Intake of Fish Oil, Oleic Acid, Folic Acid, and Vitamins B-6 and E for 1 Year Decreases Plasma C-Reactive Protein and Reduces Coronary Heart Disease Risk Factors in Male Patients in a Cardiac Rehabilitation Program

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Abstract

Certain nutrients have been shown to be effective in preventing coronary heart disease. We hypothesized that a daily intake of low amounts of a number of these nutrients would exert beneficial effects on risk factors and clinical variables in patients that suffered from myocardial infarction (MI) and were following a cardiac rehabilitation program. Forty male MI patients were randomly allocated into 2 groups. The supplemented group consumed 500 mL/d of a fortified dairy product containing eicosapentaenoic acid, docosahexaenoic acid, oleic acid, folic acid, and vitamins A, B-6, D, and E. The control group consumed 500 mL/d of semi-skimmed milk with added vitamins A and D. The patients received supervised exercise training, lifestyle and dietary recommendations, and they were instructed to consume the products in addition to their regular diet. Blood extractions and clinical examinations were performed after 0, 3, 6, 9, and 12 mo. Plasma concentrations of eicosapentaenoic acid, docosahexaenoic acid, oleic acid, folic acid, vitamin B-6, and vitamin E increased after supplementation (P < 0.05). Plasma total and LDL-cholesterol, apolipoprotein B, and high-sensitivity C-reactive protein concentrations decreased in the supplemented group (P < 0.05), and plasma total homocysteine decreased in both groups. There were no changes in heart rate, blood pressure, or cardiac electrocardiographic parameters in either group. Therapeutic lifestyle changes, effected through a CR program comprising regular exercise and the intake of a combination of dietary nutrients, reduced a variety of risk factors in MI patients, which supports the rationale for nutritional programs in the secondary prevention of coronary heart disease.

Introduction

Acute myocardial infarction (MI), a manifestation of atherosclerosis caused by the occlusion of coronary arteries, is the leading cause of death for both men and women all over the world. It is also a major cause of physical disability, particularly in the rapidly growing population of elderly persons (1). Cardiac rehabilitation (CR) programs are recommended for patients who have received a diagnosis of MI. The overall objectives of CR are to optimize patients’ functioning by decreasing disabilities resulting from coronary heart disease (CHD), enhancing the quality of life, and minimizing the risk of recurrent cardiac events and hospitalization (2,3). Comprehensive rehabilitation programs usually combine supervised exercise training and behavioral changes (smoking cessation, control of excess weight, and increased physical activity) with lifestyle and nutritional counseling, thus aiming to reduce the cardiovascular (CV) risk factors that are generally increased in these patients (4).

Cholesterol-lowering therapies remain the main strategies for primary and secondary prevention of CHD (4). Numerous studies report a direct relation between levels of total or LDL-cholesterol (LDL-C) and the rate of CHD in healthy individuals and in people with established CHD (5–8). Moreover, several prospective studies indicate that plasma levels of high-sensitivity C-reactive protein (hs-CRP), a marker of systemic inflammation, are a strong independent predictor for the risk of future CV events among individuals, including MI patients (9–11).

1 Supported in part by a PhD education grant from the University of Granada (J.J.C.). Dairy products were supplied by Puleva Food S.L., Granada, Spain. 2Abbreviations used: AA, arachidonic acid; ApoB, apolipoprotein B; C, control group; CHD, coronary heart disease; CR, cardiac rehabilitation; CV, cardiovascular; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HDL-C, HDL cholesterol; hs-CRP, high-sensitivity C-reactive protein LDL-C, LDL cholesterol; MI, myocardial infarction; S, supplemented group; sICAM-1, soluble intercellular cell adhesion molecule 1; sVCAM-1, soluble vascular cell adhesion molecule 1; TC, total cholesterol; TG, triacylglycerols; tHcy, total plasma homocysteine. *To whom correspondence should be addressed. E-mail: elopezhuertas@puleva.es.

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There is a wealth of evidence regarding the benefits produced by changing lifestyle habits, dietary patterns, and nutrients in the primary and secondary prevention of CHD. Among these, a Mediterranean dietary pattern, characterized by a high intake of olive oil (rich in oleic acid and antioxidants), fish (rich in (n-3) long-chain PUFA), vegetables and fruits (rich in folic acid and other vitamins), has been associated with a lower CHD incidence and total mortality (12–14).

