Original Research

Effect of Omega-3 Fatty Acids Supplementation on Depressive Symptoms and on Health-Related Quality of Life in the Treatment of Elderly Women with Depression: A Double-Blind, Placebo-Controlled, Randomized Clinical Trial

Mariangela Rondanelli, PhD, Attilio Giacosa, MD, Annalisa Opizzi, Claudio Pelucchi, ScD, Carlo La Vecchia, MD, Gigliola Montorfano, Manuela Negroni, Bruno Berra, PhD, Pierluigi Politi, PhD, Angela Maria Rizzo, PhD

Department of Applied Health Sciences, Section of Human Nutrition and Dietetics (M.R.), Section of Psychiatry (P.P.), Faculty of Medicine, Endocrinology and Nutrition Unit, Azienda di Servizi alla Persona di Pavia (M.R., A.O.) University of Pavia, Pavia, Department of Gastroenterology and Nutritional Science, Policlinico di Monza (A.G.), ''Mario Negri'' Institute for Pharmacological Research (C.P., C.L.V.), Institute of General Physiology and Biochemistry ''G. Esposito'', University of Milan (G.M., M.N., B.B., A.R.), Milan, ITALY

Key words: n-3 long-chain polyunsaturated fatty acids, depression, elderly, AA/EPA

Objective: In elderly individuals, depression is one of the most frequently missed diagnoses with negative effects on quality of life. The authors investigated whether a supplement containing long-chain omega-3 polyunsaturated fatty acids (n-3 LCPUFA) improves depressive symptoms and health-related quality of life (HRQoL) in depressed elderly patients.

Design: Eight-week, randomized, double-blind, placebo-controlled trial.

Setting: Nursing home in Pavia, Italy.

Participants: Forty-six depressed women, aged 66–95 years.

Intervention: Twenty-two depressed women were included in the intervention group (n-3 group, which received 2.5 g/d of n-3 LCPUFA, with 1.67 g of eicosapentaenoic acid [EPA] and 0.83 g of docosahesaenoic acid [DHA]), and 24 patients were included in the placebo group. The primary endpoint was the improvement of depressive symptoms, as evaluated by the Geriatric Depression Scale (GDS). Secondary endpoints were the evaluation of HRQoL, by using the Short-Form 36-Item Health Survey (SF-36), and modifications of erythrocyte membrane phospholipids fatty acid profile. All variables were assessed before and after the treatment period of 8 weeks.

Results: The mean GDS at 8 weeks was significantly lower compared with the n-3 group. The SF-36 physical and mental components were significantly increased in the intervention group. Compliance was good, as confirmed by erythrocyte membrane phospholipid FA concentrations, with a significant increase of EPA and DHA in the intervention group.

Conclusion: Supplementation with n-3 LCPUFA is efficacious in the amelioration of depressive symptoms and quality of life in the treatment of depressed elderly female patients.

INTRODUCTION

In individuals older than 65 years, depression is one of the most frequently missed diagnoses [1–3]. Depression is frequently associated with cardiovascular diseases [4]; cerebrovascular pathology, such as stroke; or chronic inflammatory diseases (i.e., chronic arthritis) [5,6]. Moreover, depression is observed in 50% of elderly patients with dementia [7].

Address reprint requests to: Mariangela Rondanelli, Department of Applied Health Sciences, Section of Human Nutrition and Dietetics, Faculty of Medicine, Azienda di Servizi alla Persona di Pavia, University of Pavia, Istituto di Cura ''Santa Margherita'', Via Emilia 32, 27100 Pavia, ITALY. E-mail: serv.nutrizione@asppavia.it or mariangela.rondanelli@unipv.it

Journal of the American College of Nutrition, Vol. 29, No. 1, 55–64 (2010)
Published by the American College of Nutrition

Depression in the elderly population is associated with a higher mortality rate, due to suicide or other causes, as compared with nondepressed individuals of similar age [8,9]. Standard treatment of depression in the elderly population is psychotherapy and/or pharmacological intervention, mainly selective serotonin reuptake inhibitors [10].

It has been hypothesized that one of the reasons for the increase of depressive disorders over the past century may be correlated with a marked decrease in the ratio of n-3 to n-6 long-chain omega-3 polyunsaturated fatty acids (LCPUFA) due to diet changes [11]. The 2 n-3 LCPUFA, eicosapentaenoic acid (EPA) and docosahesaenoic acid (DHA), play an important role in the functioning of the central nervous system, and several studies have demonstrated that patients suffering from depression or mood disorders have significantly lower dietary intake and serum levels of n-3 LCPUFA [12,13]. Moreover, recent observations have shown that n-3 LCPUFA represent a potential treatment of depression [14–16]. Experimental evidence from animal studies demonstrates a positive correlation between a decrease of n-3 fatty acids and abnormal activity of dopaminergic, noradrenergic, and serotoninergic systems [17–25]. These observations are relevant because several depressive events could be associated with impairment of the serotoninergic and—at least in part—the adrenergic system [26,27].

Furthermore, depression affects the quality of life of seniors, particularly by restricting their social life and gradually reducing their independence, with increased risk of physical and functional decline [28–30].

The aim of the present study is to evaluate the effect of n-3 LCPUFA supplementation on depressive symptoms and health-related quality of life in depressed elderly patients, by means of a double-blind controlled study versus placebo.

MATERIAL AND METHODS

Participants

The eligible participants were women aged between 65 and 95 years, with a body mass index (BMI) higher than 19 and lower than 30 kg/m^2. Cases were recruited from a nursing home in Pavia, where they had been institutionalized for at least 3 months prior to enrollment. The protocol was approved by the Ethics Committee of the Azienda Sanitaria Locale of Pavia, and all participants gave their written consent to the study.

Data were gathered from the end of January 2006 to the end of December 2007. All patients presenting depressive symptoms, that is, with a Geriatric Depression Scale (GDS) score >10 at the last comprehensive geriatric assessment, which is performed as a routine procedure every 4 months, and having a Mini-Mental State Examination score higher than 24 [31] underwent a psychiatric evaluation made by a senior psychiatrist after an in-depth clinical interview. All participants admitted to treatment met the Diagnostic and Statistical Manual of Mental Disorders, fourth edition, text revision (*DSM-IV-TR*) [32] full criteria for major depression or dysthymia. Exclusion criteria were the following: (1) presence of a current comorbid psychiatric diagnosis other than major depression or dysthymia, (2) presence of active suicide ideation, (3) presence of psychotic symptoms, (4) current use of psychotropic drugs other than benzodiazepines (antidepressants, mood stabilizers, antipsychotics), or (5) presence of bipolar I and II disorder. Moreover, participants with a clinically uncontrolled organic disease or with clinically relevant laboratory abnormalities were excluded from the study. The ongoing pharmacological treatment at the inclusion time (such as drugs for insomnia, hypertension, diabetes, etc.) was maintained during the study. No intervention other than the administration of n-3 fatty acids was performed for depression.

