
Conclusions: Betaine had no effect on serum lipid profile in long term in young healthy subjects. The lowering effect on plasma homocysteine concentration was weak.
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Keywords: betaine; hemostasis; homocysteine; human; lipid

Introduction

Betaine is a methylamine, which is formed from choline in the human body. It is an important methyl-group donor in methionine biosynthesis. Betaine is demethylated to form dimethylglycine and further sarcosine and glycine. Betaine has been used in animal nutrition for years. In humans, there is little safety data regarding the use of betaine in healthy individuals. In hyperhomocysteinemic patients, betaine is used to lower plasma homocysteine concentrations (Wilcken *et al.*, 1983; Singh *et al.*, 2004), which is suggested to be an independent risk factor for coronary heart disease (El-Khairy *et al.*, 1999; Chambers *et al.*, 2000; Humphrey *et al.*, 2008). Hyperhomocysteinemia is also associated with hyperinsulinemia and insulin resistance, which in turn increases the risk of cardiovascular diseases (Meigs *et al.*, 2001). Low plasma betaine concentration, on the contrary, has been shown to be related to an unfavorable cardiovascular risk factor profile (Konstantinova *et al.*, 2008). In obese subjects, betaine has been found to decrease significantly plasma homocysteine concentration (Schwab *et al.*, 2002). In healthy individuals, orally administered betaine has been shown to result in a significant acute

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decrease in plasma homocysteine concentration (Schwab *et al*., 2006). In a recent study, there was a tendency of betaine being associated with a memory improvement in elderly subjects (Eussen *et al*., 2007).

In contrast to betaine's reported favorable effect on plasma homocysteine concentration, there are studies showing that betaine supplementation may cause an increase in serum low-density lipoprotein (LDL)-cholesterol concentration in healthy (Olthof *et al*., 2005) and obese (Schwab *et al*., 2002) subjects as well as in patients with chronic renal failure (McGregor *et al*., 2002). Serum concentration of total triglycerides has also been shown to increase in some studies (Olthof *et al*., 2005). These unfavorable effects on serum lipid profile may undo the favorable effect of betaine on plasma homocysteine concentration regarding the risk of cardiovascular diseases.

The aim of this study was to examine long-term effects of betaine on homocysteine metabolism, serum total and lipoprotein lipid concentrations, blood pressure, hemostatic factors and sensitive C-reactive protein (CRP) in healthy subjects without increased risk for atherosclerotic vascular diseases.

Subjects and methods

Subjects

Sixty-three non-obese (body mass index $<27\,kg/m^2$) volunteers aged 20–50 years without atherosclerotic vascular diseases or metabolic disturbances and normal kidney, liver and thyroid functions were recruited by newspaper advertisements and from the students and the staff of the University of Kuopio. The subjects were free of any significant chronic disease that could have caused difficulties in following the study protocol. The exclusion criteria were body mass index $>26.9\,kg/m^2$, serum total triglyceride concentration >2 mmol/l, serum total cholesterol concentration >6 mmol/l, serum high-density lipoprotein (HDL)-cholesterol concentration <0.9 mmol/l or total-to-HDL-cholesterol ratio $>5{:}1$, symptomatic coronary heart disease, cancer, hypo- or hyperthyreosis, kidney or liver diseases, alcohol abuse (>40 g/day), drug treatment for mental disturbances and lipid-lowering medication. The subjects were asked to discontinue possible consumption of fish oil or evening primrose oil capsules or any vitamin or mineral supplements 1 month before the beginning of the intervention. The baseline characteristics of the subjects are presented in Table 1.

Products

The daily betaine dose was 4 g mixed with 500 ml of low-sodium mineral water. The subjects were advised to ingest the daily dose at least in two separate doses. The control group consumed low-sodium mineral water without betaine. The subjects followed their habitual diet during the study, and they were asked to keep their physical activity unchanged during the study.

Study design

The study was carried out according to a randomized, double-blinded parallel design. The subjects were matched for body weight, age and gender. Before the enrollment, the subjects gave their written informed consent. The study plan was approved by the Ethics Committee of the Hospital District of Northern Savo.

