

6c.

LONG-TERM EFFECT OF OMEGA-3 FATTY ACID SUPPLEMENTATION IN ACTIVE RHEUMATOID ARTHRITIS

A 12-Month, Double-Blind, Controlled Study

PIET GEUSENS, CARINE WOUTERS, JOS NIJS, YEBIN JIANG, and JAN DEQUEKER

Objective. To study the long-term effects of supplementation with omega-3 fatty acids (ω 3) in patients with active rheumatoid arthritis.

Methods. Ninety patients were enrolled in a 12-month, double-blind, randomized study comparing daily supplementations with either 2.6 gm of ω 3, or 1.3 gm of ω 3 + 3 gm of olive oil, or 6 gm of olive oil.

Results. Significant improvement in the patient's global evaluation and in the physician's assessment of pain was observed only in those taking 2.6 gm/day of ω 3. The proportions of patients who improved and of those who were able to reduce their concomitant anti-rheumatic medications were significantly greater with 2.6 gm/day of ω 3.

Conclusion. Daily supplementation with 2.6 gm of ω 3 results in significant clinical benefit and may reduce the need for concomitant antirheumatic medication.

Omega-3 fatty acids (ω 3) are essential polyunsaturated fatty acids found in fish oils and in marine mammals (1). They competitively inhibit the formation of the 2-series prostanoids and of the 4-series leukotrienes, eicosanoids derived from arachidonic acid with potent proinflammatory effects, and they are precursors of the 3-series prostanoids and the 5-series leukotrienes (2,3). Ingestion of ω 3 therefore results in

the production of compounds with altered and diminished biologic activity.

Beneficial effects of dietary supplementation with ω 3 have been demonstrated in some (4-7), but not all (8), animal models of inflammatory disease and in (mainly short-term) clinical studies in patients with rheumatoid arthritis (RA) (9-17). Long-term data in humans are presently scarce (18).

We therefore studied the effect of fish oil supplementation in patients with active RA who were being treated with nonsteroidal antiinflammatory drugs (NSAIDs) and/or disease-modifying antirheumatic drugs (DMARDs).

PATIENTS AND METHODS

Study design and patient population. According to the short-term observations by Kremer et al (9,10) concerning global improvement with ω 3 in RA patients, a sample size of 23 patients for each of 3 treatment groups was calculated to be adequate, with an α of 0.05 and a β of 0.20. Considering possible dropouts, we included 30 patients per group.

Patients with definite or classic RA, who had class I, II, or III disease, according to the criteria of the American College of Rheumatology (formerly, the American Rheumatism Association) (19,20), entered the study after giving informed consent. All patients had active disease, and had been receiving a stable dosage of NSAIDs and/or DMARDs for at least 3 months prior to study entry. The study protocol had been approved by the institutional ethics committee.

Patients were randomly assigned to 1 of the following 3 daily regimens: 6 capsules containing 1 gm of olive oil each (placebo), or 3 capsules containing 1 gm of fish oil (1.3 gm ω 3) each plus 3 placebo capsules, or 6 capsules containing 1 gm of fish oil each (2.6 gm of ω 3). Treatment continued for 12 months.

The fish oil capsules contained 1 gm of oil that was rich in ω 3 polyunsaturated fatty acids (42.5%), consisting primarily of eicosapentaenoic acid (EPA; 28%) and docosa-

Piet Geusens, MD, PhD: Katholieke Universiteit Leuven, Universitaire Ziekenhuizen Pellenberg, Belgium; Carine Wouters, MS: K. U. Leuven; Jos Nijs, MS: K. U. Leuven; Yebin Jiang, MD, PhD: K. U. Leuven; Jan Dequeker, MD, PhD, FRCP Edin: K. U. Leuven.

Address reprint requests to Piet Geusens, MD, PhD, Arthritis and Metabolic Bone Disease Research Unit, K.U. Leuven, U.Z. Pellenberg, Weligerveld 1, B-3212 Pellenberg, Belgium.

Submitted for publication July 14, 1992; accepted in revised form December 9, 1993.

Table 1. Reasons for study dropout, by treatment group

	Placebo	1.3 gm/day of ω3	2.6 gm/day of ω3
Adverse events	1	1	1
Medical reasons not related to study treatment	1	2	2
Nonmedical reasons (refusal, reduced mobility, relocation, etc.)	3	1	4
Noncompliance	5	5	4
Total	10	9	11

hexaenoic acid (DHA; 6%). Each capsule also contained C22:5 n3 (3.2%) and C18:4 n3 (2.3%), smaller amounts of C20:4 n3 and C18:3 n3, a low cholesterol content (0.5–1 mg/capsule), and vitamin E (2 mg/capsule). Fish oil and placebo capsules were provided by Sanofi-Pharma (Brussels, Belgium).

Kremer et al reported that a 1.9 gm/day dose of EPA for 6 months (for a patient weighing 70 kg) is effective, but suggested that longer-term studies were needed (13). We selected a dose of 2.6 gm/day of ω3, given as 6 capsules containing 1 gm of fish oil, which corresponds to a comparable daily dose of EPA (1.7 gm/day). A higher dose was not used, because it has been observed that in patients with renal hypertension and hyperlipidemia, daily ingestion of 9 capsules containing 1 gm of fish oil (same preparation we used, corresponding to 3.9 gm of ω3) resulted in digestive symptoms in the majority of the patients (21).

A normal, stable diet with approximately 30% fat, 12–15% protein, and 50–58% carbohydrate was prescribed during the study. Patients were advised to consume fish once a week. The intake of animal fat was <100 gm/day. Patients were asked to apply this diet throughout the study, so that there would be a stable and similar diet in all study groups.

Compliance. Compliance with the treatment protocol was monitored by capsule counts.