Depressive symptoms were assessed through the use of the GDS [33] before and after the treatment period (weeks 0 and 8). The GDS Long Form is the most widely used scale for the evaluation of depression in elderly persons; it is a 30-item questionnaire in which participants are asked to respond by answering yes or no with reference to how they felt over the past week. Scores of 0–9 are considered normal, depending on age, education, and complaints; scores of 10–19 indicate mild-moderate depression; and a score greater than 20 is suggestive of severe depression. The GDS may be used with healthy, unhealthy, and mild to moderately cognitively impaired elderly persons. It has been extensively used in community, acute, and long-term care settings. The validity and reliability of the test have been supported through both clinical practice and research evidence [34,35]. The scale is commonly used as a routine part of a comprehensive geriatric assessment.

In addition, the studied participants were tested with the Short-Form 36-Item Health Survey (SF-36) [34], in order to evaluate their quality of life. The SF-36 questionnaire is a valid generic measure for rating health-related quality of life in several research fields, on the basis of its validity, high internal consistency, and high test-retest reliability [36]. The SF-36 is easy to administer and to compile for respondents, it has extensive psychometric validation, and is responsive to treatment in several medical conditions [36]. Response items are usually arranged in 8 domains reflecting physical and mental health-related quality of life: physical functioning (10 items), role limitations due to physical functioning (role-physical limitation; 4 items), bodily pain (2 items), general health (5 items), vitality (4 items), social functioning (2 items), role limitations due to emotional functioning (role-emotional limitation; 3 items), and mental health (5 items). The 8 scales were scored from 0 to 100 (worst to best possible health status). For each dimension, the score represented the mean of item values obtained by the participant when all of the items were completed or when the number of missing values was no

more than half of the total items. Otherwise, the score was recorded as missing. Moreover, the scales of the SF-36 were summarized into 2 dimensions. The first 5 scales make up the physical health dimension, and the last 5 form the mental health dimension. The vitality and general health scales are parts of both dimensions. Hence, each dimension includes 3 specific and 2 overlapping scales. The standardized summary scores for physical and mental components were computed and separately used as outcome measures. Also, the Qol SF-36 was assessed before and after the treatment period. The capacity of the patients to care for themselves was assessed by the Katz Index of Independence in Activities of Daily Living [37] prior to enrollment.

Body Composition and Nutritional Status

Nutritional status was assessed using anthropometric measurements. Body weight and height were measured, and BMI (kg/m^2) was calculated. Skinfold thicknesses (biceps, triceps, suprailiac, subscapular) were measured twice by a Harpender skinfold caliper at 5-minute intervals at each site using a standardized technique [38]. The Mini Nutritional Assessment (MNA) was also performed in all of the studied participants [39]. MNA, which comprises simple measurements and a brief questionnaire, involves an anthropometric assessment (weight, height, and weight loss), a general assessment (lifestyle, medication, and mobility), and a dietary assessment (number of meals, food and fluid intake, autonomy of eating self-assessment, self-perception of health and nutrition). Patients ate 3 meals daily, with breakfast between 7:00 and 8:00 AM, lunch between 12:00 and 1:00 PM, and dinner between 6:00 and 7:00 PM. The food intake was based on a well-balanced diet (with standard caloric and macronutrient and micronutrient content) provided by the hospital kitchen.

Biochemical Analyses

Fasting venous blood samples were drawn between 8:00 and 10:00 AM with the participants in a sitting position, for evaluation of white blood cell and red blood cell (RBC) count, hemoglobin and hematocrit, platelets, total lymphocytes, total proteins, iron, albumin, gamma-GT, total bilirubin, liver enzymes, triglycerides, total cholesterol and high-density lipoprotein cholesterol, glucose, uric acid, creatinine, electrolytes, C-reactive protein, thyroid-stimulating hormone (TSH), free thyroxin (FT4), free triiodothyronine (FT3), and RBC membrane lipid profile. Serum for clinical chemistry parameters was rapidly frozen and stored at $-80^{\circ}C$ until analysis (less than 1 month later). Whole blood (EDTA as anticoagulant) was used for hematological parameters. Clinical chemistry parameters were detected on the Roche Cobas Integra 400 plus analyzer (Roche Diagnostics, Basel, Switzerland), with dedicated commercial kits provided by the manufacturer. Low-density lipoprotein cholesterol was calculated according to the Friedewald formula [40]. Hematological parameters were measured using a Coulter automated cell counter MAX-M (Beckman Coulter, Inc., Fullerton, CA). TSH, FT4, and FT3 levels were detected in serum on a Roche Elecsys 2010 analyzer (Roche Diagnostics, Basel, Switzerland). To evaluate the RBC membrane lipid profile, RBC were separated from plasma by centrifugation and stored at $-70^{\circ}C$ until used for analysis. The analysis was carried out blind to the participant status. Cell membranes of RBC (ghosts) were prepared to eliminate hemoglobin residues. Ghost lipids were extracted with chloroform/methanol according to Folch et al. [41] and fractionated by silicic acid chromatography (200–400 mesh BIORAD) into nonpolar lipids, glycolipids, and phospholipids. Fatty acids from purified membrane phospholipids were determined by gas-chromatographic analysis. The fatty acid methylesters were obtained after derivatization with sodium methoxide in methanol 3.33% w/v and injected into a gaschromatograph (Agilent Technologies 6850 Series II) equipped with a flame ionization detector (FID) under the following experimental conditions: capillary column, AT Silar length 30 m; film thickness, 0.25 μm; gas carrier, helium; and temperature, injector $250^{\circ}C$, detector $275^{\circ}C$, oven $50^{\circ}C$ for 2 minutes, rate of $10^{\circ}C\ min^{-1}$ until $200^{\circ}C$ for 20 minutes. A standard mixture containing methyl ester fatty acids was injected for calibration.

The AA/EPA ratio and the omega-3 index were calculated [42].

