Betaine supplementation decreases plasma homocysteine concentrations but does not affect body weight, body composition, or resting energy expenditure in human subjects[1–3]

Ursula Schwab, Anneli Törrönen, Leena Toppinen, Georg Alfthan, Markku Saarinen, Antti Aro, and Matti Uusitupa

ABSTRACT

Background: Betaine (trimethylglycine) is found in several tissues in humans. It is involved in homocysteine metabolism as an alternative methyl donor and is used in the treatment of homocystinuria in humans. In pigs, betaine decreases the amount of adipose tissue.

Objective: The aim of the study was to examine the effect of betaine supplementation on body weight, body composition, plasma homocysteine concentrations, blood pressure, and serum total and lipoprotein lipids.

Design: Forty-two obese, white subjects (14 men, 28 women) treated with a hypoenergetic diet were randomly assigned to a betaine-supplemented group (6 g/d) or a control group given placebo for 12 wk. The intervention period was preceded by a 4-wk run-in period with a euenergetic diet.

Results: Body weight, resting energy expenditure, and fat mass decreased significantly in both groups with no significant difference between the groups. Plasma homocysteine concentrations decreased in the betaine group ($\bar{x}$ ± SD: 8.76 ± 1.63 μmol/L at 4 wk, 7.93 ± 1.52 μmol/L at 16 wk; $P = 0.030$ for the interaction of time and treatment). Diastolic blood pressure decreased without a significant difference between the groups. Serum total and LDL-cholesterol concentrations were higher in the betaine group than in the control group ($P < 0.05$).

Conclusion: A hypoenergetic diet with betaine supplementation (6 g daily for 12 wk) decreased the plasma homocysteine concentration but did not affect body composition more than a hypoenergetic diet without betaine supplementation did. *Am J Clin Nutr* 2002;76:961–7.

KEY WORDS Betaine, blood pressure, body weight, body composition, cholesterol, energy expenditure, folate, homocysteine, humans, lipids, liver enzymes, obesity

INTRODUCTION

Betaine (trimethylglycine) is found naturally in most living organisms. It protects plants (1), microbes (2), and marine and freshwater invertebrates (3) under osmotic stress and acts as an osmolyte in mammalian tissues (4, 5). Betaine is formed in cells as an oxidation product of choline and can be obtained externally from food, eg, spinach and beets (6). The physiologic amount of betaine in man varies in different tissues. The concentration of betaine in human serum is ≈20–60 μmol/L, whereas in the liver and kidney, some cells have accumulated greater concentrations (7, 8).

Betaine has been used safely in animal feeds for > 25 y. Betaine was first used in fish feeds, especially for salmon and trout, as an attractant and an osmoprotectant during the freshwater-seawater transfer stage (9). Currently, it is also used as an additive in poultry and pig feed, where it functions as a methyl donor and osmoprotectant. There is evidence that betaine can decrease the amount of fat tissue in pigs without affecting the amount of lean tissue (10). The effect of betaine on body composition in humans has not been investigated.

High total concentrations of plasma homocysteine have been shown to be a potential risk factor for cardiovascular diseases (11, 12). Betaine is involved in homocysteine metabolism by the action of the enzyme betaine homocysteine methyltransferase (EC 2.1.1.5) (13). Betaine donates methyl groups to homocysteine, which in turn is metabolized to methionine. Betaine itself is metabolized to glycine via dimethyglycine and sarcosine.

Because betaine may help in the treatment of excess body fat and elevated serum homocysteine concentrations, the aim of the present study was to examine the effect of betaine supplementation on body weight, body composition, and resting energy expenditure in healthy obese subjects. We also examined the effects of betaine supplementation on concentrations of plasma homocysteine and serum total and lipoprotein lipids as well as on blood pressure.

SUBJECTS AND METHODS

Subjects

Forty-six obese, white subjects (14 men, 32 women) without any chronic disease and normal liver, kidney, and thyroid function were recruited for the study. Three subjects dropped out during the run-in period and one at the beginning of the intervention

[1] From the Department of Clinical Nutrition, University of Kuopio, Kuopio, Finland (US, LT, and MU); Danisco Ltd, Espoo, Finland (AT); the National Public Health Institute, Helsinki (GA and AA); and Danisco Cultor Innovation, Kantvik, Finland (MS).

[2] Supported by Cultor Research Foundation, Espoo, Finland.

[3] Address reprint requests to U Schwab, Department of Clinical Nutrition, University of Kuopio, PO Box 1627, 70211 Kuopio, Finland. E-mail: ursula.schwab@uku.fi.

Received May 2, 2001.

Accepted for publication December 12, 2001.