Evaluation criteria. Criteria were ranked at the outset of the study, in order of importance for evaluation. The following features were assessed at baseline and after 3, 6, 9, and 12 months: physician's global assessment of disease activity (0–4 scale, 0 = symptom-free and 4 = very severe), patient's global assessment of disease activity (0–10-cm visual analog scale, 0 = symptom-free and 10 = very severe), physician's and patient's assessment of pain (0–4 scale, 0 = no pain and 4 = very severe), duration of morning stiffness (in minutes), grip strength (in mm Hg; mean of 3 measurements), Ritchie articular index for pain (22) (60 joints; scored 0–3 for each joint, 0 = no pain and 3 = very severe), number of painful joints and number of swollen joints (maximum of 60 each), and concomitant medications (NSAIDs and/or DMARDs).

NSAID and/or DMARD doses were recorded as decreased, increased, or unchanged at each visit, and compared with baseline doses. All patients were followed up by same investigator (PG), who, throughout the study, employed the same empiric and judgmental criteria for deciding whether changes in these medications should be

made. Laboratory assessments included the erythrocyte sedimentation rate (ESR) and rheumatoid factor (RF) titer.

The "combined effect size" (23) was calculated as the percentage of change from baseline of the combination of changes in the Ritchie articular index, grip strength, and ESR.

Radiologic evaluations were done for the proximal and distal interphalangeal articulations, the metacarpophalangeal articulations, and the wrists of both hands. Rates of erosions were scored according to the Kellgren scale (24).

Statistical analysis. Results are expressed as the mean ± SEM. Changes from baseline were compared between treatment groups, using the Mann-Whitney test. For discrete variables, comparisons of proportions of patients having the given attribute were made with a chi-square test. Friedman's one-way analysis of variance test was applied for the effect of time in each group (25). A 2-tailed probability of less than 0.05 was considered to be statistically significant. The statistical analyses were performed using BMDP (BioMedical Data Package) programs (1988 version).

RESULTS

Ninety patients were enrolled in the study. Sixteen patients were excluded from data analysis because of premature discontinuation of study treatment and another 14 because of poor compliance. The reasons for dropout, by treatment group, are given in Table 1. There were no significant between-group

Table 2. Characteristics of the patients at study entry, by treatment group*

	Placebo	1.3 gm/day of ω3	2.6 gm/day of ω3
No. of patients	20	21	19
Age (years)	56 ± 2	57 ± 2	59 ± 2
No. of males/females	4/16	5/16	4/15
Height (cm)	161 ± 2	162 ± 2	161 ± 1
Weight (kg)	70 ± 3	69 ± 2	64 ± 2
Duration of disease (months)	123 ± 15	119 ± 15	120 ± 27
Global assessment			
Physician	1.90 ± 0.12	2.00 ± 0.10	1.79 ± 0.16
Patient	5.58 ± 0.34	5.24 ± 0.39	5.37 ± 0.41
Pain score			
Physician	1.85 ± 0.11	2.00 ± 0.12	1.90 ± 0.19
Patient	1.95 ± 0.15	2.14 ± 0.13	2.00 ± 0.17
Ritchie articular index for pain	35.6 ± 4.9	24.8 ± 3.0	27.8 ± 5.1
No. of painful joints	24 ± 2	18 ± 2	20 ± 4
No. of swollen joints	4 ± 1	2 ± 1	4 ± 2
Grip strength (mm Hg)	261 ± 33	223 ± 21	201 ± 18
Morning stiffness (minutes)	44 ± 14	61 ± 21	43 ± 18
Erythrocyte sedimentation rate (mm/hour)	23 ± 3	22 ± 3	33 ± 6
Rheumatoid factor (titer)	25 ± 7	37 ± 9	24 ± 6

* Values are the mean ± SEM.

Table 3. Changes in selected clinical parameters and rheumatoid factor, compared with baseline, in the 3 treatment groups*

	Placebo, mean (\pm SEM)				1.3 gm/day of ω 3, mean (\pm SEM)				2.6 gm/day of ω 3, mean (\pm SEM)			
	3 mos.	6 mos.	9 mos.	12 mos.	3 mos.	6 mos.	9 mos.	12 mos.	3 mos.	6 mos.	9 mos.	12 mos.
Global assessment												
Physician	-0.25 (\pm 0.14)	-0.12 (\pm 0.14)	-0.15 (\pm 0.15)	-0.28 (\pm 0.16)	-0.19 (\pm 0.08)	-0.36 (\pm 0.10)	-0.50 (\pm 0.13)	-0.30 (\pm 0.19)	-0.21 (\pm 0.14)	-0.32 (\pm 0.17)	-0.47 (\pm 0.20)	-0.53 (\pm 0.21)
Patient	+0.10 (\pm 0.20)	+0.58 (\pm 0.35)	+0.62 (\pm 0.41)	+0.14 (\pm 0.55)	-0.64 (\pm 0.22)	-0.31 (\pm 0.36)	-0.33 (\pm 0.42)	+0.05 (\pm 0.54)	-0.92† (\pm 0.25)	-1.10† (\pm 0.35)	-1.32† (\pm 0.46)	-1.38†‡ (\pm 0.42)
Pain score												
Physician	-0.10 (\pm 0.60)	-0.05 (\pm 0.14)	-0.10 (\pm 0.14)	-0.19 (\pm 0.15)	-0.17 (\pm 0.13)	-0.33 (\pm 0.15)	-0.48 (\pm 0.15)	-0.40 (\pm 0.21)	-0.32 (\pm 0.13)	-0.45 (\pm 0.14)	-0.63 (\pm 0.18)	-0.61 (\pm 0.16)
Patient	+0.02 (\pm 0.09)	+0.15 (\pm 0.15)	+0.02 (\pm 0.17)	-0.11 (\pm 0.18)	-0.21 (\pm 0.11)	-0.19 (\pm 0.16)	-0.29 (\pm 0.18)	-0.32 (\pm 0.20)	-0.16 (\pm 0.15)	-0.26 (\pm 0.19)	-0.47 (\pm 0.22)	-0.47 (\pm 0.20)
Ritchie articular index for pain	-11 (\pm 4)	-9 (\pm 3)	-9 (\pm 4)	-15 (\pm 4)	-6 (\pm 3)	-8 (\pm 4)	-10 (\pm 4)	-9 (\pm 3)	-8 (\pm 4)	-10 (\pm 4)	-12 (\pm 5)	-14 (\pm 4)
No. of painful joints	-5 (\pm 3)	-4 (\pm 2)	-5 (\pm 2)	-8 (\pm 3)	-3 (\pm 2)	-5 (\pm 2)	-7 (\pm 2)	-7 (\pm 2)	-5 (\pm 2)	-5 (\pm 2)	-8 (\pm 3)	-9 (\pm 2)
Grip strength (mm Hg)	-5 (\pm 9)	-38 (\pm 10)	-41 (\pm 14)	-1 (\pm 13)	-5 (\pm 9)	-14 (\pm 9)	-2 (\pm 15)	+8 (\pm 4)	+3 (\pm 8)	-2‡ (\pm 9)	+16‡ (\pm 12)	+25 (\pm 12)
Rheumatoid factor titer	+29 (\pm 9)	+36 (\pm 16)	+36 (\pm 18)	+24 (\pm 8)	+5 (\pm 3)	+25 (\pm 12)	+37 (\pm 16)	+51 (\pm 18)	+10 (\pm 6)	+1 (\pm 4)	+5 (\pm 8)	+11 (\pm 7)