The length of the study was 6 months. The subjects visited the research unit at the beginning of the intervention (0 weeks) and at 4, 8, 12, 16, 20 and 24 weeks. Body weight and blood pressure was measured at each visit. Serum concentrations of total lipids and HDL and LDL cholesterol as well as plasma homocysteine concentration were measured at 0, 4, 8, 12 and 24 weeks. Plasma concentrations of sensitive CRP (hs-CRP), folate and vitamin B_{12}, and serum

Table 1 Baseline characteristics of the subjects[a]

	Betaine (n = 32)	*Control* (n = 31)
Gender (M/F)	7/25	6/25
Age (years)	27 ± 8 (19–50)	27 ± 8 (20–50)
Weight (kg)[b]	64.8 ± 9.1 (47.0–85.0)	66.0 ± 9.2 (47.6–82.8)
BMI (kg/m^2)[b]	22.6 ± 2.4 (18.1–28.3)	22.6 ± 2.3 (19.1–27.1)
Blood pressure (mm Hg)[b]		
Systolic	115 ± 8 (99–134)	117 ± 8 (102–132)
Diastolic	77 ± 6 (62–69)	77 ± 5 (68–85)
fP-glucose (mmol/l)[b]	5.2 ± 0.3 (4.6–6.0)	5.1 ± 0.4 (4.4–6.6)
fS-cholesterol (mmol/l)	4.4 ± 0.8 (3.1–6.0)	4.7 ± 0.8 (3.0–6.5)
HDL-cholesterol (mmol/l)	1.4 ± 0.3 (0.9–2.3)	1.6 ± 0.3 (1.1–2.3)
LDL-cholesterol (mmol/l)	2.64 ± 0.70 (1.62–4.17)	2.63 ± 0.72 (1.51–2.63)
fS-triglycerides (mmol/l)	0.87 ± 0.27 (0.48–1.48)	0.92 ± 0.30 (0.30–1.72)

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
[a]Mean ± s.d. (range).
[b]Measured at screening.

concentration of betaine were measured at 0, 8, 12 and 24 weeks. Blood count, concentrations of serum creatinine, alanine aminotransferase, γ-glutamyltransferase and hemostatic factors (plasma plasminogen activator inhibitor 1 (PAI-1), factor VII, fibrinogen, serum D-dimer and von Willebrand factor) were measured at 0, 12 and 24 weeks. Apolipoproteins A-I and B and antibodies against oxidized LDL were measured at 0 and 24 weeks.

Subjects filled in a questionnaire about physical activity, medication use and dietary supplements at the baseline and the end of the study. A 4-day food record was kept once before the beginning of the intervention and three times during the intervention preceding the visits at 4, 12 and 20 weeks.

Laboratory analyses

Blood samples were drawn after a 12-h overnight fast from an antecubital vein. Body weight was measured with a standardized electronic scale in light clothing. Height was measured at the beginning of the study in the Frankfurt position. Blood pressure was measured by a laboratory nurse and a sphygmomanometer was used. Two measurements in a sitting position 5 min apart were measured, and the mean was used for statistical analyses.

Plasma glucose concentration was analyzed with an enzymatic photometric method (Granutest 250, Diagnostica Merck, Darmstadt, Germany) and plasma insulin concentration with luminometric immunoassay (reagent ACS:180 IRI, Bayer A/S, USA, equipment ACS:180 PLUS, Bayer/Chiron, Tarrytown, NY, USA). Concentrations of serum total cholesterol and triglycerides were analyzed with the enzymatic colorimetric method using reagents Cholesterol CHOD-PAP and Triglycerides GPO-PAP (Roche Diagnostics, Mannheim, Germany) and KonePro Clinical Chemistry Analyzer (Thermo Clinical Labsystems, Konelab, Finland). HDL-cholesterol concentration was measured after dextransulphate-Mg^{2+} precipitation, with the same method as total cholesterol and triglyceride concentrations. Concentrations of apolipoprotein A-I and B were analyzed with an immunoturbidometric method (reagents Apolipoprotein A1 and Apolipoprotein B; Thermo Clinical Labsystems) using KonePro Clinical Chemistry Analyzer. Serum creatinine concentration was measured with an enzymatic method in the laboratory of Kuopio University Hospital. Concentration of oxidized LDL was measured as the baseline level of conjugated dienes in lipids extracted from LDL (Ahotupa *et al*., 1996).