TABLE 1
Baseline characteristics of the subjects[1]

	Control group (n = 7 M, 13 F)	Betaine group (n = 7 M, 15 F)
Age (y)	44.9 ± 8.6	43.5 ± 9.1
Body weight (kg)	95.0 ± 10.2	95.9 ± 11.4
BMI (kg/m^2)	33.4 ± 3.1	33.6 ± 3.1
Systolic blood pressure (mm Hg)	130 ± 20	126 ± 10
Diastolic blood pressure (mm Hg)	88 ± 12	87 ± 7
Serum lipids (mmol/L)[2]		
Cholesterol	5.10 ± 0.60	5.72 ± 0.97
Triacylglycerols	1.59 ± 0.72	1.81 ± 0.85
Plasma glucose (mmol/L)[2]	5.85 ± 0.57	5.78 ± 0.59
Serum alanine aminotransferase (U/L)[2]	34.8 ± 20.6	37.2 ± 27.7
Serum alkaline phosphatase (U/L)[2]	153.6 ± 34.6	155.3 ± 36.7
Serum glutamyl transferase (U/L)[2]	36.8 ± 36.1	32.2 ± 18.1
Serum creatinine (μmol/L)[2,3]	84.0 ± 12.0	87.1 ± 9.7
Serum thyroid-stimulating hormone (mU/L)[2,3]	1.8 ± 1.0	1.8 ± 0.7

[1] $\bar{x}$ ± SD.
[2] Fasting sample.
[3] Measured at screening. There were no significant differences between the groups.

period because of a lack of motivation. Therefore, the final number of subjects was 42 (14 men, 28 women). The baseline characteristics of the subjects are presented in **Table 1**.

For body mass index (BMI, in kg/m^2), the inclusion criterion was BMI 28–40; for age, it was 25–60 y. The inclusion criteria for fasting serum concentrations were < 3.5 mmol/L for total triacylglycerols, < 7.5 mmol/L for total cholesterol, and < 6.7 mmol/L for plasma glucose. Subjects taking lipid-lowering medication were excluded, as were subjects who had recently (< 3 mo) attempted to lose weight. Perimenopausal women were also excluded. Four women in the control group and 6 in the betaine group were postmenopausal. None of the participants were taking antihypertensive medication. The subjects were not allowed to use nutrient supplements during the study or for 1 mo before the beginning of the study.

The subjects gave written, informed consent. The study protocol was approved by the Ethics Committee of the University of Kuopio and Kuopio University Hospital.

Study design

The study was a placebo-controlled, randomized, double-blind parallel study. Before the 12-wk intervention period, the subjects participated in a 4-wk run-in period during which they consumed 2 daily servings of 100 g orange juice with 6 g grapefruit juice (one serving in the morning and the other in the evening). The subjects consumed a euenergetic diet during the run-in period. For the intervention period, during which the subjects consumed a hypoenergetic diet (providing 2100 kJ, or 500 kcal, less than their euenergetic diet), the subjects were randomly assigned to 1 of 2 groups; 22 subjects were placed in the betaine group and 20 in the control group. The subjects were matched for BMI, sex, and menstrual cycle (menopausal status). The hypoenergetic diet consumed during the intervention period was used for motivational purposes, because the subjects were obese and willing to lose weight.

The control group consumed 100 g orange juice twice a day and the betaine group consumed 100 g betaine-enriched orange juice twice a day. The amount of betaine in the enriched juice was 3 g per 100 g orange juice, for a betaine dose of 6 g/d.

The subjects visited the research unit at the beginning of the study (0 wk), at the beginning of the intervention period (4 wk), and 4 times during the intervention period: at 6, 8, 12, and 16 wk. Body weight was measured at each visit. We measured resting energy expenditure and body composition at the 4- and 16-wk visits. Blood pressure, anthropometric measurements, serum total and lipoprotein lipids, and liver enzymes were measured at the 4-, 8-, 12-, and 16-wk visits. Plasma glucose and homocysteine concentrations, serum betaine concentration, and concentrations of erythrocyte and plasma folate were measured at the 4-, 8-, and 16-wk visits. The subjects met with a nutritionist at the 4-, 6-, and 8-wk visits and also at 12 wk if necessary. The nutrition counseling concentrated on reducing the energy content of the diet, modifying the quality of fat, and increasing the intake of dietary fiber.

To monitor the diet, the subjects kept a 4-d food record during the second week of the run-in period and during the second and fourth weeks of the intervention period (weeks 5 and 7). The food records were calculated by use of MICRO-NUTRICA dietary analysis software (The Social Insurance Institution, Helsinki) on the basis of Finnish food analyses and international food-composition tables (14).