Study Design

The study was a single-center, 8-week, double-blind randomized comparison of n-3 LCPUFA at 2.5 g/d versus placebo (paraffin oil). A total of 46 cases were included in the study. The sample size was computed on the basis of the primary endpoint (GDS). To detect a 20% difference in the GDS between the n-3 and the placebo groups, with an SD of the GDS equal to 4, a minimum of 23 participants per group were needed, with alpha = 0.05 and beta = 0.2 [15,43]. The participants were randomly assigned to 1 of the 2 groups in a double-blind parallel study. The participants were supplemented with fish oil or placebo (paraffin oil). Both the intervention and placebo group were treated with liquid products. The intervention treatment was obtained with 2.5 g/d of n-3 LCPUFA oil (1.67 g of EPA and 0.83 g of DHA) with lemon flavor. The nutritional composition of the product is reported in Table 1. The control group was treated with a placebo made of paraffin oil with the same lemon flavor as the intervention product. Participants were randomized to receive 1 serving containing 2.5 g of n-3 LCPUFA oil, orally, once a day, before lunch, or an identical serving of placebo for 8 weeks. The n-3 LCPUFA oil was manufactured by Also S.p.A., Div. Also-Enervit, Zelbio (CO), Italy. Bottles of identical oily preparation for each treatment group were assigned a participant number according to a coded (AB) block randomization table, prepared by an

Table 1. Nutritional Composition of n-3 LCPUFA Oil[1]

Nutrition Fact	100 g of Intervention Product	1 Daily Serving of Intervention Product
Calories	900 kcal 3700 kJ	37.53 kcal 154.3 kJ
Protein (g)	0	0
Total carbohydrate (g)	0	0
Total fat (g)	100	4.17
Saturated fat	3.1	0.13
Monounsaturated fat	11.5	0.48
Polyunsaturated fat	85.4	3.56
Total omega-3 fatty acids (g)	75	3.13
Eicosapentaenoic acid	40	1.67
Docosahexaenoic acid	20	0.83
Other omega-3	15	0.63

[1] Ingredients: fish oil standardized in n-3 LCPUFA (75%); lemon flavor; antioxidants: vitamin E (tocopherol), ascorbyl palmitate; acidifier: citric acid.

independent statistician. Investigators were blinded to the randomization table, the code assignments, and the procedure. As participants were enrolled, they were assigned a progressive participant number. The safety was based on the absence of serious side effects due to fish oil supplementation, which were represented by increased risk of bleeding (due to the antiaggregatory effect of fish oil on blood platelets) and by gastrointestinal symptoms, such as severe nausea and diarrhea [44]. Each day, the encharged caregivers, after the supplement supply, asked about the occurrence of unwanted side effects.

Primary Endpoint. The effect of n-3 LCPUFA supplementation on depressed mood, assessed by comparing the adjusted posttreatment means of the GDS total score in the n-3 and placebo groups, was considered the main outcome measure.

Secondary Endpoint. The secondary endpoints, addressed by comparing the adjusted posttreatment means of the n-3 and placebo groups, were the effect of n-3 LCPUFA supplementation on (1) health-related quality of life, evaluated with SF-36; (2) body composition; (3) nutritional status; and (4) hematological parameters.

Compliance. The evaluation of the compliance was performed by means of the analysis of the RBC membrane phospholipids fatty acid profile.

Statistical Analysis

Covariance analysis was performed to obtain adjusted posttreatment means and differences between treatment and placebo groups for GDS, SF-36 functions, and a number of biological outcomes. Adjustment was made for baseline values of a priori selected variables, including age (continuous term), self-sufficiency (3 levels, nonsufficient, partially, and totally self-sufficient), arthritis (no vs. yes), and, in turn, baseline measurement of each outcome considered (i.e., GDS, SF-36 physical function score, SF-36 mental function score, AA/EPA ratio, C20:4, C20:5, C22:5, C22:6, MNA score, BMI and arm muscle area, arm muscle area [AMA]); covariate adjustment for arthritis was considered due to the frequent imbalance in omega-6/omega-3 content in blood of participants with chronic inflammation pathologies [45,46]. All the analyses were 2 tailed, and p values of 0.05 or less were considered significant. Data analysis was performed using SAS software, version 9.1 (Cary, NC).

RESULTS

Twenty-one participants out of 271 institutionalized elderly women were excluded *a priori* because they were receiving antidepressant medications at the time of interview; the reason for including female patients only is based on the fact that the female to male ratio in our Institute of 530 beds is 5 to 1, and this is similar to what found in all Italian nursing homes.

Sixty-out of the remaining 226 participants fulfilled the diagnostic criteria of major depression or dysthymia according to *DSM-IV-TR*. All were institutionalized for more than 3 months and were not receiving antidepressant drugs at the time of observation. Nine of these 60 depressed patients were not eligible for the study due to creatinine >2 mg/dL in 2 cases, severe ischemic heart disease in 1 case, uncontrolled diabetes in 3 cases, hypertension not properly controlled by pharmacological treatment at the observation time in 1 case, and increased transaminase values as compared with baseline in 2 cases. Finally, 5 eligible patients refused to participate in the study, so the final number of randomized patients was 46, as shown in Figure 1. All participants were fully informed about other treatment options, but they chose to participate.

Twenty-two patients were randomly included in the intervention group (n-3 group) and 24 in the placebo group (placebo group). Population characteristics were similar in both groups (Table 2). At baseline, 21 of 22 (95.5%) participants in the n-3 group and 21 of 24 (87.5%) participants in the placebo group had a GDS >11.

Table 3 shows the complete fatty acids profile in the n-3 group and the placebo group before and after 2 months of treatment.