In this sense, the AHA recommends to MI patients a daily intake of 1 g of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from fish or fish oils (15). Dietary intakes of folate and/or vitamin B-6 have been described as the main nutrients responsible for lowering hyperhomocysteinemia, which is considered an independent risk factor for CVD (16). Consequently, European Recommended Dietary Allowances for these vitamins have been well established (17). Evidence has been recently gathered by the latest WHO report, which recommends 1) regular fish consumption to provide ~200–500 mg of EPA + DHA/wk, 2) a replacement of saturated fat by monounsaturated fat (to increase the oleic acid intake), and 3) an increase in the consumption of fruit and vegetables to achieve proper antioxidant and folate status (18). Despite the recommendations, modern Western societies tend to include little fish, fruit, and vegetables in their diets (18). In addition, whereas CR programs emphasize the adherence to healthy dietary patterns, their implementation is often unsuccessful and usually involves major lifestyle changes (2,3,19).

An effective approach to increasing the intake of healthy dietary nutrients that may lead to a decrease of CHD risk is the enrichment of foods that are regularly consumed by the majority of the population (20–24). We recently conducted a nutritional intervention with this nutritional approach in patients suffering from peripheral vascular disease, showing improvements in clinical outcomes while undertaking reductions in a variety of risk factors (23). With MI being the most frequent manifestation of CV disease, we hypothesized that the inclusion of a supplement in the daily diet of MI patients would help improve nutritional status and risk profile in the context of a CR program, and we carried out a longitudinal, controlled, randomized, double-blind, 12-mo intervention with a dairy product containing low amounts of EPA and DHA, oleic acid, folic acid, and vitamins A, D, E, and B-6.

### Materials and Methods

**Subject recruitment.** Subject recruitment was conducted at the Cardiac Rehabilitation Unit of the University Hospital of Granada (Spain). Hospital records and medical history of the patients were consulted before their inclusion in the study. All male patients diagnosed with at least 1 episode of acute MI and followed and successfully completed phase II of the CR program, and patients already on phase III, were candidates for inclusion in the study. Phase II of the CR program typically started after hospital discharge and usually lasted for 3 mo. During this phase, the patients were assessed for cardiovascular risk and received lifestyle and nutritional counseling, supervised exercise training, drug therapy, and psychological support. In phase III, the patients were referred to their medical centers for follow-up, where general practitioners supervised the CR of the patients in accordance with the guidelines received from the CR unit (25). For this study, the patients included in the study were asked to visit the CR unit twice/wk for the 12-mo duration of the study. Patients were not admitted to the study if any of the following criteria were present: 1) at high-risk after hospital discharge (New York Heart Association class III and IV), low functional capacity, ventricular arrhythmia, severe valvular disease, severe heart failure, or presenting with additional CV events during the hospital stay or during the cardiac rehabilitation program; 2) endocrine or metabolic disturbances; 3) blood concentrations of LDL-C > 3.4 mmol/L, or HDL-C < 1 mmol/L, or triacylglycerols > 1.7 mmol/L; 4) using a prescription of lipid lowering drugs (statins); 5) liver insufficiency; 6) residence outside the recruitment area of the study; 7) unable to come to the CR unit; 8) taking fish oil supplements.

**Study protocol and diets.** We carried out a longitudinal, randomized, controlled, double-blind intervention study to investigate the effects of a nutritional supplement in markers of CHD of a group of men following a cardiac rehabilitation program after an episode of MI.

From June to October 2003, 71 possible candidates were recruited; 27 of them did not fulfill the inclusion criteria because of residence outside the metropolitan area of Granada (n = 7), taking prescription statins 1 mo before the time of the inclusion due to high blood LDL-C concentration (n = 4), clinical history of stroke or peripheral vascular disease (n = 3), or a lack of willingness to participate (n = 13). The remaining candidates were randomly assigned to 2 intervention groups of 20 subjects each, using a table of random numbers (Fig. 1).