Methods

Body weight was measured on the same calibrated electronic scale throughout the study. Resting energy expenditure was measured with the use of indirect calorimetry (Deltatrac metabolic monitor; Datex/Instrumentarium Corp, Helsinki) after a 12-h fast. The results were adjusted for overnight nitrogen excretion. Body composition was measured by bioelectrical impedance (BIA 101S with BODYGRAM software; Akern Srl Bioresearch, Florence, Italy) and as a sum of 4 skinfold thicknesses (biceps, triceps, subscapular, and suprailiac) measured with skinfold calipers. The waist circumference was measured halfway between the lowest rib and the iliac crest, and the hip circumference was measured at the broadest part of the hip.

Blood pressure was measured with the use of a mercury sphygmomanometer on the right arm after the subject rested for 5 min in the sitting position. A second measurement was performed at 10 min, and the mean of the 2 measurements was calculated and used for further analyses.

All venous blood samples were drawn after an overnight (12-h) fast. For the analysis of serum total and lipoprotein lipid concentrations, samples underwent ultracentrifugation for 18 h at 4 °C, 144 000 × g, and a density of 1.006 kg/L to remove VLDL. HDL in the infranatant fluid was separated from LDL by the precipitation of LDL with dextran sulfate and magnesium chloride (15). The LDL-cholesterol concentration was calculated as the difference between the mass of cholesterol in the infranatant fluid and the HDL. Enzymatic colorimetric methods and commercial kits (Monotest cholesterol and Triacylglycerol GPO-PAP; Boehringer Mannheim GmbH Diagnostica, Mannheim, Germany) were used to measure cholesterol and triacylglycerol concentrations from whole serum and separated lipoproteins with an automated instrument (Kone Pro; Kone Instruments, Espoo, Finland).

Serum betaine concentration was analyzed by the use of HPLC as a 4-bromophenacyl triflate derivative (16). Calibration was performed by the standard addition method by spiking a pooled serum sample with standards. The method described by Mar et al (17) was used in synthesizing 4-bromophenacyl triflate.

TABLE 2
Intakes of energy, energy nutrients, dietary fiber, cholesterol, and folic acid during the run-in and intervention periods[1]

	Run-in period[2]		Intervention period[3]	
	Control group (n = 20)	Betaine group (n = 22)	Control group (n = 20)	Betaine group (n = 22)
Energy				
(MJ)	8.9 ± 2.0	8.5 ± 2.4	6.5 ± 1.5	6.5 ± 1.4
(kcal)	2125 ± 476	2045 ± 565	1560 ± 352	1545 ± 337
Fat (% of energy)	32.2 ± 5.2	30.9 ± 5.2	27.4 ± 4.3	27.4 ± 4.2
Fatty acids (% of energy)				
Saturated	13.8 ± 2.7	12.7 ± 2.3	10.6 ± 1.7	10.1 ± 1.6
Monounsaturated	10.2 ± 2.4	9.7 ± 2.3	9.2 ± 2.3	9.3 ± 2.0
Polyunsaturated	4.2 ± 1.1	4.7 ± 1.4	4.5 ± 0.9	4.9 ± 1.2
Linoleic	2.2 ± 0.9	2.5 ± 1.0	2.5 ± 0.7	2.6 ± 0.7
α-Linolenic	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.2	0.5 ± 0.2
Protein (% of energy)	17.3 ± 3.6	17.1 ± 2.7	17.5 ± 3.1	18.1 ± 1.9
Carbohydrates (% of energy)	47.6 ± 6.8	49.2 ± 5.5	51.4 ± 5.8	52.1 ± 4.3
Alcohol (% of energy)	1.5 ± 4.7	1.4 ± 2.4	2.3 ± 6.1	1.0 ± 1.9
Fiber (g/MJ)	2.6 ± 0.8	3.1 ± 0.9	3.7 ± 0.8	4.1 ± 1.0
Cholesterol (mg/MJ)	33.4 ± 7.7	32.0 ± 9.9	27.3 ± 5.8	28.2 ± 8.4
Folic acid (μg)	357 ±100	346 ± 83	303 ± 55	335 ± 89

[1] $\bar{x}$ ± SD. There were no significant differences between the groups.
[2] One 4-d food record.
[3] Mean of two 4-d food records.

Total plasma homocysteine was determined with a modification of the HPLC method described by Ubbink et al (18). The modified mobile phase consisted of 0.37 mol acetate/L and 0.5% methanol, pH 4.15. The peak heights were calibrated with the use of a secondary serum standard. The precision between batches (n = 5) for an in-house serum pool was 4.8% at a concentration of 12.3 μmol serum homocysteine/L. The accuracy was verified by participating in an interlaboratory quality-control scheme in which the mean bias was 2.2% for 12 sera ranging from 9.4 to 83 μmol serum homocysteine/L (19).

Folate concentration of plasma and erythrocytes was determined by the fluorescence polarization immunometric method (IMX; Abbott Laboratories, North Chicago, IL). The precision between batches (n = 13) was 5.0%.