Primary Endpoint

The mean GDS score at week 8 was significantly lowered for the n-3 group ($p < 0.017$; Table 4). Since n-3 LCPUFA supplementation could not be considered as an antidepressive therapy, we assumed that a 33% reduction in GDS score from

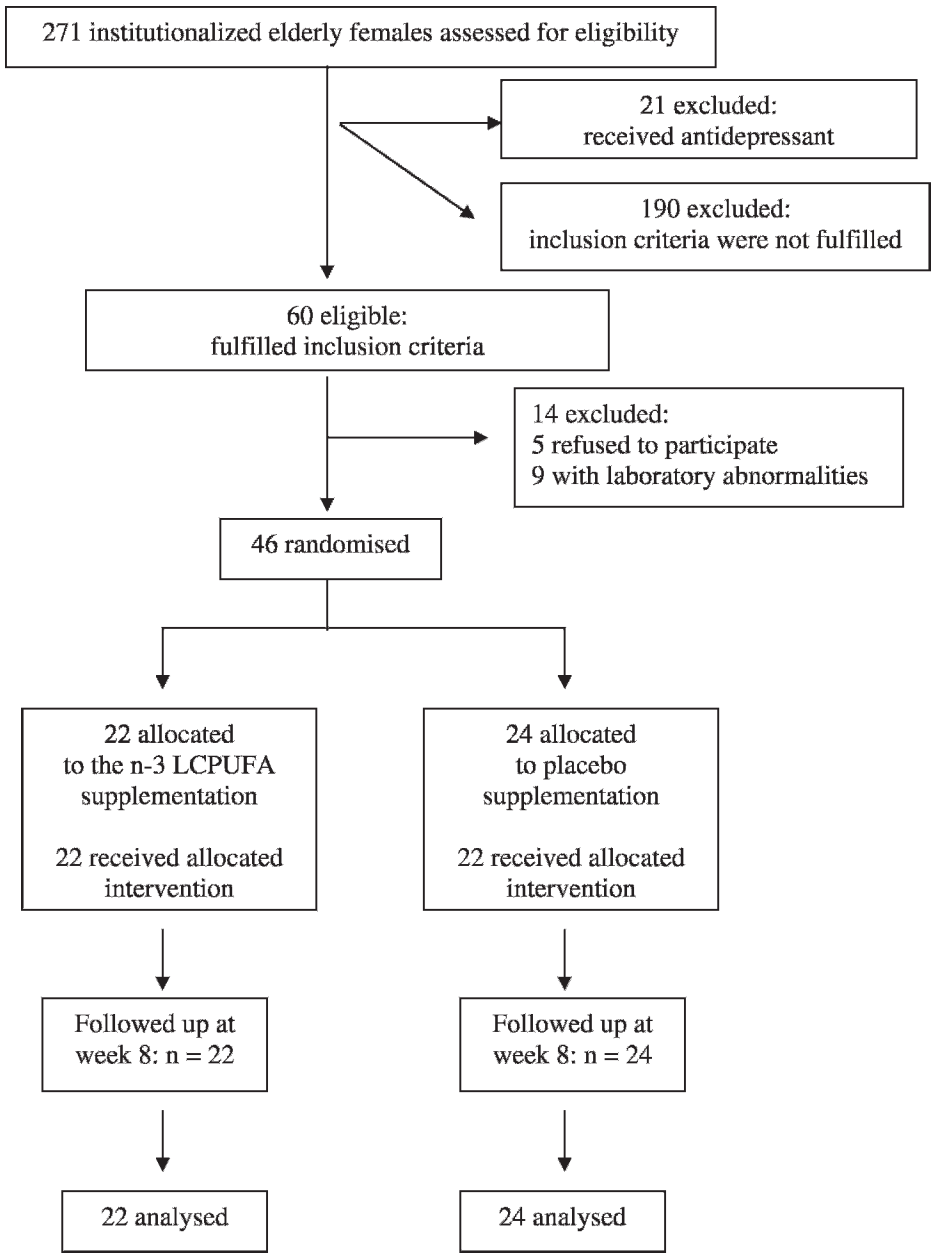

Fig. 1. Flow diagram of a trial of supplementation with n-3 LCPUFA versus placebo in the treatment of elderly patients affected by depression. The diagram includes the number of patients analyzed for the main outcome (effect on depressed mood).

baseline could be acceptable in evaluating response to supplementation. On the contrary, remission rate was defined as achieving a GDS score <11. The response rates were 45.5% in the n-3 group and 8.3% in the placebo group (χ^2 test, $p = 0.004$). The remission rates were 40.9% in the n-3 group and 16.7% in the placebo group (χ^2 test, $p = 0.07$).

Secondary Endpoints

The covariance analysis showed a significant difference ($p < 0.001$) between the n-3 group and the placebo group, as far as the SF-36 physical function score and the SF-36 mental function score were concerned (Table 4).

With regard to cell membrane fatty acids composition, the mean AA/EPA ratio at week 8 was significantly decreased for only the n-3 group (Table 4). Compliance after the supplementation period was confirmed by a significant increase in EPA in erythrocyte cell membrane phospholipids. DHA levels were increased as well in the intervention group (3.22 ± 1.33% before treatment and 4.06 ± 1.16% after 8 weeks of treatment, $p < 0.05$). All of the other studied fatty acids and the omega-3 index remained unchanged among the n-3 group. There was no

Table 2. Baseline Characteristics of Studied Participants[1]

	n-3 Group	Placebo Group
No. of subjects studied	22	24
Age (y)	84.9 ± 6.9	83.0 ± 7.3
Level of schooling (y)	12.7 ± 2.9	12 ± 3
Marital status		
Married	3	4
Widowed	19	20
Body mass index (kg/m^2)	24.1 ± 4.0	25.2 ± 5.5
Arm muscle area (cm^3)	27.7 ± 6.1	28.8 ± 8.6
Arthritis		
No	15	19
Yes	7	5
Self-sufficiency[2]		
Non–self-sufficient	1	2
Partially self-sufficient	10	16
Totally self-sufficient	11	5
Mini-Mental State Examination score	25.9 ± 2.0	26.2 ± 1.8
Mini Nutritional Assessment score	20.7 ± 4.2	20.4 ± 4.2
Activity Daily Living score	5.6 ± 0.4	5.4 ± 0.5
Geriatric Depression Scale score	17.1 ± 3.6	16.7 ± 4.3
SF-36 physical function score	45.0 ± 19.0	37.9 ± 22.1
SF-36 mental function score	61.2 ± 15.6	50.4 ± 20.0

[1] Values are means ± standard deviation.

[2] The sum does not add up to the total because of 1 missing value.

change in fatty acids level or in the omega-3 index in the placebo group.

The parameters of body composition and nutritional status (MNA, BMI, and AMA) did not change after 2 months of treatment in both the n-3 group and the placebo group (Table 4).

As far as the mean changes in the studied biochemical parameters are concerned, no significant differences were found between the n-3 group and the placebo group after 8 weeks of treatment (data not shown).