* See Results for significance of within-group changes.

† $P < 0.01$ versus placebo.

‡ $P < 0.05$ versus 1.3 gm/day ω 3.

§ $P < 0.05$ versus placebo.

differences in baseline features in the 60 patients who completed the study (Table 2).

Changes in clinical parameters and RF titers among the 3 study groups are shown in Table 3. Improvement from baseline in the physician's global assessment was noted at each clinical visit for all 3 treatment groups. These changes were not significant within any treatment group or between the ω 3-treated groups and the placebo-treated group. Significant improvement from baseline in the patient's global assessment was observed only in the group taking 2.6 gm/day of ω 3 ($P < 0.05$, by Friedman's test), and throughout the study, these changes were significantly different from those noted in the placebo group ($P < 0.01$, by Mann-Whitney test). At 12 months, this parameter was significantly improved in the group taking 2.6 gm/day compared with those taking 1.3 gm/day ($P < 0.05$). These results are presented as percentages of change from baseline in Figure 1.

The pain score, as assessed by the physician, consistently improved from baseline in all 3 treatment groups, but the changes were significant only in the group taking 2.6 gm/day of ω 3 ($P < 0.05$, by Fried-

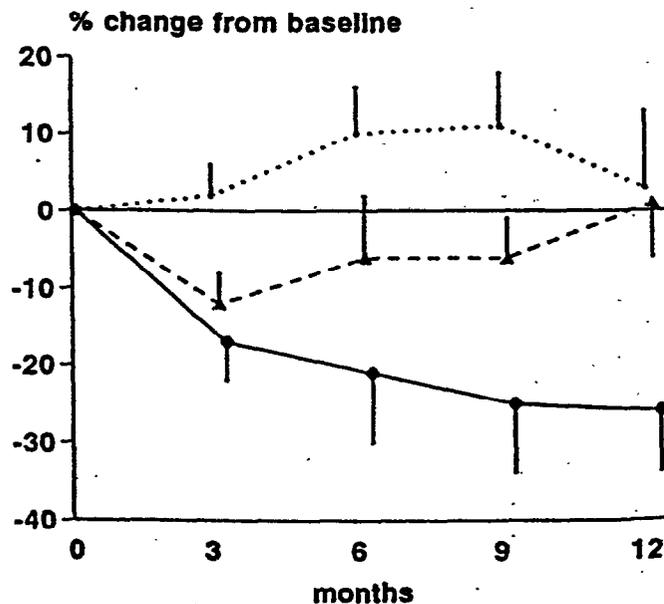


Figure 1. Percentage change from baseline in patient's global assessment of disease activity, demonstrating the superior effect of daily supplements of 2.6 gm of ω 3 fatty acids (●) compared with 1.3 gm of ω 3 fatty acids (▲) and with (olive oil) placebo (□), at each evaluation. Values are the mean and SEM of 19, 21, and 20 patients, respectively.

Table 4. Number (%) of patients with overall improvement during the study, by treatment group

	Placebo (n = 20)	1.3 gm/day of ω3 (n = 21)	2.6 gm/day of ω3 (n = 19)
Global assessment			
Physician	6 (30)	9 (43)	10 (53)
Patient	2 (10)	7 (33)	10 (53)*
Pain score			
Physician	6 (30)	8 (38)	12 (63)*
Patient	6 (30)	7 (33)	9 (47)

* P < 0.05 versus placebo.

man's test). The pain score, as assessed by the patient, improved at all visits in both ω3 dosage groups, but these changes from baseline were not significant and were not statistically different from those in the placebo group.

There were no significant changes in the duration of morning stiffness in any group (results not shown).

In each treatment group, there were significant reductions from baseline for the Ritchie articular index of pain and for the number of painful joints (P < 0.01, by Friedman's test). No significant differences between the treatment groups were found.

Friedman's test indicated a significant worsening of grip strength in the placebo treatment group (P < 0.01), and a significant improvement in the 2.6 gm/day ω3 treatment group (P < 0.05). The increase in grip strength in this group was significantly different from the decrease in the placebo group at 6 and 9 months (P < 0.05, by Mann-Whitney test).

An important increase in the RF titer was observed in the placebo treatment group (difference not significant because of a very large intragroup variability) and in the 1.3 gm/day ω3 treatment group (P < 0.05, by Friedman's test), but not in the 2.6

Table 5. Number (%) of patients whose NSAIDs and/or DMARDs could be decreased or not, by treatment group*

	Placebo (n = 20)	1.3 gm/day ω3 (n = 21)	2.6 gm/day ω3 (n = 19)
Decrease in NSAIDs and/or DMARDs	3 (15)	6 (29)	9 (47)†
No decrease in NSAIDs and/or DMARDs	17 (85)	15 (71)	10 (53)

* NSAIDs = nonsteroidal antiinflammatory drugs; DMARDs = disease-modifying antirheumatic drugs.
† P < 0.05 versus placebo.

