Choline supplemented as phosphatidylcholine decreases fasting and postmethionine-loading plasma homocysteine concentrations in healthy men1–3

Margreet R Olthof, Elizabeth J Brink, Martijn B Katan, and Petra Verhoef

ABSTRACT
Background: A high homocysteine concentration is a potential risk factor for cardiovascular disease that can be reduced through betaine supplementation. Choline is the precursor for betaine, but the effects of choline supplementation on plasma total homocysteine (tHcy) concentrations in healthy humans are unknown.

Objective: The objective was to investigate whether supplementation with phosphatidylcholine, the form in which choline occurs in foods, reduces fasting and postmethionine-loading concentrations of plasma tHcy in healthy men with mildly elevated plasma tHcy concentrations.

Design: In a crossover study, 26 men ingested ≈2.6 g choline/d (as phosphatidylcholine) or a placebo oil mixture for 2 wk in random order. Fatty acid composition and fat content were similar for both treatments. A methionine-loading test was performed on the first and last days of each supplementation period.

Results: Phosphatidylcholine supplementation for 2 wk decreased mean fasting plasma tHcy by 18% (−3.0 μmol/L; 95% CI: −3.9, −2.1 μmol/L). On the first day of supplementation, a single dose of phosphatidylcholine containing 1.5 g choline reduced the postmethionine-loading increase in tHcy by 15% (−4.8 μmol/L; 95% CI: −6.8, −2.8 μmol/L). Phosphatidylcholine supplementation for 2 wk reduced the postmethionine-loading increase in tHcy by 29% (−9.2 μmol/L; 95% CI: −11.3, −7.2 μmol/L). All changes were relative to placebo.


KEY WORDS Choline, phosphatidylcholine, methionine loading, homocysteine, humans

INTRODUCTION
Choline is an important nutrient throughout life. It is the precursor for the neurotransmitter acetylcholine and for phosphatidylcholine, a structural component of VLDL, which is essential for normal lipid-cholesterol transport. Furthermore, choline is a source of labile methyl groups (1). For a long time, choline was considered a dispensable nutrient because it can be endogenously synthesized through sequential methylation of phosphatidylethanolamine to form phosphatidylcholine, with S-adenosylmethionine as the methyl donor (2, 3). However, studies have shown that humans who ingest a choline-deficient diet develop liver and kidney problems (4–6). Thus, choline is an important dietary nutrient, and an adequate intake of choline is defined as 425 mg/d for women and 550 mg/d for men (7).

Choline is present in the human diet primarily as lecithin, which is the common name for phosphatidylcholine. Intake of the choline moiety from foods is estimated at 0.3–1 g/d, and the main food sources are eggs, liver, soybeans, and pork (3, 8, 9). Choline becomes a source of labile methyl groups when it is converted into betaine (Figure 1). This conversion occurs mainly in the liver and kidney and is irreversible (1, 10). Betaine donates its methyl group to homocysteine to form methionine in a reaction catalyzed by the enzyme betaine-homocysteine methyltransferase. A high plasma homocysteine concentration is associated with a greater risk of cardiovascular disease (CVD), but whether this relation is causal is still uncertain (11). Supplementation with betaine lowers plasma homocysteine concentrations in hyperhomocysteinemic subjects (12, 13) and in subjects with normal homocysteine concentrations (14–16). Choline has been used in the past as a homocysteine-lowering therapy for hyperhomocysteinemic patients with genetic defects in their homocysteine metabolism who had not responded to treatment with vitamin B-6 or folic acid (17). Dudman et al (18) found that choline or betaine treatment normalized concentrations of homocysteine after methionine loading in some but not all patients with CVD and impaired homocysteine metabolism. The effects

1 From the Wageningen Centre for Food Sciences and the Division of Human Nutrition, Wageningen University, Wageningen, Netherlands (MRO, MBK, and PV), and the Department of Physiological Sciences, Netherlands Organisation for Applied Scientific Research (TNO) Quality of Life, Zeist, Netherlands (EJB).
2 Supported by the Wageningen Centre for Food Sciences, an alliance of Dutch food industry and research institutes [the University of Maastricht, the Netherlands Organisation for Applied Scientific Research (TNO) Quality of Life, and Wageningen University and Research Centre] that receives funding from the Dutch government. Unilever Research Laboratory donated the placebo oil used in the study.
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of choline supplementation on plasma homocysteine concentrations in healthy subjects are unknown. Therefore, we investigated the effect of supplementation with choline (as phosphatidylcholine) on fasting and postmethionine-loading plasma concentrations of homocysteine in healthy men.