The patients received tailor-made exercise instructions and attended the CR unit twice/wk for exercise classes. They also were encouraged to exercise on their own every day (walk for 30 min to 1 h). Participating subjects gave their written consent. The protocol was approved by the Ethical Committee of S. Cecilio University Hospital and it was conducted in accordance with the Helsinki Declaration. The study lasted 12 mo, from November 2003 to December 2004. Details of the drugs prescribed to the subjects were recorded at baseline (Table 1).

The supplement (S) group (n = 20) was supplied with 300 mL/d of a fortified dairy product (Puleva Omega3, Puleva Food) containing the

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**Figure 1** Flow of participants in the study.
TABLE 1  Baseline characteristics of the participating subjects

<table>
<thead>
<tr>
<th></th>
<th>Group S</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>52.6 ± 1.9</td>
<td>57.4 ± 1.8</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>29.5 ± 0.9</td>
<td>27.9 ± 0.9</td>
</tr>
<tr>
<td>Current smokers</td>
<td>2 (10)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Ex-smokers</td>
<td>15 (75)</td>
<td>12 (60)</td>
</tr>
<tr>
<td>Type-II diabetes</td>
<td>2 (10)</td>
<td>3 (15)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>11 (55)</td>
<td>12 (60)</td>
</tr>
<tr>
<td>Drug prescription</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B-blocker</td>
<td>17 (85)</td>
<td>17 (85)</td>
</tr>
<tr>
<td>ACEI</td>
<td>14 (70)</td>
<td>11 (55)</td>
</tr>
<tr>
<td>Vasodilator</td>
<td>2 (10)</td>
<td>6 (30)</td>
</tr>
<tr>
<td>Antiplatelet</td>
<td>11 (55)*</td>
<td>18 (90)</td>
</tr>
<tr>
<td>Antidiabetic</td>
<td>2 (10)</td>
<td>1 (5)</td>
</tr>
</tbody>
</table>

1  Data are presented as means ± SEM or n (%); n = 20/group. * Different from C, P < 0.05.

following nutrients: EPA, DHA (from fish oils), oleic acid, folic acid, and vitamins A, B-6, D, and E. The dairy supplement was prepared by adding a mixture of fish and vegetable oils to skimmed milk, yielding a product containing a total fat comparable to that of standard semi-skimmed milk (19 g/L), but with a different fatty acid profile. Folic acid and vitamins A, B-6, D, and E were also added to the final product. The control (C) group (n = 20) was supplied with 500 mL/d of regular semi-skimmed milk with added vitamins A and D (Table 2). The dairy products were produced and packaged in white 500 mL Tetra Bricks by Puleva Biotech S.A., so that neither the patients nor the researchers would know what was consumed. The patients were instructed to consume the dairy products, in addition to their regular diets, in 2 × 250 mL doses at the beginning and at the end of the day. The dairy products were home-delivered to the patients monthly. Compliance with the consumption protocol during the intervention period was ensured and monitored by regular telephone calls and collection of the emptied containers. At the beginning of the CR program-Phase II, and also 2 wk before the 1st delivery of the study’s dairy beverages (in phase III), the subjects and their partners attended a dietary counseling session on general aspects of food composition, food processing, adequate portions, the effects of alcohol consumption, and the beneficial effects of the Mediterranean diet. Subjects received a leaflet with information about the CV benefits produced by fish consumption and were also advised to increase the consumption of fruit, legumes, and vegetables to ensure adequate intake of fiber and vitamins. They were advised not to eat fast food or precooked meals and to avoid smoky places or to stop smoking. Dietary intake was assessed at baseline and again at the end of the study with a 7-d self-administered food-frequency questionnaire, following instructions from the principal investigator. Spanish food composition tables were used to estimate dietary intake (26).

Blood extraction and clinical examination. The patients were interviewed at the hospital at the beginning of the study (T₀) and after 3, 6, 9, and 12 mo (T₁, T₂, T₃, and T₄). At every visit, after an overnight fast of at least 10 h, blood samples (30 mL) were collected by venipuncture into vacutainer tubes containing EDTA. Samples were kept on ice before centrifugation at 1700 × g for 15 min at 4°C to obtain plasma. To ensure analytical consistency, plasma samples T₀ - T₂ from each subject were processed at the same time and analyzed in 1 batch.