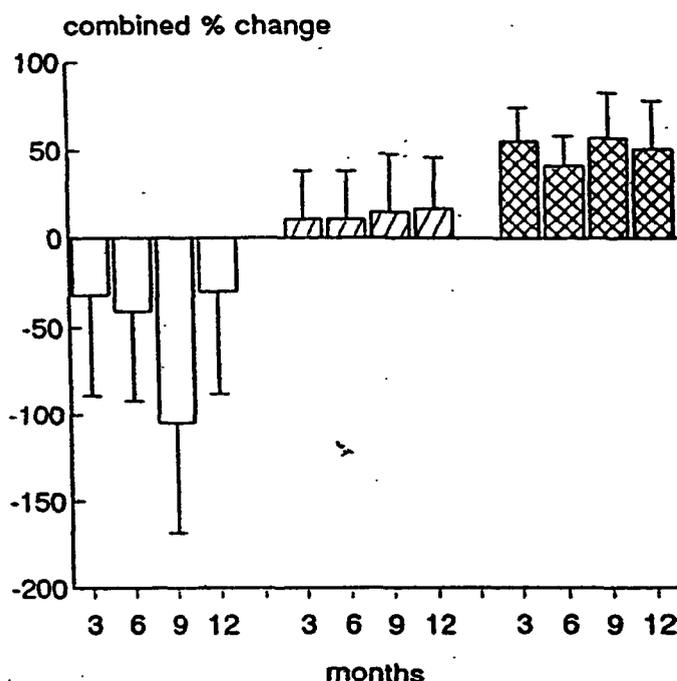


Figure 2. Combined effect size (combined percentage change from baseline) for changes in the Ritchie articular index of pain, grip strength, and erythrocyte sedimentation rate in patients taking daily supplements of 2.6 gm of ω3 fatty acids (cross-hatched bars), 1.3 gm of ω3 fatty acids (hatched bars), or (olive oil) placebo (open bars). Values are the mean ± SEM of 19, 21, and 20 patients, respectively.

gm/day ω3 treatment group. No significant between-group differences were found.

The ESR tended to increase in the placebo treatment group, was not significantly altered in the 1.3 gm/day ω3 treatment group, and tended to decrease in the 2.6 gm/day ω3 treatment group. No significant differences between the groups were found (results not shown).

Table 4 shows the proportions of patients in each group who had improvement in global evaluation

Table 6. Adverse events in the 90 rheumatoid arthritis patient enrolled in the study

Adverse event	1.3 gm/day 2.6 gm/day		
	Placebo (n = 30)	ω3 (n = 30)	ω3 (n = 30)
Gastric discomfort	2	4	6
Temporary	1	3	4
Continuous	0	0	1
Continuous, treatment withdrawn	1	1	1
Skin erythema	1	0	0
Total	3	4	6

and pain assessment. In the 2.6 gm/day ω 3 treatment group, but not in the 1.3 gm/day group, significantly greater proportions of patients reported global improvement and were found to have a reduction in their pain score as assessed by the physician, compared with the placebo group. This is consistent with the observed proportions of patients whose dosages of NSAIDs and/or DMARDs could be reduced (Table 5). In the 2.6 gm/day ω 3 group, 47% of patients were able to decrease these medications, versus 15% in the placebo group ($P < 0.05$). In the 1.3 gm/day ω 3 group, 29% of patients could decrease these medications (not significantly different from the other treatment groups).

The combined effect size revealed a marked improvement in the group taking 2.6 gm of ω 3 per day. There were no relevant changes in the 1.3 gm/day ω 3 treatment group and a distinct deterioration in the placebo treatment group (Figure 2).

No consistent significant differences were found in the evolution of the erosion rate in any group.

Adverse events for all 90 patients enrolled in the study are shown in Table 6. Most of these events were mild; only 3 patients, 1 in each treatment group, discontinued the study treatment because of gastrointestinal symptoms. Routine laboratory determinations did not show any significant changes (results not shown).

DISCUSSION

Findings of this double-blind study of the effect of fish oil supplementation in patients with active RA confirm the positive results obtained in previous short-term studies (9-17) and extend these observations to the long-term followup of 1 year of treatment.

The most striking finding was the global improvement reported by the patients treated with 2.6 gm/day of ω 3. At the outset of the study, this parameter was ranked as one of the main evaluation criteria. This 20-25% improvement was significantly different from the change observed in the group of patients receiving placebo. This difference was already observable after 3 months of supplementation and was sustained, tending to further increase, throughout the 12-month treatment period. This amelioration is superior to that reported by Kremer et al (9,10,13), and is comparable to the results reported by Sperling et al (11) in their studies of fish oil supplementation lasting 24 weeks. This long-term global improvement as assessed by the patient is of particular interest since it

relates to the patient's quality of life. The beneficial effects on the other symptoms and clinical signs of disease activity tended also to be more pronounced in the group taking 2.6 gm/day ω 3.

The proportions of patients in whom improvement was observed were significantly higher in the 2.6 gm/day ω 3 group than in the placebo group, and there was a significant reduction in the need for NSAIDs and/or DMARDs in the 2.6 gm/day ω 3 group compared with the placebo group. Interestingly, considerable increases in the RF titer were observed in the placebo group and in the 1.3 gm/day ω 3 group but not in the 2.6 gm/day ω 3 group. It has been shown that NSAIDs are able to decrease the production of IgM-RF in vitro (26). Whether supplementation with ω 3 fatty acids may have a similar effect should be further studied. Changes in the ESR also tended to be more favorable in the 2.6 gm/day ω 3 group than in either of the other groups.

A recent study (13) showed a dose-dependent effect on clinical and biologic parameters with even higher doses of fish oil of different EPA and DHA composition, without apparent additive toxicity.

Olive oil could have biologic and clinical effects (12,13) because it also contains mainly unsaturated fats. Therefore, olive oil may not be a true placebo, and this could well have had a significant favorable influence on the results in the patients treated with olive oil "placebo." Although no significant improvement in the global assessment or pain scores was found in this treatment group, the Ritchie articular index of pain and the number of painful joints improved significantly over baseline, and to a degree similar to that in the patients treated with ω 3. Significantly fewer patients taking olive oil were able to decrease their antirheumatic medication, however (compared with the 2.6 gm/day ω 3 group).