Betaine and dimethylglycine were analyzed as 4-bromophenacyl triflate derivatives according to the method by Lever *et al*. (1992) with modifications. 4-Bromophenacyl triflate was synthesized using the method described by Mar *et al*. (1995). Calibration was performed by using the standard addition method by spiking a pooled serum sample with standards. The samples were prepared according to Lever *et al*. (1992). The derivatives were separated using Chrompack Si column (3 μm, $150 \times 4.6\,mm^2$, Chrompack, Middelburg, Netherlands). The mobile phase was 4 mmol/l triethanolamine–1.6 mmol/l citric acid in water–isopropanol (1:3).

Homocysteine was assessed by measuring concentrations of plasma homocysteine (tHcy). In addition, 24-h urine excretion of betaine and dimethylglycine were measured. Plasma folate and vitamin B_{12} concentrations were measured as well, as they affect homocysteine metabolism. Plasma total homocysteine (tHcy) was determined by a modification of the high-pressure liquid chromatographic method described by Ubbink *et al*. (1991). The modified mobile phase consisted of 0.37 mol/l acetate and 0.5 % methanol, pH 4.15. Plasma folate concentration was determined by the fluorescence polarization immunometric method (IMX; Abbott Laboratories, North Chicago, IL, USA). Plasma vitamin B_{12} concentration was analyzed by the Quantaphase II B_{12} radioassay (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Regarding hemostatic processes, factors included both in thrombotic and fibrinolytic processes were measured (factor VII, fibrinogen, D-dimer, PAI-1, von Willebrand factor). Hemostatic factors were analyzed by the laboratory of Red Cross, Helsinki, Finland. The analysis of plasma fibrinogen was performed with a functional method based on the bleeding time by using Fibri-Prest Automate 2 reagent (Diagnostica Stago, Asnières, France) and Thrombolyzer Compact analyzer (Behnk Elektronik, Norderstedt, Germany). Plasma D-dimer was analyzed by a turbidometric method (reagent Auto-Dimer, Biopool International, Umeå, Sweden; analyzer Konelab 60i Clinical Chemistry System, Labsystems CLD, Konelab, Finland). von Willebrand factor antigen was measured with STA-Liatest von Willebrand factor from Diagnostica Stago with STA Compact. STA Unicalibrator from Diagnostica Stago was used as a standard. PAI-1 was measured with Stachrom PAI using STA Compact. Factor VII was measured with the one-stage method using STA-Neoplastine CI plus and STA Deficient VII from Diagnostica Stago as reagents. The assays were performed with STA Compact analyzer using STA Unicalibrator from Diagnostica Stago as a standard.

The analysis of the food records was performed using the Micro-Nutrica software, v. 2.5 (Rastas *et al*., 1997), which is based on Finnish analyses and international food composition tables.

Statistical analyses

SPSS statistical analysis software was used to perform the statistical analyses (v. 14.0, SPSS Inc., Chicago, IL, USA). The normal distribution of the variables was analyzed by the Kolmogorov–Smirnov test with a Lilliefors' correction. General linear model was used to analyze the interactions of time and group during the study. Paired samples *t*-test and Student's *t*-test were used for further analyses. Variables, which did not reach normal distribution after arithmetic procedures, were analyzed using Wilcoxon signed ranks test (comparisons within the groups) and Mann–Whitney's *U*-test (comparisons between the groups). Spearman's correlation coefficient was calculated for correlations and

Cronbach's α for coefficient of reliability. Results are presented as mean ± s.d.

Results

In the betaine group, body weight was 64.7 ± 9.0 kg at 0 weeks and 64.5 ± 9.0 kg at week 24. The respective values for body mass index were 22.6 ± 2.4 vs 22.5 ± 2.5 kg/m^2, for systolic blood pressure 115 ± 7 vs 113 ± 7 mm Hg and for diastolic blood pressure 77 ± 5 vs 72 ± 7 mm Hg. The respective values in the control group were 65.7 ± 9.2 vs 66.3 ± 9.6 kg for body weight, 22.5 ± 2.2 vs 22.7 ± 2.2 kg/m^2 for body mass index, 117 ± 7 vs 114 ± 7 mm Hg for systolic blood pressure and 77 ± 5 vs 72 ± 7 mm Hg for diastolic blood pressure, respectively. There were no significant differences within or between the groups in these variables.