Plasma glucose concentration was analyzed by the glucose hydrogenase method (Granutest 250; Merck, KgaA, Darmstadt, Germany). The Kone Pro automated instrument was used to measure the concentrations. Plasma insulin concentration was analyzed by a radioimmunoassay method (Phadeseph Insulin RIA 100; Pharmacia Diagnostics, Uppsala, Sweden).

The laboratory personnel were unaware of the randomization group. The subjects were given forms for self-reporting on the level of their physical activity and on the occurrence of side effects. Both were reported 4 times during the intervention period (every 4 wk). The adverse side effects monitored included gastrointestinal illnesses, thirst, feeling of dry mouth, increased urination, and increased preference for salt or salty food.

Statistical analyses

The data were analyzed with the SPSS/PC+ statistics program (V8.0; SPSS Inc, Chicago). Before further analyses, normal distribution of the variables was checked with the Kolmogorov-Smirnov test with Lilliefors significance correction. Variables with abnormal distribution [thyroid-stimulating hormone (TSH), γ-glutamyl transferase (EC 2.3.2.9), alanine aminotransferase (EC 2.6.1.2), asparagine aminotransferase (EC 2.6.1.1), and triacylglycerol] were log transformed, and the log values were used in further analyses. Two-factor repeated-measures analysis of variance (general linear model in SPSS) was used to examine the interaction between time and treatment and to test for changes within time and treatment. When the interaction of time and treatment was significant, the two-tailed t test was used for comparisons within the groups. Student's t test was used for between-group comparisons. When the interaction of time and treatment was not significant, the main effect of time was reported. Two-factor repeated-measures analysis of variance (general linear model) with ranks was used to test the changes in fat-free mass (FFM, in kg), FFM (%), and fat mass (%) as measured by bioelectrical impedance because the distribution of these variables did not become normal by log transformation or other arithmetic procedures. The Bonferroni correction was used for multiple comparisons to control the overall alpha level. All data are expressed as mean ± SD. $P < 0.05$ was considered significant.

RESULTS

Dietary data

The dietary goals were reached. The decrease in energy intake was ≈2 MJ (500 kcal). The proportion of energy obtained from fat decreased, and the amount of saturated fat, in particular, decreased. There were no significant differences in the nutrient intake between the groups during the intervention period (**Table 2**).

Body weight, body composition, and resting energy expenditure

The main effect of time was a significant ($P < 0.001$) difference in body weight, BMI, waist circumference, and resting energy expenditure in both groups during the intervention (**Table 3**). The main effect of time was a significant ($P < 0.002$) difference in triceps, biceps, and suprailiac skinfold thicknesses. Fat mass and FFM calculated from skinfold thicknesses decreased

TABLE 3
Body weight, BMI, anthropometric measures, body composition, and resting energy expenditure (REE) at the beginning (4 wk) and the end (16 wk) of the intervention period in both groups[1]

	Control group (n = 20)		Betaine group (n = 22)		P for the main effect of time[2]
	4 wk	16 wk	4 wk	16 wk	
Body weight (kg)	94.6 ± 9.9	91.1 ± 8.7	95.7 ± 11.3	93.5 ± 11.1	0.0001
BMI (kg/m^2)	33.2 ± 3.2	32.1 ± 3.0	33.5 ± 3.2	32.8 ± 3.7	0.0001
Waist circumference (cm)	107.3 ± 9.6	103.2 ± 9.9	107.7 ± 9.7	105.1 ± 9.5	0.0001
Hip circumference (cm)	112.8 ± 6.3	111.2 ± 5.9	113.5 ± 7.8	112.7 ± 8.1	0.001
Skinfold thickness (mm)					
Triceps	27.7 ± 6.3	25.1 ± 7.0	27.2 ± 7.4	25.0 ± 7.6	0.0001
Biceps	18.7 ± 7.0	16.4 ± 7.4	20.1 ± 7.1	19.2 ± 8.6	0.002
Subscapular	31.0 ± 6.1	32.0 ± 6.8	28.5 ± 7.0	28.4 ± 8.0	NS
Suprailiac	26.0 ± 6.5	20.8 ± 5.7	26.0 ± 7.8	22.1 ± 7.4	0.0001
Body composition					
Skinfold thickness					
Fat mass (kg)	35.4 ± 6.3	32.9 ± 6.5	35.5 ± 7.2	33.7 ± 8.5	0.0001
Fat-free mass (kg)	59.1 ± 9.9	58.2 ± 9.7	59.1 ± 9.6	58.5 ± 9.0	0.001
Bioimpedance					
Fat mass					
(kg)	34.3 ± 6.6	31.8 ± 6.8	35.7 ± 8.2	33.9 ± 8.7	0.0001
(%)	36.5 ± 6.8	35.1 ± 7.6	37.3 ± 7.2	36.2 ± 7.9	NS
Fat-free mass					
(kg)	60.2 ± 10.6	59.3 ± 10.3	60.0 ± 9.8	59.6 ± 9.6	NS
(%)	63.5 ± 6.8	64.9 ± 7.6	62.7 ± 7.2	63.8 ± 7.9	NS
REE					
(kJ/24 h)	7244 ± 941	6826 ± 836	7231 ± 869	6776 ± 656	0.0001
(kcal/24 h)	1733 ± 225	1633 ± 200	1730 ± 208	1621 ± 157	0.0001