In conclusion, the observations of this long-term study in patients with active RA being treated with NSAIDs and/or DMARDs indicate that dietary supplementation with ω 3 fatty acids results in significant beneficial clinical effects and may lessen the need for NSAIDs or DMARDs.

REFERENCES

1. Leaf A, Weber PC: Cardiovascular effects of n-3 fatty acids. *N Engl J Med* 318:549-557, 1988
2. Von Shacky C: Prophylaxis of atherosclerosis with marine omega-3 fatty acids: a comprehensive strategy. *Ann Intern Med* 107:890-899, 1987
3. Kinsella JE, Lokesh B, Stone RA: Dietary n-3 polyunsaturated

EFFECTS OF HIGH-DOSE FISH OIL ON RHEUMATOID ARTHRITIS AFTER STOPPING NONSTEROIDAL ANTIINFLAMMATORY DRUGS

Clinical and Immune Correlates

JOEL M. KREMER, DAVID A. LAWRENCE, GAYLE F. PETRILLO, LAURA L. LITTS, PATRICK M. MULLALY, RICHARD I. RYNES, RALPH P. STOCKER, NOUROLLAH PARHAMI, NEAL S. GREENSTEIN, BETSY R. FUCHS, ANUPUM MATHUR, DWIGHT R. ROBINSON, RICHARD I. SPERLING, and JEAN BIGAQUETTE

Objective. To determine the following: 1) whether dietary supplementation with fish oil will allow the discontinuation of nonsteroidal antiinflammatory drugs (NSAIDs) in patients with rheumatoid arthritis (RA); 2) the clinical efficacy of high-dose dietary ω 3 fatty acid fish oil supplementation in RA patients; and 3) the effect of fish oil supplements on the production of multiple cytokines in this population.

Methods. Sixty-six RA patients entered a double-blind, placebo-controlled, prospective study of fish oil supplementation while taking diclofenac (75 mg twice a day). Patients took either 130 mg/kg/day of ω 3 fatty acids or 9 capsules/day of corn oil. Placebo diclofenac was substituted at week 18 or 22, and fish oil supplements were continued for 8 weeks (to week 26 or 30). Serum levels of interleukin-1 β (IL-1 β), IL-2, IL-6, and IL-8 and tumor necrosis factor α were measured by enzyme-linked immunosorbent assay at baseline and during the study.

Results. In the group taking fish oil, there were significant decreases from baseline in the mean (\pm SEM) number of tender joints (5.3 ± 0.835 ; $P < 0.0001$),

duration of morning stiffness (-67.7 ± 23.3 minutes; $P = 0.008$), physician's and patient's evaluation of global arthritis activity (-0.33 ± 0.13 ; $P = 0.017$ and -0.38 ± 0.17 ; $P = 0.036$, respectively), and physician's evaluation of pain (-0.38 ± 0.12 ; $P = 0.004$). In patients taking corn oil, no clinical parameters improved from baseline. The decrease in the number of tender joints remained significant 8 weeks after discontinuing diclofenac in patients taking fish oil (-7.8 ± 2.6 ; $P = 0.011$) and the decrease in the number of tender joints at this time was significant compared with that in patients receiving corn oil ($P = 0.043$). IL-1 β decreased significantly from baseline through weeks 18 and 22 in patients consuming fish oil (-7.7 ± 3.1 ; $P = 0.026$).

Conclusion. Patients taking dietary supplements of fish oil exhibit improvements in clinical parameters of disease activity from baseline, including the number of tender joints, and these improvements are associated with significant decreases in levels of IL-1 β from baseline. Some patients who take fish oil are able to discontinue NSAIDs without experiencing a disease flare.

Omega-3 fatty acids are highly polyunsaturated long-chain fatty acids derived primarily from marine sources, including fish and shellfish. Eicosapentaenoic acid (EPA), which has 20 carbons and 5 double bonds, may compete with arachidonic acid, which has 20 carbons and 4 double bonds, as a substrate for oxygenation by both the cyclooxygenase and 5-lipoxygenase pathways. These two pathways lead to the production of highly metabolically active eicosanoids, including prostaglandins (PGs) and leukotrienes (LTs), respectively (1). In the absence of fish consumption, the modern Western diet generally lacks a significant con-

Joel M. Kremer, MD, David A. Lawrence, PhD, Gayle F. Pettillo, BS, Laura L. Litts, BS, Patrick M. Mullaly, BA, Richard I. Rynes, MD, Ralph P. Stocker, MD, Nourollah Parhami, MD, Neal S. Greenstein, MD, Betsy R. Fuchs, MD, Anupum Mathur, MD: Albany Medical College, Albany, New York; Dwight R. Robinson, MD: Massachusetts General Hospital, Boston, Massachusetts; Richard I. Sperling, MD: Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts; Jean Bigaouette, MA, RD: Albany, New York.

Address reprint requests to Joel M. Kremer, MD, Division of Rheumatology, Albany Medical College A-100, New Scotland Avenue, Albany, NY 12208.

Submitted for publication October 14, 1994; accepted in revised form February 24, 1995.

tent of ω 3 fatty acids, a reversal of the pattern through most of human history, when ω 3 fatty acids were ingested in the fat of game animals (2).

Dietary supplementation with ω 3 fatty acids is associated with significant decreases in neutrophil production of LTB₄ (3), a highly potent chemotactic substance, as well as a decrease in the production of interleukin-1 (IL-1) from monocytes (4,5). EPA will also compete with arachidonate for cyclooxygenase, with a consequent decrease in the production of PGE₂ (6).

The beneficial effects of dietary supplementation with ω 3 in inflammatory disease have been demonstrated in some (7,8), but not all (9), animal models of inflammatory disease. Because of this and the beneficial changes in eicosanoids, we and others (5,10-19) have studied the effects of dietary fish oil supplements in patients with rheumatoid arthritis (RA).

We describe here the effects of dietary supplementation with doses of ω 3 fatty acids that are higher than any previously reported. We also describe the effects of discontinuing therapy with nonsteroidal anti-inflammatory drugs (NSAIDs) on the efficacy of ω 3 dietary supplements and expand our observations of potential alterations in immune function by reporting on the effects of these supplements on the *in vivo* production of multiple cytokines.