The food record data presented in Table 2 did not show any significant differences in nutrient intakes between the groups during the study.

Plasma betaine concentrations increased markedly in the betaine group during the first 8 weeks and stayed elevated until the end of the study. The concentration of plasma dimethylglycine, a metabolite of betaine, increased also in the betaine group. The excretion of betaine in urine increased in the betaine group as well (Table 3). No changes in these variables were found in the control group. The correlation between plasma and urine betaine was $r = 0.005$, $P = 0.970$ at the beginning of the intervention and $r = 0.813$, $P < 0.001$ at the end of the intervention. The coefficients of reliability of plasma and urine betaine were calculated as well. Cronbach's α was 0.838 for plasma betaine and 0.022 for urine betaine.

The interaction of time and group was of borderline significance (general linear model, $P = 0.058$) in plasma homocysteine concentration. It did not change in the betaine group (8.7 ± 2.3 vs 8.4 ± 1.5 μmol/l, 0 vs 24 weeks, respectively), but it tended to increase in the control group (8.9 ± 3.3 vs 9.4 ± 2.7 μmol/l, 0 vs 24 weeks, respectively, $P = 0.064$), resulting in a 12% difference of a borderline statistical significance between the groups at the end of the study ($P = 0.097$). Plasma hs-CRP concentration was 2.69 ± 5.56 vs 1.74 ± 1.89 mg/l in the betaine group and 1.59 ± 1.42 vs 2.54 ± 2.74 ml/l in the control group (0 vs 24

Table 3 Concentrations of plasma betaine and dimethylglycine, and urine betaine during the study

	Betaine (n = 32)	*Control (n = 31)*	P[a]
Plasma betaine (μmol/l)			
0 weeks	25.6 ± 9.6	22.6 ± 10.0	
8 weeks	166.0 ± 71.0	21.8 ± 11.5	
12 weeks	178.5 ± 81.7	22.7 ± 11.6	
24 weeks	187.3 ± 107.8[b]	24.4 ± 12.9[c]	<0.001
Plasma DMG (μmol/l)			
0 weeks	15.8 ± 3.3	16.2 ± 4.4	
24 weeks	51.8 ± 55.6[b]	17.6 ± 4.9[c]	<0.001
d-Urine betaine (μmol/l)			
0 weeks	82.0 ± 52.6	96.5 ± 64.3	
24 weeks	954.1 ± 1186.8[b,d]	70.4 ± 59.2[c,e]	NA
d-Urine betaine (μmol/mmol creatinine)			
0 weeks	9.2 ± 5.9	10.2 ± 6.6	
24 weeks	130.9 ± 199.4[b,d]	7.9 ± 8.4[c,e]	NA

Abbreviations: GLM, general linear model; NA, not analyzed due to an abnormal distribution of the variable.
[a]GLM, interaction of time and group.
[b]$P < 0.001$, difference within the group, 0 vs 24 weeks.
[c]$P < 0.001$, difference between the groups at week 24.
[d]Wilcoxon signed ranks test.
[e]Mann–Whitney's *U*-test.

Table 2 Nutrient intake of the subjects

	Betaine (n = 32)		*Control (n = 31)*	
	Before the study[a]	*During the study*[b]	*Before the study*[a]	*During the study*[b]
Energy (kJ)	8254 ± 1767	8268 ± 2069	8049 ± 1996	7959 ± 1537
Protein (E%)[c]	15.7 ± 2.8	15.8 ± 2.5	16.9 ± 3.0	16.9 ± 2.5
Carbohydrates (E%)	51.3 ± 7.0	50.1 ± 5.9	47.7 ± 7.0	48.0 ± 6.0
Fat (E%)	30.5 ± 6.5	31.6 ± 5.8	31.0 ± 6.7	32.2 ± 4.3
Saturated	11.2 ± 2.7	12.2 ± 3.0	11.7 ± 3.6	12.2 ± 2.5
Monounsaturated	10.6 ± 3.1	10.5 ± 2.4	10.6 ± 2.5	10.9 ± 2.1
Polyunsaturated	5.2 ± 1.7	5.3 ± 1.5	5.4 ± 1.5	5.6 ± 1.2
Alcohol (E%)	2.6 ± 4.0	1.8 ± 2.9	4.5 ± 5.1	2.9 ± 3.7
Fiber (g)	21 ± 9	21 ± 8	24 ± 10	25 ± 10
Cholesterol (mg)	209 ± 105	209 ± 92	217 ± 101	206 ± 83
Folate (μg)	289 ± 105	292 ± 84	313 ± 92	299 ± 92
Vitamin B_6 (mg)	1.8 ± 0.5	1.9 ± 0.5	1.9 ± 0.5	1.9 ± 0.5
Vitamin B_{12} (μg)	6.5 ± 5.2	5.1 ± 2.4	6.0 ± 3.7	5.8 ± 2.9