[1] $\bar{x}$ ± SD. There were no significant differences between the groups.
[2] There was no significant interaction of time and treatment.

significantly in both groups ($P < 0.001$ for the main effect of time). The measurements performed with bioelectrical impedance showed a significant decrease ($P < 0.001$ for the main effect of time) in the fat mass expressed in kg. There were no significant differences between the groups in any of these variables during the intervention.

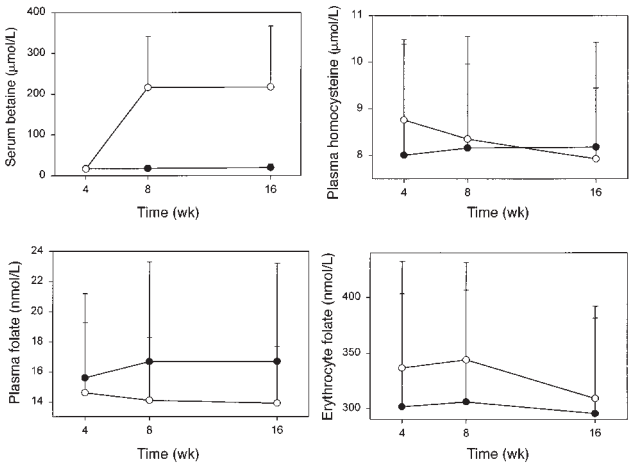

FIGURE 1. Mean (±SD) concentrations of serum betaine, plasma homocysteine, and plasma and erythrocyte folate during the intervention. Betaine group (n = 22), ○; control group (n = 20), ●. For the interaction of time and treatment, $P < 0.0001$ for serum betaine concentrations and $P = 0.030$ for plasma homocysteine concentrations. In the betaine group, $P < 0.001$ for betaine concentrations and $P < 0.05$ for homocysteine concentrations at 4 and 16 wk, respectively. There was no significant interaction of time and treatment in plasma or erythrocyte folate concentrations.

Homocysteine and folate concentrations

In the betaine group, mean (±SD) plasma homocysteine concentrations were 8.76 ± 1.63 μmol/L (at 4 wk) and 7.93 ± 1.52 μmol/L (at 16 wk; $P < 0.05$), and in the control group, the concentrations were 8.01 ± 2.47 and 8.18 ± 2.25 μmol/L, respectively ($P = 0.030$ for the interaction of time and treatment). There was no significant difference in the fasting plasma or erythrocyte folate concentration between the groups. There were no significant changes within the groups in these variables (**Figure 1**).

Blood pressure and serum total and lipoprotein lipids

Diastolic blood pressure decreased significantly ($P < 0.0001$ for the main effect of time; **Table 4**), but there was no significant difference between the groups during the intervention period. Systolic blood pressure did not change significantly in either of the groups or differ significantly between the groups during the intervention (Table 4).

No significant changes in serum total or lipoprotein lipid concentrations were found in either of the groups, but a significant ($P < 0.05$) difference in serum total and LDL-cholesterol concentrations during the intervention was found between the groups (Table 4).

Serum betaine concentration

Fasting serum betaine concentration increased in the betaine group ($P < 0.001$) but did not change significantly in the control group ($P < 0.0001$ for the interaction of time and treatment). In the betaine group, the serum betaine concentration was > 10-fold that in the placebo group. The concentrations of betaine remained stable, at ≈20 μmol/L, in subjects in the control group (Figure 1).

TABLE 4
Blood pressure and serum total lipid and lipoprotein cholesterol concentrations at the beginning (4 wk) and the end (16 wk) of the intervention period in both groups[1]

	Control group (n = 20)		Betaine group (n = 22)		P for the interaction of time and treatment	P for the main effect of time[2]
	4 wk	16 wk	4 wk	16 wk		
Blood pressure (mm Hg)						
Systolic	127.4 ± 17.5	126.8 ± 18.1	122.5 ± 9.5	121.1 ± 9.4	NS	NS
Diastolic	86.1 ± 10.8	83.7 ± 11.7	85.4 ± 7.9	80.5 ± 7.1	NS	0.0001
Serum cholesterol (mmol/L)						
Total	5.2 ± 0.8	4.9 ± 0.6	5.4 ± 0.9	5.5 ± 1.1[3]	0.009	
HDL	1.2 ± 0.2	1.2 ± 0.3	1.2 ± 0.3	1.2 ± 0.3	NS	NS
LDL	3.3 ± 0.6	3.0 ± 0.5	3.5 ± 0.8	3.7 ± 1.0[3]	0.011	
Serum triacylglycerols (mmol/L)	1.5 ± 0.6	1.4 ± 0.7	1.7 ± 0.5	1.6 ± 0.8	NS	NS

[1] $\bar{x}$ ± SD.
[2] There were no significant differences between the groups.
[3] Significantly different from control group at week 16, $P < 0.05$.