Safety

Both the omega-3 and placebo supplementation were well tolerated, and no serious adverse event was observed over the 8 weeks of the study. On the contrary, the following minor side effects were reported in the intervention group: eructation in 1 case, increase of usual constipation in 2 cases, and bloating in 3 cases. In the control group, 1 patient complained of mild headache, 2 of constipation, and 3 of eructation.

None of the studied patients complained of fish smell or fish eructation or made any comment about the content of the supplement she was taking or about the perception of having been included in 1 of the 2 groups, thus indicating the concealment of treatment allocation.

DISCUSSION

This double-blind intervention study shows that the supplementation with 2.5 g/d of n-3 LCPUFA for 2 months is associated with a significant reduction in depressive symptoms among elderly patients with major depression or dysthymia. This result has not been influenced by inadequacy of blinding because none of the patients made any comments about the type of oil (intervention or placebo) they were taking and none of the caregivers could identify the type of oil being administered. The main motivation was the similarity of taste of the intervention product and placebo, with both being masked by the same lemon flavor.

The covariance analysis of the GDS score between the n-3 and placebo group after treatment, adjusted for baseline value, age, arthritis, and self-sufficiency at baseline, shows a significant difference (i.e., −3.2, 95% confidence interval: −5.9, −0.6) between the 2 groups.

In this study with n-3 PUFA supplementation, no improvement in GDS or in SF-36 mental function score was found in the placebo group (i.e., placebo effect). This result might raise some doubts about treatment allocation concealment. However, the number of participants investigated was small, and random variation might be an explanation for the lack of effect. Furthermore, it is still not clear whether placebo has a greater effect than no treatment [47], and according to Walsh et al. [48], the response to placebo in published trials of antidepressant medication for major depression is highly variable.

The relation with supplementation of an LCPUFA is confirmed by a significant decrease in the AA/EPA ratio in RBC membrane, which indicates compliance with treatment, as this difference is mainly due to the increase of EPA value.

The evaluation of the RBC membrane lipid profile appears to be an interesting aspect of this study because the erythrocyte phospholipids fatty acid status reflects the usual dietary intake. Moreover, phospholipids are a model of fatty acid incorporation into a cellular membrane, and, finally, erythrocyte phospholipids are in equilibrium with structural phospholipids of tissues.

The positive effect of n-3 LCPUFA supplementation in depressed elderly patients appears of relevant clinical importance because depression is common in late life [1–3]. The prevalence of depressive symptoms in free-living elderly persons ranges from 10% to 20% [49], and in elderly hospitalized patients, it ranges from 22% to 34% [50,51]. Depression is not a natural part of aging and is often reversible with prompt and appropriate treatment. However, if left untreated, depression may result in the onset of physical, cognitive, and social impairment, as well as delayed recovery from medical illness and surgery, increased health care utilization, and suicide [52,53].

The results of the present study confirm various epidemiological and intervention studies already published in this field [14–16]. Hibbeln [54] showed a strong inverse association

Table 3. Fatty Acids Profile of Participants at Baseline and after the Treatment Period[1]

	n-3 Group, at Baseline	n-3 Group, after Treatment	n-3 Group, Mean Change from Baseline	Placebo Group, at Baseline	Placebo Group, after Treatment	Placebo Group, Mean Change from Baseline
AA/EPA, whole blood	35.7 ± 18.4	19.1 ± 12.8	−16.6	31.4 ± 17.2	28.2 ± 15.1	−3.18
AA/EPA, membrane	70.8 ± 37.4	41.9 ± 24.2	−28.9	82.1 ± 40.8	69.3 ± 30.7	−12.8
Membrane/whole blood	2.32 ± 1.15	2.95 ± 2.45	0.63	2.74 ± 0.85	2.60 ± 0.99	−0.14
C 16, whole blood	26.1 ± 2.1	25.4 ± 2.6	−0.66	25.7 ± 2.3	25.3 ± 1.9	−0.42
C 16:1, whole blood	2.86 ± 0.72	3.12 ± 0.86	0.26	2.69 ± 0.83	2.69 ± 1.03	−0.01
C 18, whole blood	10.8 ± 0.9	10.6 ± 1.3	−0.23	10.6 ± 1.2	10.1 ± 1.4	−0.42
C 18:1, whole blood	25.2 ± 2.0	24.9 ± 2.4	−0.30	26.0 ± 3.7	26.0 ± 3.7	0.00
C 18:2, whole blood	18.8 ± 2.9	19.7 ± 3.3	0.94	18.6 ± 3.5	19.7 ± 3.8	1.11
C 18:3, whole blood	0.35 ± 0.17	0.39 ± 0.16	0.04	0.32 ± 0.12	0.47 ± 0.71	0.15
C 20:3, whole blood	1.95 ± 0.51	1.97 ± 0.37	0.02	1.90 ± 0.35	1.95 ± 0.64	0.05
C 20:4, whole blood	11.0 ± 1.7	10.2 ± 1.2	−0.88	11.1 ± 2.1	10.6 ± 1.9	−0.47
C 20:5, whole blood	0.39 ± 0.20	0.77 ± 0.48	0.38	0.44 ± 0.21	0.46 ± 0.21	0.02
C 22:5, whole blood	0.92 ± 0.16	1.27 ± 0.41	0.35	0.98 ± 0.23	1.00 ± 0.25	0.02
C 22:6, whole blood	1.63 ± 0.39	1.71 ± 0.61	0.08	1.68 ± 0.44	1.64 ± 0.42	−0.04
C 16, membrane	28.2 ± 3.8	26.5 ± 4.0	−1.67	28.0 ± 3.6	26.8 ± 3.0	−1.25
C 16:1, membrane	0.94 ± 0.53	1.28 ± 0.74	0.34	0.75 ± 0.63	0.62 ± 0.37	−0.13
C 18, membrane	18.6 ± 2.1	16.7 ± 4.1	−1.80	18.7 ± 1.3	18.7 ± 1.2	−0.03
C 18:1, membrane	19.0 ± 2.6	18.9 ± 1.8	−0.08	18.1 ± 2.2	18.0 ± 2.1	−0.13
C 18:2, membrane	9.3 ± 1.1	9.5 ± 1.3	0.15	9.8 ± 1.4	9.8 ± 1.6	0.00
C 18:3, membrane	0.29 ± 0.30	0.61 ± 0.43	0.32	0.31 ± 0.34	0.54 ± 0.77	0.23
C 20:3, membrane	1.90 ± 0.71	2.16 ± 0.60	0.26	1.90 ± 0.49	1.88 ± 0.48	−0.03
C 20:4, membrane	16.5 ± 4.7	17.5 ± 3.3	1.01	16.6 ± 4.0	17.5 ± 3.0	0.94
C 20:5, membrane	0.26 ± 0.14	0.51 ± 0.21	0.25	0.27 ± 0.15	0.31 ± 0.16	0.04
C 22:5, membrane	1.79 ± 0.65	2.19 ± 0.59	0.40	1.85 ± 0.51	1.99 ± 0.46	0.13
C 22:6, membrane	3.22 ± 1.33	4.06 ± 1.16	0.83	3.68 ± 1.29	3.92 ± 0.96	0.24