PATIENTS AND METHODS

Patients. Sixty-six patients with definite or classic RA, according to the criteria of the American College of Rheumatology (formerly, the American Rheumatism Association) (20), were recruited from the outpatient clinic of the Division of Rheumatology of Albany Medical College, the Albany Veterans Administration Medical Center, and a private practice of rheumatology in Albany, NY. Patients had active disease, as demonstrated by the presence of 3 of the following 4 criteria: \geq 6 tender joints, \geq 3 swollen joints, \geq 30 minutes of morning stiffness, and a Westergren erythrocyte sedimentation rate of \geq 28 mm/hour.

All patients were receiving NSAIDs prior to study inception, 56 patients were also receiving slow-acting anti-rheumatic drugs (SAARDs; hydroxychloroquine in 16, intramuscular gold in 11, methotrexate in 15, auranofin in 4, D-penicillamine in 3, sulfasalazine in 6, and azathioprine in 1), and 18 patients were receiving prednisone at a dosage of \leq 5 mg/day, which was held constant through the duration of the study. The demographic features of the 49 patients completing evaluations at least through week 18 or 22 are presented in Table 1.

Between baseline and either week 18 or week 22 (the maximum duration of diclofenac therapy), there were 10 dropouts from the group receiving fish oil supplements and 7 dropouts from the group receiving corn oil supplements. Four patients receiving fish oil and 3 receiving corn oil

Table 1. Demographic and clinical features of rheumatoid arthritis study patients at baseline, by dietary supplement group*

	Fish oil (n = 23)	Corn oil (n = 26)
Age, mean	58	57
Disease duration, mean years	11	10
Females:males	13:10	14:12
Medication, no		
Prednisone (mean mg/day)	11 (4.9)	6 (4.5)
Methotrexate	9	3
Hydroxychloroquine	8	9
Intramuscular gold	4	3
Auranofin	2	1
D-penicillamine	1	2
Sulfasalazine	3	3
Azathioprine	0	1
Hemoglobin, gm/dl	13.0 \pm 0.26	12.0 \pm 0.29
Westergren ESR, mm/hour	31 [†] \pm 3.9	41 \pm 8.1
Tender joint count	15.1 \pm 8.5	12.1 \pm 8.2
Swollen joint count	10.2 \pm 5.6	9.3 \pm 6.0
AM stiffness, minutes	108.1 \pm 121	128.1 \pm 248
Physician's assessment of pain, 0-4 scale	1.8 \pm 0.56	1.6 \pm 0.64
Physician's global assessment of arthritis activity, 0-4 scale	1.9 \pm 0.54	1.6 \pm 0.51 [†]
Patient's assessment of pain, 0-4 scale	1.8 \pm 0.70	1.7 \pm 0.78
Patient's global assessment of arthritis activity, 0-4 scale	2.1 \pm 0.74	1.8 \pm 0.68
Time to onset of fatigue, hours	8.7 \pm 3.9	8.3 \pm 3.0
Grip strength, mm Hg	105.8 \pm 49.3	124.4 \pm 65.0

* Except where noted otherwise, values are the mean \pm SEM. ESR = erythrocyte sedimentation rate.

[†] $P = 0.049$.

dropped out of the study during the 4 weeks after starting the diclofenac placebo. A total of 6 patients in the fish oil group and 5 in the corn oil group had dropped out during the 8-week interval between cessation of diclofenac and discontinuation of fish oil (week 26 or 30). In addition, 4 patients who had received fish oil and 3 who had received corn oil dropped out of the study during the period between discontinuation of fish oil and termination of the study (week 48).

Study design. This was a double-blind, placebo-controlled, prospective study. Patients were randomized to receive ω 3 fatty acid or corn oil supplements according to age, sex, disease duration, and 3 categories of disease severity: total joint count \leq 10, 11-20, and \geq 21.

All patients discontinued their previous NSAID for a period of at least 5 half-lives of the drug before being evaluated at a screening visit. Patients who were taking SAARDs were allowed to continue the medication. Immediately after the screening visit, patients were started on diclofenac, 75 mg twice a day, and were reevaluated 2 weeks later (baseline). At the baseline visit, either 130 mg/kg/day of ω 3 fatty acid or corn oil was added to the diclofenac and the background SAARD.