Liver enzymes

Fasting serum asparagine aminotransferase, alanine aminotransferase, and γ-glutamyl transferase concentrations decreased in both groups without a significant difference between the groups during the intervention period ($P < 0.05$ for the main effect of time; **Table 5**). The fasting serum alkaline phosphatase concentration showed a tendency to increase in the control group but not in the betaine group ($P = 0.008$ for the main effect of time).

Other laboratory measurements

Fasting plasma glucose concentration did not change significantly in either of the groups during the intervention period. The mean (± SD) values in the betaine group were 5.77 ± 0.50 and 5.74 ± 0.54 mmol/L, and those in the control group were 5.91 ± 0.71 and 5.75 ± 0.66 mmol/L at 4 and 16 wk, respectively.

Serum TSH and creatinine concentrations were measured at the screening and at the end of the study (16 wk). There were no significant differences in these variables in either of the groups. Mean (± SD) serum TSH values for the control group were 1.8 ± 1.0 mU/L at screening and 1.6 ± 0.7 mU/L at 16 wk, and those for the betaine group were 1.8 ± 0.7 and 1.6 ± 0.7 mU/L, respectively. Mean (± SD) serum creatinine values were 84.0 ± 12.0 and 85.2 ± 11.8 μmol/L in the control group and 87.1 ± 9.7 and 87.1 ± 10.6 μmol/L in the betaine group at screening and at 16 wk, respectively.

Physical activity and side effects

There was no significant difference in the level of physical activity between the groups. The mean frequency was 2–3 times/wk during the intervention period in both groups. No adverse side effects were systematically reported by any of the subjects in either of the groups.

DISCUSSION

The results of the present study show that a daily supplement of 6 g betaine does not have any effect beyond that of a hypoenergetic diet on body weight, BMI, anthropometric measures, body composition, or resting energy expenditure in obese subjects. No previous studies of the effect of betaine on body composition were performed in humans. Betaine has been used as a dietary supplement in pigs, and the amount of fat tissue has been shown to decrease in these animals without an effect on FFM (10). It has been suggested that carnitine might be one factor behind this finding in pigs. The metabolisms of carnitine, which is involved in mitochondrial transport and the oxidation of fatty acids (20), and of betaine are closely linked (21).

Betaine supplementation was found to induce a significant decrease in plasma homocysteine concentration. The present study shows that betaine alone can reduce plasma homocysteine concentrations significantly in healthy subjects whose folate status remained unchanged. Brouwer et al (22) also showed that a daily supplement of 6 g betaine for 3 wk reduced homocysteine values in healthy subjects, yet neither the folate nor the betaine status of the subjects was reported in their study. Patients with homocystinuria are traditionally treated with folic acid and vitamin B-12. However, some patients have received a daily dose of betaine in combination with their vitamin therapy. Tangerman et al (23) reported that betaine alone reduced the plasma homocysteine values in selected patients with homocystinuria. The reduction was

TABLE 5
Serum concentrations of liver enzymes at the beginning (4 wk) and the end (16 wk) of the intervention period in both groups[1]

	Control group (n = 20)		Betaine group (n = 22)		P for the main effect of time[2]
	4 wk	16 wk	4 wk	16 wk	
Asparagine aminotransferase (U/L)	25.7 ± 7.2	21.1 ± 5.3	28.3 ± 13.2	21.8 ± 5.5	0.0001
Alanine aminotransferase (U/L)	34.1 ± 19.1	26.8 ± 15.8	28.9 ± 17.7	22.3 ± 8.5	0.005
Alkaline phosphatase (U/L)	149.4 ± 36.0	154.2 ± 36.1	154.3 ± 32.8	154.2 ± 32.1	0.008
γ-Glutamyl transferase (U/L)	34.3 ± 32.8	32.8 ± 31.1	27.6 ± 12.3	27.1 ± 9.2	0.021

[1] $\bar{x}$ ± SD.
[2] There was no significant interaction of time and treatment and no significant differences between the groups.