[1] Values are means ± standard deviation.

between the prevalence of depression across 13 countries, and Tanskanen et al. [55] reported a higher prevalence of depressive symptoms in infrequent than in frequent fish consumers in Finland. An inverse correlation between n-3 PUFA intake and depressed mood has also been reported in various clinical studies. Lower concentrations of n-3 PUFA have been reported in plasma or in RBC membranes of patients with a *DSM-IV* major depressive disorder diagnosis compared with matched nondepressed control subjects [56,57]. In addition to this, the positive effect of omega-3 fatty acids in the treatment of depression has been shown in a recent meta-analysis published by Lin and Su [58], although findings were limited by publication bias and heterogeneity between the studies considered. In fact, the positive effect was observed only in patients with clearly defined *DSM-IV* major depressive disorder.

Another relevant advantage observed in this study is that the supplementation with n-3 LCPUFA is associated with a significant amelioration of quality of life. After 2 months of treatment, the group of patients who received the supplement showed a significant amelioration of physical and mental function, as demonstrated by the SF-36 score. This observation has never been achieved before, and it appears of great value from a clinical point of view, due to the importance of these aspects in the elderly population. The concept of quality of life is defined as a perceived global satisfaction and satisfaction within a number of key domains, with special emphasis on well-being [28]. Therefore, the amelioration of quality of life in depressed elderly patients after supplementation with n-3 LCPUFA is an important finding.

Moreover, the intervention with n-3 LCPUFA appears to be safe. No relevant side effects were observed in the intervention group, not even adverse gastrointestinal effects, as reported in previous studies [59]. On the contrary, the antidepressant drugs usually prescribed in these patients are frequently associated with unwanted symptoms and complications.

In addition to these observations, a wealth of evidence indicates that consumption of fish or dietary fish oils containing long-chain omega-3 polyunsaturated fatty acids, such as EPA and DHA, is associated with cardiovascular benefits, including a reduction in circulating triglycerides and reduced mortality from coronary heart disease [60]. These data represent an additional advantage of the use of n-3 LCPUFA in elderly depressed female patients.

In conclusion, the treatment of female elderly depression with LCPUFA supplementation appears to be efficacious in reducing depressive symptoms and ameliorating quality of life.

The results of this study appear of relevant clinical interest, but the small number of studied patients represents a limitation. In view of the of high incidence of depression in

Table 4. N-3 and Placebo Group Posttreatment Means and Their Differences (95% CI) Both Unadjusted and Adjusted for Baseline Value of the Corresponding Endpoint, Age, Arthritis, and Self-Sufficiency at Baseline[1]

	Posttreatment Mean ± SD, n-3 Group (n = 22)	Posttreatment Mean ± SD, Placebo Group (n = 24)	Unadjusted Difference between n-3 and Placebo Group (95% CI)	Significance	Adjusted Difference between n-3 and Placebo Group (95% CI)	Significance
Primary endpoint						
Geriatric Depression Scale score	12.6 ± 4.3	15.9 ± 5.4	−3.2 (−6.2, −0.3)	0.032	−3.2 (−5.9, −0.6)	0.017
Secondary endpoints						
SF-36 physical function score	52.0 ± 15.6	30.8 ± 16.0	21.1 (10.8, 31.4)	<0.001	15.9 (9.9, 21.9)	<0.001
SF-36 mental function score	69.8 ± 11.0	44.6 ± 15.6	25.2 (16.3, 34.1)	<0.001	18.3 (12.9, 23.7)	<0.001
AA/EPA ratio	41.9 ± 24.2	69.3 ± 30.7	−27.4 (−43.9, −10.8)	0.002	−28.2 (−44.4, −11.9)	0.001
C 20:4 (arachidonic acid)	17.5 ± 3.3	17.5 ± 3.0	−0.02 (−2.2, 2.1)	0.98	−0.5 (−2.6, 1.6)	0.63
C 20:5 (eicosapentaenoic acid)	0.51 ± 0.21	0.31 ± 0.16	0.20 (0.07, 0.32)	0.004	0.20 (0.07, 0.33)	0.003
C 22:5 (docosapentaenoic acid)	2.19 ± 0.59	1.99 ± 0.46	0.20 (−0.15, 0.56)	0.25	0.19 (−0.18, 0.55)	0.30
C 22:6 (docosahenoic acid)	4.06 ± 1.16	3.92 ± 0.96	0.14 (−0.58, 0.86)	0.70	0.26 (−0.46, 0.99)	0.46
Omega-3 index membrane	4.56 ± 1.28	4.23 ± 1.05	0.33 (−0.46, 1.13)	0.40	0.49 (−0.29, 1.28)	0.21
Mini Nutritional Assessment score	20.1 ± 3.8	20.1 ± 4.4	0.0 (−2.5, 2.5)	0.99	−0.2 (−1.2, 0.9)	0.77
Body mass index (kg/m^2)	23.6 ± 3.7	25.2 ± 5.2	−1.6 (−4.4, 1.1)	0.23	−0.6 (−1.3, 0.1)	0.08
Arm muscle area (cm^3)	27.9 ± 6.1	27.9 ± 8.7	−0.0 (−4.5, 4.5)	0.99	0.8 (−0.8, 2.3)	0.33

[1] Adjusted difference between n-3 and placebo group was calculated using covariance analysis, including terms for age, self-sufficiency, arthritis, and, in turn, baseline measurement of each outcome considered.

elderly persons, with high social and economic costs, further intervention studies with n-3 PUFA of longer duration and in larger groups of patients are warranted.

ACKNOWLEDGMENTS

This study was supported by a research grant from Regione Lombardia. The grant was used to support the team coordinated by Professor B. Berra (biochemical assays), Dr C. Pelucchi (statistical analysis), and Dr A. Opizzi (clinical management of the trial). None of the authors have conflicts of interest.