The subjects also received a complete clinical examination, including an anamnesis. Blood pressure and heart rate were determined. Electrocardiogram tests were performed and recorded at the times of the study, P-wave, QRS complex, and T-wave abnormalities were measured and registered.

Biochemical measurements. The plasma concentrations of triacylglycerols (TGₘ), total cholesterol (TC), and HDL cholesterol (HDL-C) were measured at the hospital central laboratory by colorimetry, using commercial kits (Biosystems). Analyses were conducted in triplicate and in 1 batch, following the protocols provided by the manufacturer. Plasma LDL-C was calculated according to the Friedewald formula (27). Plasma fatty acid profiles were determined by GLC (28). Plasma apolipoprotein B (ApoB) was measured using an immuno-turbidimetry test (Olympus Diagnostica). Plasma concentrations of total homocysteine (tHcy), vitamin E, and malondialdehyde were quantified by HPLC with fluorescence detection (29–31, respectively). Plasma vitamin B-6 concentration was also measured by HPLC, using instructions from a commercial kit (Immunoagnostik). Plasma and RBC folate concentrations were measured using commercial immunoassay kits (ICN Pharmaceuticals). Soluble vascular adhesion molecule 1 (sVCAM-1) and soluble intercellular cell adhesion molecule 1 (sICAM-1) concentrations were measured by ELISA commercial kits (Biosource International). HS-CRP concentrations in plasma were quantified by an immuno-nephelometric commercial kit (Dade Behring). Oxidized LDL in plasma was quantified using an ELISA kit (Mercodia). ApoB, hs-CRP, and the vitamins described above were measured in 1 batch at Balaguer Center Laboratories.

Statistical analysis. The data were analyzed using SPSS software (version 12.0). Data are expressed as means ± SEM, and differences of P < 0.05 were considered significant. Normality was assessed by the Kolmogorov-Smirnov test. Between-group comparisons at the beginning of the study were assessed by an independent t test or Mann-Whitney test for the non-Gaussian variables. The longitudinal effect of each dairy product within each group at the various time points of the study was analyzed by 1-way repeated-measures ANOVA followed by Tukey’s honestly significant difference post hoc test (within-group comparison). Statistical differences produced by the consumption of each dairy product were analyzed using 2-way repeated-measures ANOVA. For the non-Gaussian variables, Wilcoxon and Krustal-Wallis comparisons were performed to assess differences within and between groups, respectively. When between-group comparisons showed significant differences, an independent t test or Mann-Whitney test was applied to determine the time points at which the groups differed. The relations among increased plasma nutrients concentrations and CHD risk-factor improvement were assessed using two-tailed Pearson’s bivariate correlations.
Results

Baseline characteristics of the subjects included in the groups (Table 1) did not change from the beginning to the end of the study (not shown). At entry, 50% of patients were overweight (BMI = 25 and <30 kg/m²) and 33% were obese (≥30 kg/m²). The dairy products used were well accepted and compliance was good. Dietary intake of nutrients did not differ between the beginning and the end of the study (not shown). None of the patients included suffered further CV events during the study. One patient in the S group did not finish the study due to taking a prescription of statins (n = 1). The rest of the patients successfully completed the study (Fig. 1).

The amounts of oleic acid, DHA, and EPA daily supplemented in 500 mL of the enriched product were 5.12 g, 0.13 g, and 0.2 g, respectively, whereas the semi-skimmed milk contained only 1.82 g oleic acid/500 mL and undetectable levels of DHA and EPA. The plasma fatty acid profile did not change in patients from the C group, but in the S group, consumption of the fortified dairy product significantly increased the proportions of EPA, DHA, and the decreased ratio of arachidonic acid (AA) to EPA (Table 3). The plasma oleic acid level was greater in the S group at T6 and T12 compared with baseline, but at no time did the S and C groups differ. Plasma total saturated fatty acid levels in the C group and monounsaturated fatty acid levels in the S group tended to increase (P = 0.06). Other plasma fatty acids did not vary (not shown).

The plasma TC and LDL-C concentrations decreased in the S group at T9 and T12. The TC, but not the LDL-C concentration, differed between the S and C groups at these times. The plasma HDL-C and TG concentrations did not change in either group during the study, whereas the plasma concentration of ApoB significantly decreased at T6 and T12 in the S group (Table 4).