SUBJECTS AND METHODS

Subjects

Subjects were recruited from the pool of volunteers registered at the Netherlands Organisation for Applied Scientific Research (TNO) Quality of Life (Zeist, Netherlands) and by advertisements in local newspapers. Eligible volunteers were healthy as assessed by physical examination, a general health and lifestyle questionnaire, blood pressure measurement, routine clinical laboratory tests, and blood analyses of tHcy and B vitamins. Plasma tHcy concentrations were <26 μmol/L. Volunteers had no history of CVD and had not used vitamin B supplements, lecithin, or supplements containing choline, choline derivatives, or betaine > 1 time/wk during the month preceding screening.

Of 48 eligible men, 26 men aged 50–71 y with the highest plasma tHcy concentrations (range: 11.0–23.1 μmol/L) were included in this study. Subject characteristics are shown in Table 1. All subjects completed the study.

Written informed consent was obtained from all subjects. The study was conducted according to Good Clinical Practice guidelines at TNO Quality of Life. The local medical ethics committee approved the protocol.

Study design

In this double-blind, placebo-controlled, crossover study, the subjects were randomly assigned to 1 of 2 treatment orders. Randomization was stratified by plasma tHcy concentrations at screening and by smoking habits. Thirteen subjects began phosphatidylcholine treatment, and the other 13 began placebo treatment, both for 2 wk. After a 2-wk washout period, the treatments were reversed. Treatments consisted of ingestion of 34.0 g of a

<table>
<thead>
<tr>
<th>Table 1 Subject characteristics at screening</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>61 ± 6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.7 ± 2.2</td>
</tr>
<tr>
<td>tHcy (μmol/L)</td>
<td>14.7 ± 3.4</td>
</tr>
<tr>
<td>Vitamin B-6 (nmol/L)</td>
<td>64 ± 44</td>
</tr>
<tr>
<td>Vitamin B-12 (pmol/L)</td>
<td>236 ± 61</td>
</tr>
<tr>
<td>Folate (nmol/L)</td>
<td>11.7 ± 3.9</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>73 ± 16</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>21 ± 8</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>21 ± 6</td>
</tr>
<tr>
<td>γ-GT (U/L)</td>
<td>25.7 ± 11.0</td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td>88 ± 11</td>
</tr>
</tbody>
</table>

1 All values are x ± SD; n = 26. All measurements in blood were taken after an overnight fast. tHcy, total homocysteine; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GT, glutamyltransferase.
TABLE 2  Composition of treatment supplements

<table>
<thead>
<tr>
<th>Dose (g/d)</th>
<th>Phosphatidylcholine(^1)</th>
<th>Placebo(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td></td>
<td>25.5</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>2.7</td>
<td>Not present</td>
</tr>
<tr>
<td>Carbohydrate (g/d)</td>
<td>2.4</td>
<td>Not present</td>
</tr>
<tr>
<td>Fat (g/d)</td>
<td>25.5</td>
<td>25.5</td>
</tr>
<tr>
<td>Choline (g/d)(^3)</td>
<td>2.6</td>
<td>Not detectable</td>
</tr>
<tr>
<td>Amount of fatty acids (g/d)(^4)</td>
<td>22.0</td>
<td>21.8</td>
</tr>
<tr>
<td>8:0 (caprylic acid)</td>
<td>3.0</td>
<td>2.0</td>
</tr>
<tr>
<td>10:0 (capric acid)</td>
<td>1.4</td>
<td>1.7</td>
</tr>
<tr>
<td>16:0 (palmitic acid)</td>
<td>2.1</td>
<td>1.0</td>
</tr>
<tr>
<td>18:0 (stearic acid)</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>18:1 n = 9 (oleic acid)</td>
<td>1.8</td>
<td>4.0</td>
</tr>
<tr>
<td>18:2 n = 6 (linoleic acid)</td>
<td>11.7</td>
<td>10.4</td>
</tr>
<tr>
<td>18:3 n = 3 (α-linolenic acid)</td>
<td>1.0</td>
<td>1.4</td>
</tr>
</tbody>
</table>

\(^1\) PhosChol (Nutrasal LLC, Oxford, CT) consisted of 80% soybean lecithin extract (Phosal 75A), 18% medium-chain triacylglycerol mix, 1.5% anethole, and traces of p-α-tocopherol and paraben blend. The soybean lecithin extract consisted of 8% moisture, 3% ash, 10% protein, 71% fat (including phosphatidylcholine), 9% carbohydrates, and traces of vitamins and minerals. Folic acid content of the extract was 195 μg/kg, which is ~15 μg/34 g phosphatidylcholine oil (calculated from data provided by the manufacturer). This amount is negligible relative to the total daily intake of folate (100–200 μg/d).