REFERENCES

1. Luijendijk HJ, van den Berg JF, Dekker MJ, van Tuijl HR, Otte W, Smit F, Hofman A, Stricker BH, Tiemeier H: Incidence and recurrence of late-life depression. Arch Gen Psychiatry 65:1394–1401, 2008.
2. Lebowitz BD, Pearson JL, Schneider LS, Reynolds CF III, Alexopoulos GS, Bruce ML, Conwell Y, Katz IR, Meyers BS, Morrison MF, Mossey J, Niederehe G, Parmelee P: Diagnosis and treatment of depression in late life: consensus statement update. JAMA 278:1186–1190, 1997.
3. Anderson DN: Treating depression in old age: the reasons to be positive. Age Ageing 30:13–17, 2001.
4. Frasure-Smith N, Lespérance F: Recent evidence linking coronary heart disease and depression. Can J Psychiatry 51:730–737, 2006.
5. Rao R: Cerebrovascular disease and late life depression: an age old association revisited. Int J Geriatr Psychiatry 15:419–433, 2000.
6. Mingo CA, McIlvane JM, Baker TA: Explaining the relationship between pain and depressive symptoms in African-American and white women with arthritis. J Natl Med Assoc 100:996–1003, 2008.
7. Olin JT, Schneider LS, Katz IR, Meyers BS, Alexopoulos GS, Breitner JC, Bruce ML, Caine ED, Cummings JL, Devanand DP, Krishnan KR, Lyketsos CG, Lyness JM, Rabins PV, Reynolds CF III, Rovner BW, Steffens DC, Tariot PN, Lebowitz BD: Provisional diagnostic criteria for depression of Alzheimer disease. Am J Geriatr Psychiatry 10:125–128, 2002.
8. Pearson JL, Conwell Y: Suicide in late life: challenges and opportunities for research. Int Psychogeriatr 7:131–136, 1995.
9. Pearson JL, Brown GK: Suicide prevention in late life: directions for science and practice. Clin Psychol Rev 20:685–705, 2000.
10. Sonnenberg CM, Deeg DJ, Comijs HC, van Tilburg W, Beekman AT: Trends in antidepressant use in the older population: results from the LASA-study over a period of 10 years. J Affect Disord 111:299–305, 2008.
11. Parker G, Gibson NA, Brotchie H, Heruc G, Rees AM, Hadzi-Pavlovic D: Omega-3 fatty acids and mood disorders. Am J Psychiatry 163:969–978, 2006.
12. Hakkarainen R, Partonen T, Haukka J, Virtamo J, Albanes D, Lönnqvist J: Is low dietary intake of omega-3 fatty acids associated with depression? Am J Psychiatry 161:567–569, 2004.
13. Conklin SM, Manuck SB, Yao JK, Flory JD, Hibbeln JR, Muldoon MF: High omega-6 and low omega-3 fatty acids are associated with depressive symptoms and neuroticism. Psychosom Med 69:932–934, 2007.
14. Nemets B, Stahl Z, Belmaker RH: Addition of omega-3 fatty acid to maintenance medication treatment for recurrent unipolar depressive disorder. Am J Psychiatry 159:477–479, 2002.
15. Peet M, Horrobin DF: A dose-ranging study of the effects of ethyl-eicosapentaenoate in patients with ongoing depression despite apparently adequate treatment with standard drugs. Arch Gen Psychiatry 59:913–919, 2002.