[a]One 4-day food record.
[b]Mean of three 4-day food records kept at weeks 3, 11 and 19.
[c]E%, percent of energy intake.

Table 4 Concentrations of serum total and lipoprotein lipids, apolipoproteins A-I and B and oxidized LDL at weeks 0 and 24

	Betaine (n = *32*)	*Control* (n = *31*)	P[a]
fS-cholesterol (mmol/l)			
0 weeks	4.4 ± 0.8	4.7 ± 0.8	
24 weeks	4.6 ± 0.8	4.6 ± 0.9	0.003
fS-HDL-chol (mmol/l)			
0 weeks	1.41 ± 0.30	1.61 ± 0.31	
24 weeks	1.47 ± 0.30	1.63 ± 0.36	NS
fS-LDL-chol (mmol/l)			
0 weeks	2.64 ± 0.70	2.63 ± 0.72	
24 weeks	2.74 ± 0.65	2.61 ± 0.74	0.003
Total-to-HDL-cholesterol ratio			
0 weeks	3.2 ± 0.7	3.0 ± 0.7	
24 weeks	3.2 ± 0.6	2.9 ± 0.6	0.062
fS-triglycerides (mmol/l)[b]			
0 weeks	0.87 ± 0.27	0.92 ± 0.30	
24 weeks	0.83 ± 0.27	0.86 ± 0.32	NS
Serum apolipoprotein A-1 (g/l)			
0 weeks	1.49 ± 0.28	1.69 ± 0.37	
24 weeks	1.56 ± 0.27[c]	1.69 ± 0.35	0.064
Serum apolipoprotein B (g/l)			
0 weeks	0.75 ± 0.18	0.76 ± 0.20	
24 weeks	0.78 ± 0.16	0.75 ± 0.18	NS
fP-oxidized LDL (U/l)			
0 weeks	55.2 ± 19.5	56.4 ± 19.6	
24 weeks	54.8 ± 15.3	53.1 ± 19.3	NS

Abbreviations: GLM, general linear model; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
[a]GLM, interaction of time and group.
[b]Logarithmized due to an abnormal distribution of the variable.
[c]Paired samples *t*-test, $P = 0.011$.

weeks, respectively) without a difference between or within the groups. In the concentrations of plasma cysteine, vitamin B_{12}, folate or urate, no changes were found during the study in either of the groups (data not shown).

There was a significant interaction of time and group in the general linear model analysis in serum total and LDL-cholesterol concentrations and total-to-HDL-cholesterol ratio without a significant difference in further statistical analyses between or within the groups. Concentrations of HDL-cholesterol, triglycerides or oxidized LDL did not change during the study (Table 4). Serum apolipoprotein A-I concentration increased in the betaine group, whereas no change was found in the control group. Apolipoprotein B concentration did not change during the study (Table 4).

Regarding hemostatic factors, there was a significant ($P = 0.035$) interaction of time and group in plasma fibrinogen concentration without a significant difference between or within the groups in further statistical analyses (betaine group 3.2 ± 0.6 vs 3.3 ± 0.7, control group 3.1 ± 0.7 vs 3.0 ± 0.4 g/l, 0 vs 24 weeks, respectively). PAI-1 concentration increased significantly in the betaine group (7.19 ± 2.91 vs 8.85 ± 3.78 AU/ml, 0 vs 24 weeks, $P = 0.028$), resulting in a significantly higher concentration in the betaine group as compared with the control group at the end of the study (8.85 + 3.78 vs 6.25 ± 2.38 AU/ml, 0 vs 24 weeks, $P = 0.006$). The proportion of plasma factor VII or serum D-dimer concentration did not change during the study (data not shown).