>40% (from 210 to 114 μmol/L). This hereditary disease, usually caused by severe cystathionine β-synthase (EC 4.2.1.22) deficiency, is very rare. Furthermore, it has no role in the characterization of the epidemiologic role of slightly elevated homocysteine concentrations at the population level.

The reduction of serum homocysteine concentrations in healthy subjects was ≈9% in the present study. This may be important, because there is evidence for a relation between the plasma homocysteine concentration and the risk of atherosclerotic vascular diseases, especially those related to thrombosis (24, 25). Mildly elevated serum homocysteine concentrations (> 15 μmol/L) may be more common in white populations than was previously believed. The homocysteine concentrations are dependent on both genetic and lifestyle factors. It is well known that folic acid is efficient in lowering plasma homocysteine concentrations, and supplementation with folic acid is widely accepted for that purpose. The reduction in homocysteine concentrations is ≈11–20% with a daily dose of 250–500 mg folic acid (26). Betaine acts via a different route in homocysteine metabolism, however, and may enhance homocysteine metabolism when the folic acid response is weak.

There was no significant change in systolic blood pressure during the interaction period. However, the main effect of time on diastolic blood pressure was significant: in normotensive subjects participating in the present study, the observed reductions were ≈4.9 mm Hg in the betaine group and ≈2.4 mm Hg in the control group. Betaine has the property of being an osmolyte, especially in kidney cells (5). The effect might be more significant in mildly hypertensive subjects than in normotensive subjects. In rats, another osmolyte, taurine, was also shown to have properties of blood pressure reduction (27).

Hyperhomocysteinemia has been reported to be involved in arterial endothelial dysfunction (28). It is associated with impaired endothelium-dependent vasodilatation, whereas no effect on endothelium-independent responses has been found (29). Furthermore, oral folate supplementation in patients with hyperhomocysteinemia has been shown to result in a decrease in plasma homocysteine concentrations and to enhance endothelium-dependent responses, whereas endothelium-independent responses remained unchanged (30). These phenomena might be associated with diastolic blood pressure.

The change in serum total and LDL-cholesterol concentrations between the groups was significant. However, the difference (0.4 mmol/L for total cholesterol, 0.3 mmol/L for LDL-cholesterol) was small and of minor clinical significance. The concomitant weight change may also contribute to this marginal difference in serum total and LDL-cholesterol concentrations between the groups.

The present study was performed with a 6-g daily dose of betaine. The amount was chosen according to previously published studies of patients with homocystinuria (31). However, the 6-g dose is supposed to represent the maximum effective dose. It induced a > 10-fold increase in plasma betaine concentrations from the normal range of ≈20 μmol/L.

No adverse effects of the 6-g daily betaine dose on liver enzymes were found during the 3-mo intervention period. The concentrations of serum alanine aminotransferase and asparagine aminotransferase decreased similarly in both groups, whereas no change was found in serum concentrations of alkaline phosphatase or γ-glutamyl transferase.

In conclusion, a daily supplement of 6 g betaine for 12 wk along with a hypoenergetic diet decreases plasma homocysteine concentration but does not affect body composition more than a hypoenergetic diet without betaine supplementation does. The study showed that the 6-g daily dose was well tolerated, and no side effects were observed. Further studies are needed to examine the dose response of betaine in the long term.

We thank Erja Huttunen, Irja Kanniainen, Kaija Kettunen, Kaija Lampi, and Anne Vähäniitty for excellent technical assistance.