\(^2\) Mixture of linola (low-linolenic, high-linoleic linedse) oil (70%), rapeseed oil (21%), and medium-chain triacylglycerol mix (9%).

\(^3\) The choline content of PhosChol was measured in 2 different laboratories. Koe et al (19) measured choline as phosphatidylcholine in 2 samples. Phosphatidylcholine concentrations in the 2 samples were 800 and 801 μmol/g sample: this represents ~20.6 g phosphatidylcholine (molecular weight 758) or ~2.8 g choline in 34 g PhosChol. Total choline content was also measured in 4 samples of PhosChol at TNO (see Subjects and Methods). Mean choline content was 74.8 mg/g (range: 71–77 mg/g); this represents 2.5 g choline in 34 g PhosChol. The mean choline content from all 6 samples analyzed was 2.6 g/d.

\(^4\) Fatty acids were measured by gas chromatography of fatty acid methyl esters (20). Trinomadecanoin (19:0) was used as a reference compound to calculate the amounts of individual fatty acids.

Blood collection

Venous blood was taken from the antecubital vein after an overnight fast on days 1, 13, and 15 of each treatment period. In addition, blood samples were obtained 6 h after methionine loading on days 1 and 15 of each treatment period. Blood for analysis of tHcy and vitamin B-6 was collected in evacuated tubes containing EDTA. Samples were mixed and put on ice immediately after collection. Within 30 min, samples were centrifuged for 15 min at 2000 × g at 4 °C. For analyses of vitamins B-12 and folate acid; blood lipids; and alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, γ-glutamyltransferase, and creatinine, blood was collected in evacuated tubes containing clot activator and a gel to separate serum and cells. Approximately 30 min after collection, samples were centrifuged for 15 min at 2000 × g at 4 °C. All samples were stored at −70 °C. Samples were coded to hide the identity and treatment of subjects. All samples obtained from one subject were analyzed in the same run.
Fasting plasma total homocysteine (tHcy) and the increase in plasma tHcy after methionine loading in 26 healthy men after their ingestion of placebo and of phosphatidylcholine (corresponding to 2.6 g choline/d) in a crossover design

### TABLE 3

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Phosphatidylcholine</th>
<th>Difference from placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting (μmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First day of supplementation</td>
<td>16.5 ± 4.2²</td>
<td>15.6 ± 4.0</td>
<td>-0.9 (1.8, -0.0)⁴,⁴⁴</td>
</tr>
<tr>
<td>After 2 wk of supplementation</td>
<td>16.6 ± 4.0</td>
<td>13.6 ± 2.5⁵</td>
<td>-3.0 (-3.9, -2.1)⁵</td>
</tr>
<tr>
<td>6-h Postmethionine load (μmol/L)</td>
<td>3.8 ± 0.8</td>
<td>2.7 ± 0.6</td>
<td>-1.1 (1.4, -0.8)⁶</td>
</tr>
<tr>
<td>First day of supplementation</td>
<td>3.2 ± 0.7</td>
<td>2.2 ± 0.3⁶,⁶⁰</td>
<td>-1.0 (1.1, -0.9)⁶</td>
</tr>
<tr>
<td>After 2 wk of supplementation³</td>
<td>3.5 ± 0.6</td>
<td>2.3 ± 0.3⁶,⁶⁰</td>
<td>-1.2 (1.1, -0.9)⁶</td>
</tr>
</tbody>
</table>