Fish oil capsules were the ethyl ester concentrate

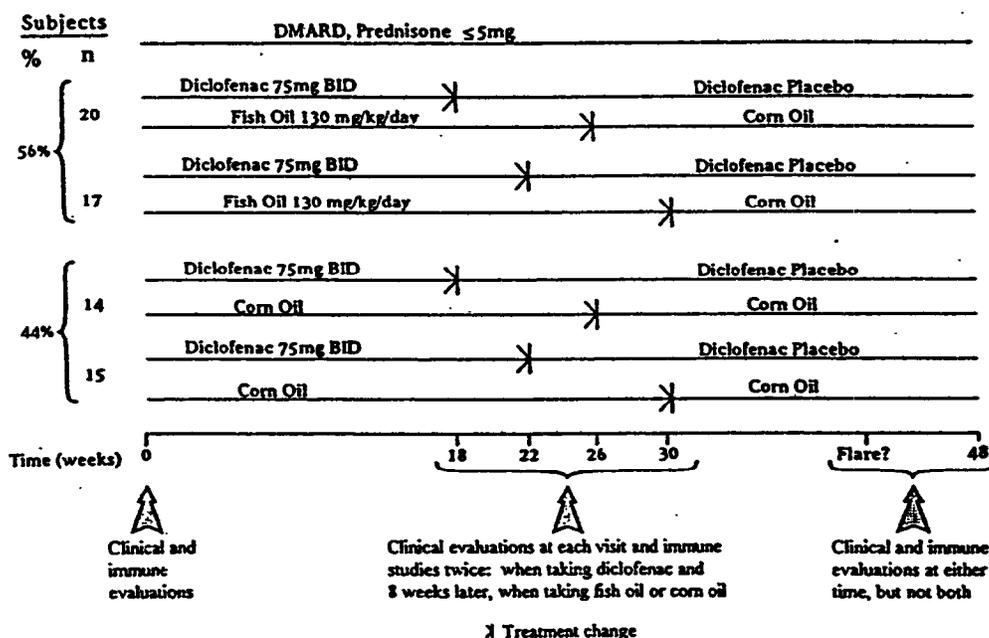


Figure 1. Study design. Prior to the screening visit (not shown), patients discontinued their nonsteroidal antiinflammatory drug for a duration of at least 5 of the drug's half-lives. Background disease-modifying antirheumatic drugs (DMARDs) and prednisone (≤ 5 mg/day) were continued throughout the study. At the screening visit, patients were given diclofenac, 75 mg twice a day (BID), and 2 weeks later (baseline visit, or week 0), they returned for reevaluation and for randomization to receive fish oil or corn oil supplements. At week 18 or week 22, active diclofenac was changed to diclofenac placebo. The time for this change was staggered, so that at the week-22 or week-26 evaluation, there would be only a 50% chance that the investigators would be able to guess which patients had been switched from the active diclofenac. Patients who were taking fish oil continued those supplements through week 26 or week 30 (8 weeks after discontinuing diclofenac), when they were switched to corn oil supplements. The final study visit was at week 48 or at the time of an arthritis flare after week 30. Immunologic studies were performed 4 times: baseline (week 0); maximum duration of diclofenac (week 18 or 22); maximum duration of fish oil, or for those taking corn oil, 8 weeks after switching to diclofenac placebo (week 26 or 30); and study end (week 48 or at arthritis flare).

supplied by the National Marine Fisheries Association for the National Institutes of Health. The $\omega 3$ ethyl ester concentrate is prepared from vacuum-deodorized menhaden oil, using transesterification, urea adduction, and short-path distillation. The concentrate contains ~80% $\omega 3$ fatty acid ethyl esters (44% EPA, 24% docosahexaenoic acid, 10–12% other $\omega 3$ fatty acid ethyl esters), 3% C18 (other than $\omega 3$), 6% C16, and the remainder as other esters. It also contains 0.2 mg/gm of TBHQ (tertiary butyl hydroquinone) as antioxidant, 2 mg/gm of tocopherols, and 2.0 mg/gm of cholesterol. The concentrate is encapsulated in 1-gm soft-gel capsules.

Patients randomized to receive fish oil continued their supplements through either week 26 or week 30, when all who remained in the study were switched to corn oil. At either week 18 or week 22, active diclofenac was replaced with an identical placebo diclofenac (supplied by Ciba-Geigy, Summit, NJ). Half of the patients were switched at week 18 and the other half at week 22 so that the investigators would not be unblinded to NSAID usage at the time of the first evaluation after week 18, when half of all patients could still be receiving active diclofenac and half would be receiving placebo diclofenac. Patients receiving fish oil con-

tinued these supplements for a full 8 weeks after discontinuing active diclofenac at either week 18 or week 22. After week 30, all subjects were taking both corn oil and placebo diclofenac, the latter having been taken since week 18 or week 22.

Clinical evaluations after baseline were done at weeks 18, 22, 26, and 30 in all patients. After week 30, evaluations were performed at the time of a disease flare, which served as a termination visit, or at week 48, which was the study termination. Individual patients were evaluated by the same investigator for the duration of the study. A schema of the study design is shown in Figure 1. The clinical evaluations performed at each visit have been described previously (5). Consistency of nutrient intake was analyzed as previously described (5).

Outcome measures were also calculated using the criteria of Paulus et al (21) and OMERACT (Outcome Measures in Rheumatoid Arthritis Clinical Trials) (22). Seven binary (0/1) improvement scores were constructed: 1 overall Paulus Index, and an OMERACT score for each of the 6 outcomes. The Paulus Index is scored as 1, if 4 of the 6 following measures show $\geq 20\%$ improvement: tender joint

count, swollen joint count, duration of morning stiffness, grip strength, and physician and patient's evaluation of global arthritis activity. The percentages of improvement necessary for a score of 1 on the OMERACT measures are as follows: 27% for tender joint count, 17% for swollen joint count, 25% for morning stiffness, 25% for grip strength, 39% for physician's global evaluation, and 35% for patient's global evaluation.

The time intervals examined were baseline to maximum duration of diclofenac, baseline to maximum duration of fish oil, and maximum duration of diclofenac to maximum duration of fish oil. The chi-square statistic was computed to test for a significant association between study group (fish oil/corn oil) and the Paulus and OMERACT scores for each time period.

Laboratory evaluations. Laboratory studies performed at baseline, at the maximum duration of diclofenac (week 18 or 22), at the maximum duration of fish oil (week 26 or 30), and at disease flare after week 30 or at study termination (week 48). Evaluations were the same as those previously described (5).

Immunologic studies. Immunologic studies were performed at the same times as the laboratory evaluations (baseline, week 18 or 22, week 26 or 30, and between week 30 and week 48 or at week 48). Serum enzyme-linked immunosorbent assays (ELISAs) were performed to assess the following levels: IL-1 β , IL-2, IL-6, IL-8, and tumor necrosis factor α (TNF α). All ELISAs were run on sera that had been stored at -80°C and processed simultaneously.

Statistical analysis. Several types of analysis were performed on the data for this study, each using SPSS Version 5.0.1 for Windows on an IBM 80386 computer. To determine whether changes in clinical and other parameters over the course of the study were statistically significant from zero, 2-tailed *t*-tests were performed with data from the fish oil and corn oil supplement groups. For these tests, a dummy variable, equal to zero for all cases, was created to use in the Paired Comparisons option in the SPSS T-Test command. A number of changes were examined in the study: changes from baseline to week 18, from baseline to week 26, from week 18 to week 26, from week 18 to week 22, and from the screening visit to week 22.

To compare the fish oil and corn oil supplement groups, 2-tailed independent-sample *t*-tests were performed using the SPSS T-Test command. For these comparisons, a dummy variable fish oil (-0 for the corn oil group and -1 for the fish oil group) was used to define the independent groups. The following changes were compared: from baseline to week 18, from baseline to week 26, from week 18 to week 26, from week 18 to week 22, and from the screening visit to week 26. Correlation coefficients were calculated using both Pearson and Spearman computations.