Regarding the safety parameters, there were no changes in the concentrations of serum creatinine, γ-glutamyltransferase or alanine aminotransferase (data not shown). There was a significant interaction of time and group ($P = 0.007$) in blood hemoglobin concentration without significant differences between or within the groups in further statistical analyses (betaine group 137 ± 12 vs 130 ± 13, control group 132 ± 13 vs 131 ± 12 g/l, 0 vs 24 weeks, respectively). Blood hematocrit or number of leucocytes or thrombocytes did not change during the study (data not shown).

Discussion

The present study was planned to give pertinent information about the long-term effects of betaine on several risk factors of atherosclerotic vascular diseases (serum total and lipoprotein lipid concentrations, blood pressure, plasma homocysteine concentration and hemostatic factors) and to assess the safety of long-term betaine intake in healthy subjects.

The increase in plasma betaine concentration and urine betaine excretion found in this study indicated excellent compliance of the subjects during the study, as the changes found in these variables in the betaine group are impossible to reach without betaine supplementation. Plasma concentrations are usually about 30 μmol/l, ranging from 9 to 90 μmol/l (Ueland *et al*., 2005). However, the intra-individual variability is small without betaine supplementation (Lever *et al*., 2004).

In some studies, betaine supplementation has been shown to increase serum LDL-cholesterol concentrations in healthy subjects (Olthof *et al*., 2005), subjects on a weight-loss diet (Schwab *et al*., 2002) and also in subjects with chronic renal failure (McGregor *et al*., 2002). The mechanism by which betaine might increase serum LDL-cholesterol concentration has been proposed to be the increased synthesis and export of lipids in VLDL from the liver into the circulation. This same mechanism is suggested to be behind the finding that betaine attenuates the development of alcoholic fatty liver in rats (Kharbanda *et al*., 2009) and decreases lipid accumulation in the liver in a transgenic mice model on hyperhomocysteinemia (Schwahn *et al*., 2007). In the present study, betaine supplementation did not increase serum LDL-cholesterol concentration statistically significantly. The concentration of apolipoprotein B, which is the only apolipoprotein of LDL, did not change in the betaine group, indicating that the number of LDL particles did not change either. The reason for a statistically non-significant finding

in this study regarding serum LDL-cholesterol concentration might be due to the normal lipid profile of the subjects, and the fact that the subjects were healthy and quite young. The possible increase in VLDL output from the liver may not cause a clinically measurable outcome in this kind of a group of subjects.

Betaine supplementation has been reported to have both acute (Schwab *et al.*, 2006) and longer term (Schwab *et al.*, 2002; Steenge *et al.*, 2003) lowering effect on plasma homocysteine concentration in humans. Doses in the range of dietary intake are also effective (Olthof *et al.*, 2003). Unlike previous results, plasma homocysteine concentration did not decrease significantly in the betaine group in this study. However, in the control group, plasma homocysteine concentration increased. This increase in plasma homocysteine concentration might be explained by the seasonal variation. So, the betaine supplementation might have prevented the increase of plasma homocysteine concentration found in the control group, resulting in a somewhat lower plasma homocysteine concentration in the betaine group at the end of the study. Furthermore, it is noteworthy that the plasma homocysteine concentration of the subjects was at the lower range. It has been shown that the association of betaine with fasting plasma homocysteine concentration is more pronounced in subjects with low-serum folate concentration (Ueland *et al.*, 2005). Furthermore, folic acid supplementation has been shown to increase plasma betaine concentration (Melse-Boonstra *et al.*, 2005). In the present study, the nutrient intake analysis showed the folate intake being close to the level recommended for men (300 μg/day), whereas the recommendation for women in fertile age (400 μg/day) was not reached.