¹ Treatment × time interaction, P = 0.001 (split-plot ANOVA): thus, the effect on the first day of supplementation was significantly different from the effect after 2 wk.
² x ± SD (all such values).
³; ⁴; ⁵; ⁶; ⁷; ⁸; ⁹; ¹⁰; ¹¹; ¹²
⁴ P = 0.04 (split-plot ANOVA).
⁵ Significantly different from fasting tHcy on day 1 of phosphatidylcholine treatment, P < 0.0001 (split-plot ANOVA).
⁶ P < 0.0001 (split-plot ANOVA).
⁷ The increase in plasma tHcy 6 h after methionine loading is the difference between the value obtained immediately before methionine loading and that obtained 6 h after methionine loading.
⁸ Treatment × time interaction, P = 0.003 (split-plot ANOVA): thus, the effect on the first day of supplementation was significantly different from the effect after 2 wk.
⁹ n = 25 because 1 sample was lost during processing.
¹⁰ Significantly different from postmethionine-loading tHcy on day 1 of phosphatidylcholine treatment, P < 0.0001 (split-plot ANOVA).

Biochemical analyses

Plasma tHcy concentrations were measured in fasting blood samples that were collected on days 1, 13, and 15 of each treatment period and in nonfasting blood samples collected after the methionine loads on days 1 and 15 of each treatment period. The concentrations of tHcy (sum of all oxidized and reduced forms of homocysteine) were measured by using HPLC with fluorescence detection (21). Within- and between-run CVs were 3.5% and 8.0%, respectively. Vitamins were measured in fasting blood samples collected on days 13 and 15 of each treatment period. Vitamin B-6 was measured by using semiautomated fluorimetric determination of pyridoxal-5'-phosphate in whole blood with HPLC (22), and folate and vitamin B-12 were measured with a competitive protein-binding assay with a commercially available reagent kit [Simultrac LP Radioassay kit; ICN, Pharmaceutical Diagnostics Division, Orangeburg, NY (23) as modified by Givas and Gutcho (24)]. Concentrations of total and HDL cholesterol, triacylglycerol, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, γ-glutamyltransferase, and creatinine were measured with a Hitachi 911 analyzer (Hitachi Instrument Division, Ibaraki-ken, Japan) according to routine laboratory procedures. LDL-cholesterol concentrations were calculated by using the formula of Friedewald (25). For the measurement of choline in the supplements at TNO, choline was liberated from the sample by boiling the sample for 4 h with nitric acid (17% vol:vol). After filtration of the sample, the pH of the extract was increased to 9.0 with sodium hydroxide solution. To an aliquot of the extract, a solution of ammonium reineckate in methanol was added, and the insoluble choline reineckate was formed. The precipitate was filtered off and dissolved in acetone. The color of the acetone solution was determined by using a spectrophotometer at a wavelength of 530 nm.

Statistical analysis

Primary outcomes were the plasma tHcy concentration in the fasting state at the end of each treatment period and the increase in plasma tHcy concentrations 6 h after each methionine loading on the first and on the last day of supplementation. For tHcy, vitamins, and kidney and liver function indicators, the values measured in fasting subjects on days 13 and 15 of each treatment period were averaged per subject per treatment period. In addition, for each subject, the increase in plasma tHcy 6 h after each methionine loading was calculated by subtracting the concentrations at 0 h (fasting sample) from the concentrations at 6 h after methionine loading.

The split-plot ANOVA procedure in SAS, with treatment as whole plots and time (days 1 and 15) as subplots, was used to investigate the main effects of treatment and time and their interaction. Carryover effects were also evaluated to assess whether treatment effects were influenced by treatment order. All statistical tests were based on a two-sided significance value of 5%, and 95% CIs were calculated. Statistical analyses were carried out by using SAS software (version 8.1; SAS Institute Inc, Cary, NC).

RESULTS

Fasting and postmethionine-loading total homocysteine

Fasting plasma tHcy was 18% lower after subjects had ingested phosphatidylcholine for 2 wk than after placebo treatment (Table 3). Treatment effects were not influenced by treatment order (P = 0.59).

On the first day of phosphatidylcholine supplementation (ie, after a single dose), the increase in plasma tHcy 6 h after methionine loading was 15% lower than that on the first day of placebo treatment (Table 3). On the last day of phosphatidylycholine supplementation, the increase in plasma tHcy 6 h after methionine loading was 29% lower than that after placebo treatment (Table 3). In addition, the effect of a dose of phosphatidylcholine with a methionine load on postmethionine-loading homocysteine concentrations on the first day was smaller than the effect on the
last day of supplementation (Table 3). Treatment effects were not influenced by treatment order \( (P = 0.35) \).