The amounts of folic acid and vitamin B-6 daily supplemented in 500 mL of enriched product were 150 μg and 1.5 mg, respectively. Plasma vitamin B-6 and plasma and RBC folate concentrations increased in the S group but not in the C group (Table 5). Plasma tHcy concentrations at baseline were within the normal range (<15 μmol/L) in both study groups (32). The S group exhibited a within-group reduction in the plasma tHcy concentration beginning at T3 that was sustained throughout the intervention, whereas the C group had only a modest reduction at T12. The vitamin E in the supplemented dairy product increased the plasma vitamin E concentration and the vitamin E:TC ratio in the S group at T12.

The plasma hs-CRP concentration at baseline was above high-risk values (>3 mg/L; 32) in both study groups. In the S group, this concentration decreased 20% at T6 and 48% at T12 (Table 5). The reductions were more pronounced when the subjects with baseline values >3 mg/L were considered separately and the changes were independent of weight reduction. Decreases in the hs-CRP of the subjects were directly correlated with the increases in plasma EPA + DHA (r = −0.52, P = 0.03) and EPA alone (r = −0.51; P = 0.04) and tended to be correlated with decreases plasma tHcy (r = 0.42; P = 0.08).

The plasma concentration of sICAM-1 increased slightly in the C group at T6 (276 ± 14 μg/L; P < 0.05) and T12 (285 ± 13 μg/L; P < 0.05) compared with initial values (247 ± 13 μg/L). In contrast, the plasma sICAM-1 concentration tended to decrease (P = 0.06) in the S group (from 289 ± 22 at T0, to 269 ± 15 at T6, and 254 ± 18 μg/L at T12). Other markers of endothelial dysfunction, such as sVCAM-1, and of plasma oxidizability, such as malondialdehyde, and oxidized LDL did not change in either group (data not shown). Blood pressure, heart rate, and results of the electrocardiogram tests did not change in either group throughout the study (data not shown).

Discussion

We found that the inclusion of a combination of nutrients in the daily diet of a group of male MI patients following a CR program may improve nutritional status and reduce CHD risk factors. The way the nutrients were administered (in a beverage consumed daily) may have contributed to the good compliance obtained with the protocol, as shown by the percentages of the plasma fatty acids and plasma concentration of vitamins that varied in response to the nutrients supplemented, which is in agreement with similar studies (21–24). In fact, the absorption of EPA and DHA from fish oil is improved when associated with other fats and spread out in small doses during the day (33), and the fact that milk fat is highly dispersed in very small micelles, increasing the surface of absorption of fats and lipid-soluble compounds (20), may explain the metabolic effects achieved when only small amounts were administered.

Although the prescription of the drugs, exercise, recommendations for healthier lifestyle, and the Mediterranean dietary pattern of the CR program resulted in the ability of C-group patients to maintain their lipid concentrations within the desirable range, the inclusion of the supplement in the diet reduced the total cholesterol concentrations by 10% and LDL-C by 14%, both of which have been associated with a lower risk for second MI events (4). Reduced ApoB in the S group suggests further beneficial effects in the reduction of CHD risk. ApoB has been reported to be a better index of CVD risk than LDL-C, as ApoB is a marker for all the potential atherogenic particles (34,35). Such reductions have been reported previously with the consumption of higher doses of fish oils (36,37) and similar food supplements (23,24). The amounts of lipid-lowering nutrients supplemented each day were quite low: 1 g of fish oil (0.33 g of EPA + DHA) and 5.2 g of oleic acid. Still, the effects observed
TABLE 4  Plasma lipid and ApoB concentrations in the C and S groups at the beginning of the study (T0) and after 3 (T3), 6 (T6), 9 (T9) and 12 (T12) mo of intervention