Inflammation is included in the atherosclerotic process and hs-CRP is a marker of low-grade systemic inflammation (Pradhan and Ridker, 2002). In the present study, betaine was found to have no effect on hs-CRP. In a recent cross-sectional survey, betaine intake was found to be negatively associated with CRP, tumor necrosis factor-α and interleukin-6 (Detopoulou *et al.*, 2008). In the rather young subjects participating in the present study, changes in indicators of low-systemic inflammation might be difficult to detect. Regarding hemostatic factors, betaine was not found to have any effect except an increase in PAI-1 concentration in the betaine group. Increased PAI-1 concentration may slow down the fibrinolytic process. The levels of PAI-1 were quite low in the present study, and the clinical significance of low levels in healthy subjects may be considered to be of limited value.

The safety parameters including blood count, and concentrations of serum creatinine, alanine aminotransferase and γ-glutamyltransferase indicated no adverse effects by the use of betaine. Betaine has been reported to lower hepatic steatosis in patients with non-alcoholic steatohepatitis and reduce liver transaminases, for example alanine aminotransferase and γ-glutamyltransferase (Miglio *et al.*, 2000; Abdelmalek *et al.*, 2001). Betaine serves as a lipotrope, that is prevents or reduces accumulation of fat in the liver (Craig, 2004). In rats, betaine has been shown to protect the liver from oxidative stress and steatosis. The mechanism might be its effect on the transsulfuration reactions (Kwon *et al.*, 2009).

In conclusion, betaine supplementation did not have a significant effect on serum lipid profile, and it is safe in a long-term use in healthy subjects.

Conflict of interest

The authors declare no conflict of interest.

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References

Abdelmalek MF, Angulo P, Jorgensen RA, Sylvestre PB, Lindor KD (2001). Betaine, a promising new agent for patients with nonalcoholic steatohepatitis: results of a pilot study. *Am J Gastroenterol* **96**, 2711–2717.

Ahotupa M, Ruutu M, Mäntylä E (1996). Simple methods of quantifying oxidation products and antioxidant potential of low density lipoproteins. *Clin Biochem* **29**, 139–144.

Chambers JC, Obeid OA, Refsum H, Ueland P, Hackett D, Hooper J *et al* (2000). Plasma homocysteine concentrations and risk of coronary heart disease in UK Indian Asian and European men. *Lancet* **355**, 523–527.

Craig SA (2004). Betaine in human nutrition. *Am J Clin Nutr* **80**, 539–549.

Detopoulou P, Panagiotakos DB, Antonopoulou S, Pitsavos C, Stefanadis C (2008). Dietary choline and betaine intakes in relation to concentrations of inflammatory markers in healthy adults: the ATTICA study. *Am J Clin Nutr* **87**, 424–430.

El-Khairy L, Ueland PM, Nygard O, Refsum H, Vollset SE (1999). Lifestyle and cardiovascular disease risk factors as determinants of total cysteine in plasma: the Hordaland Homocysteine Study. *Am J Clin Nutr* **70**, 1016–1024.

Eussen SJPM, Ueland PM, Clarke R, Blom HJ, Hoefnagels WHL, van Staeveren WA *et al.* (2007). The association of betaine, homocysteine and related metabolites with cognitive function in Dutch elderly people. *Br J Nutr* **98**, 960–968.

Humphrey LL, Fu R, Rogers K, Freeman M, Helfand M (2008). Homocysteine level and coronary heart disease incidence: a systematic review and meta-analysis. *Mayo Clin Proc* **83**, 1203–1212.

Kharbanda KK, Todero SL, Ward BW, Cannella III JJ, Tuma DJ (2009). Betaine administration corrects ethanol-induced defective VLDL secretion. *Mol Cell Biochem* **327**, 75–78.

Konstantinova SV, Tell GS, Vollset SE, Nygård O, Bleie Ø, Ueland PM (2008). Divergent associations of plasma choline and betaine with components of metabolic syndrome in middle age and elderly men and women. *J Nutr* **138**, 914–920.

Kwon DY, Jung YS, Kim SJ, Park HK, Park JH, Kim YC (2009). Impaired sulfur-amino acid metabolism and oxidative stress in nonalcoholic fatty liver are alleviated by betaine supplementation in rats. *J Nutr* **139**, 63–68.

Lever M, Bason L, Leaver C, Hayman CM, Chambers ST (1992). Same-day batch measurement of glycine, betaine, carnitine and other betaines in biological material. *Anal Biochem* **205**, 14–21.