**B vitamins**

Serum folate concentrations were significantly lower after phosphatidylcholine \((± SD: 10.9 ± 3.4 \text{ nmol/L})\) than after placebo \((11.8 ± 3.1 \text{ nmol/L})\) treatment \( (P = 0.04) \). Concentrations of vitamin B-6 were significantly higher after phosphatidylcholine \((53 ± 23 \text{ nmol/L})\) than after placebo \((49 ± 17 \text{ nmol/L})\) treatment \( (P = 0.05) \). Vitamin B-12 concentrations did not differ significantly between the 2 treatment periods.

**Liver and kidney function indicators, serum lipids, and body weight**

Serum alkaline phosphatase concentrations were significantly lower after phosphatidylcholine \((64 ± 13 \text{ U/L})\) than after placebo \((68 ± 14 \text{ U/L})\) treatment \( (P = 0.001) \). Concentrations of alanine aminotransferase, aspartate aminotransferase, \( γ \)-glutamyltransferase, and creatinine did not differ significantly between treatments (data not shown). Serum triacylglycerol concentrations were significantly higher after subjects ingested phosphatidylcholine for 2 wk \((2.00 ± 1.27 \text{ mmol/L})\) than after they ingested placebo \((1.77 ± 1.15 \text{ mmol/L})\) (statistical analyses performed on log-transformed values, \( P = 0.001) \). Serum concentrations of total, LDL, and HDL cholesterol did not differ significantly between treatment periods.

Body weight increased by 0.4 kg during each of the 2-wk treatment periods \( (P = 0.007) \), and the increase did not differ significantly between treatments \( (P = 0.95) \). During the 2-wk washout period, subjects lost the weight that they had gained during the preceding 2-wk treatment, so that, at the start of the second supplementation period, they were back to their starting weights (data not shown).

**DISCUSSION**

**Effects on total homocysteine**

We showed that supplementation with phosphatidylcholine lowers fasting and postmethionine-loading plasma \( \text{tHcy} \) concentrations in healthy men with mildly elevated plasma \( \text{tHcy} \) concentrations. A single dose of phosphatidylcholine together with a methionine load acutely reduced the increase in \( \text{tHcy} \) after methionine loading, which indicates that production of betaine from supplemental choline is quick \((10, 26, 27) \). Because we matched the fatty acid content and composition of the supplements, we may assume that the effects we found are solely due to the choline moiety of phosphatidylcholine. Both fasting and postmethionine-loading \( \text{tHcy} \) concentrations are predictors of CVD risk \((28–30) \). If homocysteine truly is causally involved, supplementation with choline from phosphatidylcholine might be a novel dietary way to decrease CVD risk \((11, 31) \).

The decrease in plasma \( \text{tHcy} \) through choline is most likely mediated through increased betaine-dependent remethylation of homocysteine into methionine. Excess choline is irreversibly converted into betaine by the enzyme choline oxidase \((10, 32, 33) \), which increases betaine-dependent remethylation and leads to homocysteine lowering (Figure 1). An alternative mechanism could involve a reduction in endogenous production of phosphatidylcholine via the phosphatidylethanolamine \( N \)-methyltransferase pathway when phosphatidylcholine is supplemented, which in turn may lead to lower homocysteine concentrations \((34) \). Approximately 30% of the phosphatidylcholine is formed through sequential methylation of phosphatidylethanolamine, which generates 3 homocysteine molecules for each phosphatidylcholine molecule synthesized \((35) \). However, whether this mechanism can explain the homocysteine-lowering effects of phosphatidylcholine is not known, and that possibility should be investigated.

**The effects of choline, betaine, and folic acid on plasma total homocysteine**

We compared the effects of phosphatidylcholine, betaine, and folic acid supplementation on plasma \( \text{tHcy} \) concentrations by using data from studies previously done by our group in the same setting and laboratory \((14, 15, 36) \). Supplementation with phosphatidylcholine, corresponding to \( 2.6 \text{ g choline/d} \), reduced fasting \( \text{tHcy} \) by 18% after 2 wk, which is similar to the reduction seen after 2-wk treatment with \( 1.5–3 \text{ g betaine/d} \) \((14) \). A single dose of phosphatidylcholine, corresponding to \( ≈1.5 \text{ g choline/d} \), reduced the postmethionine-loading increase in \( \text{tHcy} \) as much as did a single 0.75-g dose of betaine \((14) \). The acute effects on postmethionine-loading plasma \( \text{tHcy} \) thus appear somewhat more efficient with betaine than with phosphatidylcholine supplementation. This seems plausible because, once absorbed, betaine is directly available as a methyl donor, whereas choline first has to be oxidized to betaine. Lowering of fasting plasma \( \text{tHcy} \) after phosphatidylcholine supplementation was similar to that after supplementation with \( \approx400 \text{ µg folic acid/d} \) \((36) \). Folic acid does not affect postmethionine-loading plasma \( \text{tHcy} \), but phosphatidylcholine and betaine do \((14, 15, 37) \). Thus, our results imply that betaine and phosphatidylcholine, given as supplements, can serve as alternatives to folic acid as a homocysteine-lowering agent. In addition, phosphatidylcholine and betaine supplementation might temper \( \text{tHcy} \) increases after a meal, whereas folic acid does not.