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>T0</th>
<th>T3</th>
<th>T6</th>
<th>T9</th>
<th>T12</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC, mmol/L</td>
<td>S</td>
<td>4.89 ± 0.21</td>
<td>5.14 ± 0.17</td>
<td>4.63 ± 0.13</td>
<td>4.43 ± 0.15*</td>
<td>4.37 ± 0.14†</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>4.74 ± 0.16</td>
<td>5.23 ± 0.19*</td>
<td>4.95 ± 0.22</td>
<td>5.07 ± 0.13</td>
<td>5.01 ± 0.15</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>S</td>
<td>3.02 ± 0.17</td>
<td>3.22 ± 0.17</td>
<td>2.73 ± 0.13</td>
<td>2.60 ± 0.17*</td>
<td>2.62 ± 0.12*</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>2.86 ± 0.14</td>
<td>3.15 ± 0.17</td>
<td>2.82 ± 0.15</td>
<td>2.87 ± 0.16</td>
<td>2.95 ± 0.17</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>S</td>
<td>1.21 ± 0.04</td>
<td>1.25 ± 0.04</td>
<td>1.22 ± 0.04</td>
<td>1.16 ± 0.05</td>
<td>1.17 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>1.31 ± 0.05</td>
<td>1.18 ± 0.05</td>
<td>1.11 ± 0.04</td>
<td>1.14 ± 0.05</td>
<td>1.10 ± 0.05</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>S</td>
<td>1.38 ± 0.12</td>
<td>1.41 ± 0.12</td>
<td>1.34 ± 0.13</td>
<td>1.43 ± 0.20</td>
<td>1.45 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>1.42 ± 0.09</td>
<td>1.54 ± 0.14</td>
<td>1.46 ± 0.11</td>
<td>1.58 ± 0.16</td>
<td>1.43 ± 0.09</td>
</tr>
<tr>
<td>ApoB, g/L</td>
<td>S</td>
<td>0.98 ± 0.04</td>
<td>ND (2)</td>
<td>0.88 ± 0.03*</td>
<td>ND (2)</td>
<td>ND (2)</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.94 ± 0.02</td>
<td>ND (2)</td>
<td>0.92 ± 0.02</td>
<td>ND (2)</td>
<td>0.92 ± 0.03</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 19 for S or n = 20 for C. *Different from T0, P < 0.05. †Different from C, P < 0.05.
2 ND, not determined.

were rather remarkable compared with other studies that involved capsules and/or tablets, but were in agreement with previous studies using this supplement (21–24) and could be attributed to the method of administration. Previous short-term studies using this food supplement (22) or a similar one (20,24) demonstrated a comparable lipid-lowering effect on TC and LDL-C, but, in contrast to those reports, our work did not find any effect on TG concentrations. A plausible explanation could be that, in those studies, TG concentrations were above normal at baseline, whereas, in our study, TG concentrations were normal (<1.7 mmol/L) (25). Indeed, in 2 other of our intervention studies with this food supplement, subjects with normal TG at baseline did not have TG reductions (21,23). These results suggest that the supplemented nutrients might contribute to blood-lipid stabilization in the context of a blood-lipid imbalance.

Local production of proinflammatory cytokines in the atheromatous plaque increases systemic markers of inflammation such as hs-CRP (38). In the present study, the hs-CRP reductions in the S group suggest a reduced risk of further coronary events by amelioration of the inflammatory status. In addition, the decreases of the AA:EPA ratio and increases of the plasma EPA and DHA concentrations in the S group suggest a less proinflammatory and thrombotic environment due to the increased production of EPA-derived eicosanoids, which possess less activity than those derived from AA (39).

At the present time, proven therapies to reduce hs-CRP levels include weight reduction (40) and, particularly, statin therapy (41,42). However, in our study, we found reductions of hs-CRP to be independent of those. A recent report showed that exercise training and a Mediterranean dietary pattern during phase II of CR produced a 41% median reduction of hs-CRP that was also independent of statin therapy and weight variation (43). The reductions of hs-CRP in the S group of our study agree with that report, and both were of similar or greater magnitude to those observed in numerous studies using statin therapy, with reductions in hs-CRP between 15 and 20% (41,42). Whereas statin therapy constitutes the major treatment option available for the reduction of hs-CRP and LDL-C (44), the combination of the supplemented beverage and statins are likely to produce further CV benefits and deserves to be explored. The CRP reduction observed with the administering of low amounts of anti-inflammatory nutrients could be attributed, again, to the increased bioavailability of the nutrients through the dairy product and perhaps to a pleiotropic effect of such nutrients combined.