16. Lucas M, Asselin G, Mérette C, Poulin MJ, Dodin S: Ethyl-eicosapentaenoic acid for the treatment of psychological distress and depressive symptoms in middle-aged women: a double-blind, placebo-controlled, randomized clinical trial. Am J Clin Nutr 89:641–651, 2009.
17. Delion S, Chalon S, Hérault J, Guilloteau D, Besnard JC, Durand G: Chronic dietary alpha-linolenic acid deficiency alters dopaminergic and serotoninergic neurotransmission in rats. J Nutr 124:2466–2476, 1994.
18. Delion S, Chalon S, Guilloteau D, Besnard JC, Durand G: Alpha-Linolenic acid dietary deficiency alters age-related changes of dopaminergic and serotoninergic neurotransmission in the rat frontal cortex. J Neurochem 66:1582–1591, 1996.
19. Zimmer L, Hembert S, Durand G, Breton P, Guilloteau D, Besnard JC, Chalon S: Chronic n-3 polyunsaturated fatty acid diet-deficiency acts on dopamine metabolism in the rat frontal cortex: a microdialysis study. Neurosci Lett 240:177–181, 1998.
20. Zimmer L, Durand G, Guilloteau D, Chalon S: n-3 polyunsaturated fatty acid deficiency and dopamine metabolism in the rat frontal cortex. Lipids 34:S251, 1999.
21. Zimmer L, Delion-Vancassel S, Durand G, Guilloteau D, Bodard S, Besnard JC, Chalon S: Modification of dopamine neurotransmission in the nucleus accumbens of rats deficient in n-3 polyunsaturated fatty acids. J Lipid Res 41:32–40, 2000.
22. Zimmer L, Delpal S, Guilloteau D, Aïoun J, Durand G, Chalon S: Chronic n-3 polyunsaturated fatty acid deficiency alters dopamine vesicle density in the rat frontal cortex. Neurosci Lett 284:25–28, 2000.
23. Chalon S, Vancassel S, Zimmer L, Guilloteau D, Durand G: Polyunsaturated fatty acids and cerebral function: focus on monoaminergic neurotransmission. Lipids 36:937–944, 2001.
24. Kodas E, Page G, Zimmer L, Vancassel S, Guilloteau D, Durand G, Chalon S: Neither the density nor function of striatal dopamine transporters were influenced by chronic n-3 polyunsaturated fatty acid deficiency in rodents. Neurosci Lett 321:95–99, 2002.
25. Zimmer L, Vancassel S, Cantagrel S, Breton P, Delamanche S, Guilloteau D, Durand G, Chalon S: The dopamine mesocortico-limbic pathway is affected by deficiency in n-3 polyunsaturated fatty acids. Am J Clin Nutr 75:662–667, 2002.
26. Brunello N, Riva M, Rovescalli AC, Galimberti R, Racagni G: Age-related changes in rat serotonergic and adrenergic systems and in receptor responsiveness to subchronic desipramine treatment. Pharmacol Toxicol 63:150–155, 1988.
27. McEntee WJ, Crook TH: Serotonin, memory, and the aging brain. Psychopharmacology 103:143–149, 1991.
28. Hörnquist JO: Quality of life: concept and assessment. Scand J Soc Med 18:69–79, 1990.
29. Gallo JJ, Lebowitz BD: The epidemiology of common late-life mental disorders in the community: themes for the new century. Psychiatr Serv 50:1158–1166, 1999.
30. Cohen GD: Depression in late life: an historic account demonstrates the importance of making the diagnosis. Geriatrics 57:38–39, 2002.
31. Folstein MF, Folstein SE, McHugh PR: ''Mini-mental state'': a practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 12:189–198, 1975.
32. American Psychiatric Association: Mood disorders. In American Psychiatric Association (eds): ''Diagnostic and Statistical Manual of Mental Disorders,'' 4th ed. Washington, DC: American Psychiatric Association, pp 317–345, 2000.
33. Yesavage JA, Brink TL, Rose TL, Lum O, Huang V, Adey M, Leirer VO: Development and validation of a geriatric depression screening scale: a preliminary report. J Psychiatr Res 17:37–49, 1983.
34. Sheikh JI, Yesavage JA: Geriatric Depression Scale (GDS): recent evidence and development of a shorter version. In Brink TL (ed): ''Clinical Gerontology: A Guide to Assessment and Intervention.'' New York, NY: The Haworth Press, pp 165–173, 1986.
35. Wancata J, Alexandrowicz R, Marquart B, Weiss M, Friedrich F: The criterion validity of the Geriatric Depression Scale: a systematic review. Acta Psychiatr Scand 114:398–410, 2006.
36. Ware JE, Snow KK, Kosinski M, Gandek B: ''SF-36 Health Survey: Manual and Interpretation Guide.'' Boston, MA: The Health Institute, 1993.
37. Katz S, Downs TD, Cash HR, Grotz RC: Progress in development of the index of ADL. Gerontologist 10:20–30, 1970.
38. Frisancho AR: New standards of weight and body composition by frame size and height for assessment of nutritional status of adults and the elderly. Am J Clin Nutr 40:808–819, 1984.
39. Guigoz Y, Vellas B, Garry PJ: Assessing the nutritional status of the elderly: the Mini Nutritional Assessment as part of the geriatric evaluation. Nutr Rev 54:S59–S65, 1996.
40. Friedewald WT, Levy RI, Fredrickson DS: Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18:499–502, 1972.
41. Folch J, Lees M, Sloane Stanley GH: A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 226:497–509, 1957
42. Harris WS, Von Schacky C: The Omega-3 Index: a new risk factor for death from coronary heart disease? Prev Med 39:212–220, 2004.
43. Sims J, Hill K, Davidson S, Gunn J, Huang N: Exploring the feasibility of a community-based strength training program for older people with depressive symptoms and its impact on depressive symptoms. BMC Geriatr 6:18, 2006.
44. Mason P: Fish oils—an update. Pharm J 265:720–723, 2000.
45. Simopoulos AP: The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Exp Biol Med 233:674–688, 2008.
46. Petersson H, Basu S, Cederholm T, Risérus U: Serum fatty acid composition and indices of stearoyl-CoA desaturase activity are associated with systemic inflammation: longitudinal analyses in middle-aged men. Br J Nutr 99:1186–1190, 2008.
47. Hrobjartsson A, Gøtzsche PC: Is the placebo powerless? An analysis of clinical trials comparing placebo with no treatment. N Engl J Med 344:1594–1602, 2001.
48. Walsh BT, Seidman SN, Sysko R, Gould M: Placebo response in studies of major depression: variable, substantial and growing. JAMA 287:1840–1847, 2002.
49. Beekman AT, Copeland JR, Prince MJ: Review of community prevalence of depression in later life. Br J Psychiatry 174:307–311, 1999.
50. Meldon SW, Emerman CL, Schubert DS: Recognition of depression in geriatric ED patients by emergency physicians. Ann Emerg Med 30:442–447, 1997.

51. Raccio-Robak N, McErlean MA, Fabacher DA, Milano PM, Verdile VP: Socioeconomic and health status differences between depressed and nondepressed ED elders. Am J Emerg Med 20:71–73, 2002.
52. Luber MP, Meyers BS, Williams-Russo PG, Hollenberg JP, DiDomenico TN, Charlson 5E, Alexopoulos GS: Depression and service utilization in elderly primary care patients. Am J Geriatr Psychiatry 9:169–176, 2001.
53. Krishnan KR, Delong M, Kraemer H, Carney R, Spiegel D, Gordon C, McDonald W, Dew M, Alexopoulos G, Buckwalter K, Cohen PD, Evans D, Kaufmann PG, Olin J, Otey E, Wainscott C: Comorbidity of depression with other medical diseases in the elderly. Biol Psychiatry 52:559–588, 2002.
54. Hibbeln JR: Fish consumption and major depression. Lancet 351:1213, 1998.
55. Tanskanen A, Hibbeln JR, Tuomilehto J, Uutela A, Haukkala A, Viinamäki H, Lehtonen J, Vartiainen E: Fish consumption and depressive symptoms in the general population in Finland. Psychiatr Serv 52:529–531, 2001.
56. Edwards R, Peet M, Shay J, Horrobin D: Depletion of docosahexaenoic acid in red blood cell membranes of depressive patients. Biochem Soc Trans 26:S142, 1998.
57. Peet M, Murphy B, Shay J, Horrobin D: Depletion of omega-3 fatty acid levels in red blood cell membranes of depressive patients. Biol Psychiatry 43:315–319, 1998.
58. Lin PY, Su KP: A meta-analytic review of double-blind, placebo-controlled trials of antidepressant efficacy of omega-3 fatty acids. J Clin Psychiatry 68:1056–1061, 2007.
59. Austria JA, Richard MN, Chahine MN, Edel AL, Malcomson LJ, Dupasquier CM, Pierce GN: Bioavailability of alpha-linolenic acid in subjects after ingestion of three different forms of flaxseed. J Am Coll Nutr 27:214–221, 2008.
60. Carroll DN, Roth MT: Evidence for the cardioprotective effects of omega-3 fatty acids. Ann Pharmacother 36:1950–1956, 2002.

Received April 7, 2009; revision accepted November 30, 2009.