REFERENCES

1. Sakamoto A, Murata N. Genetic engineering of glycine betaine synthesis in plants: current status and implications for enhancement of stress tolerance. J Exp Bot 2000;51:81–8.
2. Rozwadowski KL, Khachatourians GG, Selvaraj G. Choline oxidase, a catabolic enzyme in *Arthrobacter pascens*, facilitates adaptation to osmotic stress in *Escherichia coli*. J Bacteriol 1991;173:472–8.
3. Konosu S, Hayashi T. Determination of beta-alanine and glycine betaine in some marine invertebrates. Bull Jpn Soc Sci Fish 1975; 41:743–6.
4. Garcia-Perez A, Burg MB. Renal medullary organic osmolytes. Physiol Rev 1991;71:1081–115.
5. Burg MB. Renal osmoregulatory transport of compatible organic osmolytes. Curr Opin Nephrol Hypertens 1997;6:430–3.
6. McCue KF, Hanson AD. Salt-inducible betaine aldehyde dehydrogenase from sugar beet: cDNA cloning and expression. Plant Mol Biol 1992;18:1–11.
7. Lever M, Sizeland PC, Bason LM, Hayman CM, Chambers ST. Glycine betaine and proline betaine in human blood and urine. Biochim Biophys Acta 1994;1200:259–64.
8. Sakura N, Ono H, Nomura S, Ueda H, Fujita N. Betaine dose and treatment intervals in therapy for homocystinuria due to 5, 10-methylenetetrahydrofolate reductase deficiency. J Inherit Metab Dis 1998;21:84–5.
9. Virtanen E, Junnila M, Soivio A. Effects of food containing betaine/amino acid additive on the osmotic adaptation of young Atlantic salmon, S*almo salar L.* Aquaculture 1989;83:109–22.
10. Virtanen E, Cambell R. Reduction of backfat thickness through supplementation of diets for fattening pigs. In: Verlag H, ed. Handbuch der tierischen Veredlung. Oesnabruek, Germany: Kamlage, 1994: 145–50.
11. McCully KS. Vascular pathology of homocysteinemia: implications for the pathogenesis of arteriosclerosis. Am J Pathol 1969;56:111–28.
12. Eikelboom JW, Lonn E, Genest J Jr, Hankey G, Yusuf S. Homocyst(e)ine and cardiovascular disease: a critical review of the epidemiologic evidence. Ann Intern Med 1999;131:363–75.
13. Breksa AP, Garrow TA. Recombinant human liver betaine-homocysteine *S*-methyltransferase: identification of three cysteine residues critical for zinc binding. Biochemistry 1999;38:13991–8.
14. Rastas M, Seppänen R, Knuts L-R, Hakala P, Karttila V, eds. Nutrient composition of foods. Helsinki: The Social Insurance Institution, 1997.
15. Penttilä IM, Voutilainen E, Laitinen O, Juutilainen P. Comparison of different analytical and precipitation methods for the direct estimation of high-density lipoprotein cholesterol. Scand J Clin Lab Invest 1981; 41:353–60.
16. Lever M, Bason L, Leaver C, Hayman CM, Chambers ST. Same-day batch measurement of glycine, betaine, carnitine and other betaines in biological material. Anal Biochem 1992;205:14–21.
17. Mar M-HH, Ridky TW, Garner SC, Zeisel SH. A method for the determination of betaine in tissues using high performance liquid chromatography. Nutr Biochem 1995;6:392–8.
18. Ubbink JB, Vermaak WJH, Bissbort S. Rapid high-performance liquid chromatographic assay for total homocysteine levels in human serum. J Chromatogr 1991;565:441–6.
19. Möller J, Rasmussen K, Christensen L. External quality assessment

of methylmalonic and total homocysteine. Clin Chem 1999;45: 1536–42.
20. Zeisel SH. Choline deficiency. J Nutr Biochem 1990;1:332–49.
21. Carter AL, Abney TO, Lapp DF. Biosynthesis and metabolism of carnitine. J Child Neurol 1995;10(suppl):S23–7.
22. Brouwer IA, Verhoef P, Urgert R. Betaine supplementation and plasma homocysteine in healthy volunteers. Arch Intern Med 2000; 160:2546–7.
23. Tangerman A, Wilcken B, Levy HL, Boers GH, Mudd SH. Methionine transamination in patients with homocystinuria due to cystathionine beta-synthase deficiency. Metabolism 2000;49:1071–7.
24. Chambers JC, Obeid OA, Refsum H, et al. Plasma homocysteine concentrations and risk of coronary heart disease in UK Indian Asian and European men. Lancet 2000:355:523–7.
25. El-Khairy L, Ueland PM, Nygard O, Refsum H, Vollset SE. Lifestyle and cardiovascular disease risk factors as determinants of total cysteine in plasma: the Hordaland Homocysteine Study. Am J Clin Nutr 1999;70:1016–24.
26. Brouwer IA, van Dusseldorp M, Thomas CM, et al. Low-dose folic acid supplementation decreases plasma homocysteine concentrations: a randomized trial. Am J Clin Nutr 1999;69:99–104.
27. Harada H, Kitazaki K, Tsujino T, et al. Oral taurine supplementation prevents the development of ethanol-induced hypertension in rats. Hypertens Res 2000;23:277–84.
28. Woo KS, Chook P, Lolin YI, et al. Hyperhomocyst(e)inemia is a risk factor for arterial endothelial dysfunction in humans. Circulation 1997;96:2542–4.
29. Tawakol A, Omland T, Gerhard M, Wu JT, Creager MA. Hyperhomocyst(e)inemia is associated with impaired endothelium-dependent vasodilation in humans. Circulation 1997;95:1119–21.
30. Bellamy MF, McDowell IF, Ramsey MW, Brownlee M, Newcombe RG, Lewis MJ. Oral folate enhances endothelial function in hyperhomocysteinaemic subjects. Eur J Clin Invest 1999;29: 659–62.
31. Wilcken DE, Dudman NP, Tyrrell PA. Homocystinuria due to cystathionine beta-synthase deficiency—the effects of betaine treatment in pyridoxine-responsive patients. Metabolism 1985;34: 1115–21.