**Effect of phosphatidylcholine on B vitamins and on kidney and liver function indicators**

Folic acid–dependent remethylation and choline- or betaine-dependent remethylation are interrelated \((38) \). In humans and animals, a choline-deficient diet led to low folate concentrations, which were restored with the administration of choline \((39–42) \). Conversely, when the diet is deficient in folate, choline concentrations are low, but choline status is restored with folate repletion \((39, 43–45) \). Contrary to our expectations, we found that phosphatidylcholine supplementation decreased serum folate concentrations and increased vitamin B-6 concentrations in blood. However, the effects were small, and this probably is a chance finding.

Choline deficiency leads to liver problems both in healthy humans and in humans on total parenteral nutrition that is choline poor \((4–6) \). We found that phosphatidylcholine supplementation did not greatly affect liver or kidney function. However, the small increase in triacylglycerol concentrations on phosphatidylcholine supplementation should be investigated further.

**Study limitations**

We tested the effects of phosphatidylcholine supplementation on plasma homocysteine only in men. From animal studies, it appears that females are more resistant to induced choline deficiency than are males, probably because of females’ enhanced
capacity to form phosphatidylcholine endogenously (46–48). It is not known whether supplementation with phosphatidylcholine will affect plasma homocysteine concentrations differently in men and in women who are choline replete.

We supplied choline in the form of phosphatidylcholine and not as free choline, mainly because phosphatidylcholine is the form in which choline occurs in foods. In addition, ingestion of phosphatidylcholine leads to a prolonged and greater increase in serum choline concentrations than does ingestion of free choline (as choline chloride) (49), and therefore the former might be more effective in lowering tHcy. Furthermore, unlike the ingestion of high doses of free choline, that of phosphatidylcholine does not lead to the undesirable formation of trimethylamines that makes persons who ingest large amounts of choline smell of fish (50). Nevertheless, for the production of foods with extra choline or for supplementation purposes, choline salts will be more feasible than will phosphatidylcholine. In addition, phosphatidylcholine provides extra energy intake due to the fatty acid component, which can be avoided when free choline is ingested.

Furthermore, we supplied a high dose of choline relative to dietary intake of choline (estimated at 0.3–1 g/d). The homocysteine-lowering potential of choline doses in the range of dietary intake is as yet unknown. Because of the substantial energy content of the supplements (~230 kcal/d), the body weight of the subjects increased by ~0.4 kg during both intervention periods. Apparently, the subjects did not (completely) compensate for the extra energy intake from the supplements. However, weight changes did not affect our results because the increases in weight were similar during the 2 intervention periods.

Conclusion

We conclude that phosphatidylcholine supplementation is as effective as betaine and folic acid in lowering fasting tHcy. We expect that betaine or choline supplementation in combination with folic acid supplementation or fortification will augment the tHcy-lowering effect of folic acid, but that possibility remains to be investigated. Both phosphatidylcholine and betaine supplements lower postmethionine-loading plasma tHcy concentrations, but folic acid does not. If homocysteine is causally related to CVD, a diet rich in (phosphatidyl)choline or supplements containing (phosphatidyl)choline might prove to be beneficial. However, the effects of phosphatidylcholine on other risk factors for CVD, including serum triacylglycerols, should first be studied in greater detail.

We thank the volunteers for their participation, all those involved at TNO Quality of Life for their dedication, and the laboratory staff at the Division of Human Nutrition, Wageningen University, for laboratory analyses.

All authors participated in the design of the study, interpretation of the data, and writing of the manuscript. EJB also participated in the conduct of the study. None of the authors had any personal or financial conflicts of interest.

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