Lever M, Sizeland PC, Frampton CM, Chambers ST (2004). Short and long-term variation of plasma glycine betaine concentrations in humans. *Clin Biochem* **37**, 184–190.

Mar MH, Ridky TW, Garner SC, Zeisel SH (1995). A method for the determination of betaine in tissues using high performance liquid chromatography. *J Nutr Biochem* **6**, 392–398.

McGregor DO, Dellow WJ, Robson RA, Level M, George PM, Chambers ST (2002). Betaine supplementation decreases post-methionine hyperhomocysteinemia in chronic renal failure. *Kidney Int* **61**, 1040–1046.

Melse-Boonstra A, Holm PI, Ueland PM, Olthof M, Clarke R, Verhoef P (2005). Betaine concentration as a determinant of fasting total homocysteine concentrations and the effect of folic supplementation on betaine concentrations. *Am J Clin Nutr* **81**, 1378–1382.

Meigs JB, Jacques PF, Selhub J, Singer DE, Nathan DM, Rifai N *et al*. (2001). Fasting plasma homocysteine levels in the insulin resistance syndrome: the Framingham offspring study. *Diabetes Care* **24**, 1403–1410.

Miglio F, Rovati LC, Santoro A, Setnikar I (2000). Efficacy and safety of oral betaine glucuronate in non-alcoholic steatohepatitis. A double-blind, randomized, parallel-group, placebo-controlled prospective clinical study. *Arzneimittelforschung* **50**, 722–727.

Olthof MR, van Vliet T, Boelsma E, Verhoef P (2003). Low dose betaine supplementation leads to immediate and long term lowering of plasma homocysteine in healthy men and women. *J Nutr* **133**, 4135–4138.

Olthof MR, van Vliet T, Verhoef P, Zock PL, Katan MB (2005). Effect of homocysteine-lowering nutrients on blood lipids: results from four randomised, placebo-controlled studies in healthy humans. *PLoS Med* **2**, e135.

Pradhan AD, Ridker PM (2002). Do atherosclerosis and type 2 diabetes share a common inflammatory basis? *Eur Heart J* **23**, 831–834.

Rastas M, Seppänen R, Knuts L-R, Hakala P, Karttila V (eds.) (1997). *Nutrient Composition of Foods*. Publications of the Social Insurance Institution: Helsinki, Finland.

Schwab U, Törrönen A, Toppinen L, Alfthan G, Saarinen M, Aro A *et al*. (2002). Betaine supplementation decreases plasma homocysteine concentrations but does not affect body weight, body composition, or resting energy expenditure in human subjects. *Am J Clin Nutr* **76**, 961–967.

Schwab U, Törrönen A, Meririnne E, Saarinen M, Alfthan G, Aro A *et al*. (2006). Orally administered betaine has an acute and dose-dependent effect on serum betaine and plasma homocysteine concentrations in healthy humans. *J Nutr* **136**, 34–38.

Schwahn BC, Wang XL, Mikael LG, Wu Q, Cohn J, Jiang H *et al.* (2007). Betaine supplementation improves the atherogenic risk factor profile in a transgenic mouse model of hyperhomocysteinemia. *Atherosclerosis* **195**, e100–e107.

Singh RH, Kruger WD, Wang L, Pasquali M, Elsas II LJ (2004). Cystathionine beta-synthase deficiency: effects of betaine supplementation after methionine restriction in B6-nonresponsive homocystinuria. *Genet Med* **6**, 90–95.

Steenge GR, Verhoef P, Katan MB (2003). Betaine supplementation lowers plasma homocysteine in healthy men and women. *J Nutr* **133**, 1291–1295.

Ubbink JB, Vermaak WJH, Bissbort S (1991). Rapid high-performance liquid chromatographic assay for total homocysteine levels in human serum. *J Chromatogr* **565**, 441–446.

Ueland PM, Holm PI, Hustad S (2005). Betaine: a key modulator of one-carbon metabolism and homocysteine status. *Clin Chem Lab Med* **43**, 1069–1075.

Wilcken DE, Wilcken B, Dudman NP, Tyrell PA (1983). Homocystinuria—the effects of betaine in the treatment of patients not responsive to pyridoxine. *N Engl J Med* **309**, 448–453.