Finally, at the end of the study, the S group of patients had improved vitamin status, increasing their vitamin E:TC ratio to optimal values (>5.2 μmol/L) (45) and changing their serum

TABLE 5  Plasma vitamins, tHcy, and CRP concentrations in the C and S groups at the beginning of the study (T0) and after 3 (T3), 6 (T6), 9 (T9), and 12 (T12) mo of intervention

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>T0</th>
<th>T3</th>
<th>T6</th>
<th>T9</th>
<th>T12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma folate, nmol/L</td>
<td>S</td>
<td>9.76 ± 0.64</td>
<td>21.08 ± 2.06*</td>
<td>18.06 ± 0.83*</td>
<td>16.90 ± 1.44*</td>
<td>19.53 ± 1.61*</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>10.47 ± 0.98</td>
<td>11.38 ± 1.08</td>
<td>12.14 ± 0.99</td>
<td>10.30 ± 0.82</td>
<td>11.48 ± 1.15</td>
</tr>
<tr>
<td>RBC folate, nmol/L</td>
<td>S</td>
<td>1196 ± 64</td>
<td>1292 ± 80†</td>
<td>1883 ± 100**</td>
<td>1768 ± 84**</td>
<td>1800 ± 105†</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>1208 ± 90</td>
<td>1037 ± 67*</td>
<td>1276 ± 78</td>
<td>1079 ± 82</td>
<td>1229 ± 116</td>
</tr>
<tr>
<td>tHcy, μmol/L</td>
<td>S</td>
<td>12.40 ± 0.81</td>
<td>10.53 ± 0.54*</td>
<td>9.97 ± 0.45*</td>
<td>9.53 ± 0.49*</td>
<td>8.98 ± 0.42*</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>11.59 ± 0.83</td>
<td>11.41 ± 0.60</td>
<td>11.06 ± 0.59</td>
<td>10.98 ± 0.60</td>
<td>10.00 ± 0.43*</td>
</tr>
<tr>
<td>hs-CRP, mg/L</td>
<td>S</td>
<td>3.90 ± 0.92</td>
<td>3.59 ± 0.49</td>
<td>3.12 ± 0.31*</td>
<td>2.48 ± 0.15*</td>
<td>2.01 ± 0.21†</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>3.64 ± 0.60</td>
<td>3.15 ± 0.81</td>
<td>3.87 ± 0.69</td>
<td>3.54 ± 0.44</td>
<td>3.23 ± 0.51</td>
</tr>
<tr>
<td>Vitamin B-6, nmol/L</td>
<td>S</td>
<td>80.64 ± 8.1</td>
<td>ND (2)</td>
<td>93.76 ± 10.51*</td>
<td>ND (2)</td>
<td>105.34 ± 10.62†</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>71.97 ± 12.14</td>
<td>ND</td>
<td>60.52 ± 8.36</td>
<td>ND</td>
<td>65.54 ± 6.75</td>
</tr>
<tr>
<td>Vitamin E, μmol/L</td>
<td>S</td>
<td>23.80 ± 2.84</td>
<td>ND</td>
<td>28.61 ± 3.25</td>
<td>ND</td>
<td>32.71 ± 3.06*</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>27.27 ± 2.7</td>
<td>ND</td>
<td>23.50 ± 2.09</td>
<td>ND</td>
<td>26.05 ± 2.09</td>
</tr>
<tr>
<td>Vitamin E:TC, μmol/L</td>
<td>S</td>
<td>4.83 ± 0.53</td>
<td>ND</td>
<td>6.31 ± 0.66*</td>
<td>ND</td>
<td>7.52 ± 0.69*</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>5.84 ± 0.58</td>
<td>ND</td>
<td>4.91 ± 0.48</td>
<td>ND</td>
<td>5.19 ± 0.39</td>
</tr>
</tbody>
</table>

1 Data are presented as means ± SEM, n = 19 for S or n = 20 for C. *Different from T0, P < 0.05. †Different from C, P < 0.05.
2 ND, not determined.


30. Thurnham DI, Smith E, Flora PS. Concurrent liquid-chromatographic assay of retinol, alpha tocopherol, beta-carotene, alpha-carotene, lycopene

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