RESULTS

The changes in clinical parameters after discontinuation of active diclofenac are reported in the following ways: 1) the change 4 weeks after discontinuation; 2) the change 8 weeks after discontinuation;

Table 2. Mean change from maximum duration of diclofenac to first visit while taking diclofenac placebo*

	Fish oil (n = 19)		Corn oil (n = 20)	
	Mean \pm SEM change	P	Mean \pm SEM change	P
Tender joint count	3.7 \pm 1.3	0.008	3.0 \pm 3.0	0.34
Swollen joint count	0.11 \pm 1.1	0.93	1.1 \pm 1.0	0.29
AM stiffness, minutes	43.1 \pm 21.4	0.06	100.2 \pm 53.0	0.08
Patient's assessment of pain, 0-4 scale	0.21 \pm 0.16	0.22	0.58 \pm 0.19	0.007
Physician's assessment of pain, 0-4 scale	0.37 \pm 0.14	0.02	0.47 \pm 0.23	0.06
Grip strength, mm Hg	-13.1 \pm 5.3	0.02	-6.4 \pm 6.1	0.31
Patient's global assessment of arthritis activity, 0-4 scale	0.37 \pm 0.16	0.03	0.53 \pm 0.21	0.02
Physician's global assessment of arthritis activity, 0-4 scale	0.15 \pm 0.12	0.19	0.26 \pm 0.10	0.02
Interval to onset of fatigue, hours	-0.19 \pm 0.40	0.64	-0.76 \pm 0.56	0.19
Diastolic BP, mm Hg	-2.0 \pm 2.1	0.35	0.4 \pm 2.1	0.84
Systolic BP, mm Hg	-5.1 \pm 3.2	0.13	1.7 \pm 2.8	0.56

* Patients received diclofenac through either week 18 or week 22. The first visit while taking diclofenac placebo occurred at either week 22 or week 26, respectively. Patients taking fish oil received these supplements for 8 full weeks after beginning diclofenac placebo (see Patients and Methods). BP = blood pressure.

3) the change from baseline to maximum duration of fish oil after stopping active diclofenac; 4) the change from the screening visit to the first visit after stopping active diclofenac.

Effect of dietary oil supplements on RA flare after discontinuation of diclofenac: change from maximum duration of active diclofenac to first visit while taking diclofenac placebo. The mean changes in clinical parameters between the time of the evaluation after the maximum duration of diclofenac to the first visit while taking the diclofenac placebo are shown in Table 2. In patients consuming fish oil, significant worsening was observed in patient's global evaluation, grip strength, physician's evaluation of pain, and the tender joint count. Patients consuming corn oil showed significant worsening in both the physician's and the patient's evaluation of global arthritis activity and in the patient's evaluation of pain, but not in the number of tender joints.

Morning stiffness showed a trend toward significant prolongation in both groups. Patients consuming fish oil also exhibited a nonsignificant decrease in both systolic and diastolic blood pressure after discontinu-

Table 3. Mean change from baseline to maximum duration of fish oil supplementation while receiving diclofenac placebo for 8 weeks*

	Fish oil (n = 15)		Corn oil (n = 14)	
	Mean \pm SEM change	P	Mean \pm SEM change	P
Tender joint count	-7.8 \pm 2.6	0.011	-6.4 \pm 2.2	0.78
Swollen joint count	-4.7 \pm 2.7	0.10	-5.6 \pm 1.7	0.004
Stiffness, minutes	-71.3 \pm 41.5	0.12	-2.1 \pm 14.9	0.89
Patient's assessment of pain, 0-4 scale	0.10 \pm 0.35	0.78	-0.08 \pm 0.31	0.80
Physician's assessment of pain, 0-4 scale	-0.40 \pm 0.22	0.10	0.08 \pm 0.26	0.75
Diastolic BP, mm Hg	17.5 \pm 13.6	0.23	-1.5 \pm 11.5	0.90
Patient's global assessment of arthritis activity, 0-4 scale	-0.10 \pm 0.28	0.73	-0.17 \pm 0.27	0.55
Physician's global assessment of arthritis activity, 0-4 scale	-0.40 \pm 0.16	0.04	-0.17 \pm 0.21	0.44
Interval to onset of fatigue, hours	0.23 \pm 0.67	0.74	-0.63 \pm 0.46	0.20
Systolic BP, mm Hg	-8.6 \pm 8.5	0.04	-2.3 \pm 2.0	0.28
Diastolic BP, mm Hg	-2.1 \pm 17.0	0.24	-0.28 \pm 4.7	0.95

*Patients in the fish oil group took the supplement through week 26 or week 30, which was 8 weeks after beginning diclofenac placebo (see Patients and Methods). BP = blood pressure. P = 0.043 versus corn oil group.

ing diclofenac. None of the clinical changes in the fish oil group versus the corn oil group during this time were significant.

Change 8 weeks after discontinuing diclofenac. After switching to diclofenac placebo at week 18 or 22, patients consuming fish oil continued these supplements for a full 8 weeks (Figure 1). Nonsignificant decreases in both systolic and diastolic blood pressure continued to be seen in those who were taking fish oil, but not in those who were taking corn oil. None of the changes during this period achieved significance when patients receiving fish oil were compared with those receiving corn oil.

Change from baseline to maximum duration of fish oil (week 26 or week 30). In patients consuming fish oil, the week-26 or week-30 change from baseline in the physician's global evaluation of disease activity achieved significance (-0.40 ± 0.16 ; $P = 0.04$), as did the decrease in the tender joint count (-7.8 ± 2.6 ; $P = 0.01$) (Table 3). The change in the number of swollen joints from the number at baseline achieved significance in patients taking corn oil (-5.6 ± 1.7 ; $P = 0.004$) (Table 3). The improvements in the tender joint count and physician's global evaluation of disease

activity from baseline in patients taking fish oil and the decrease in the swollen joint count in those taking corn oil were achieved despite their having taken placebo diclofenac for 8 weeks at the time of this evaluation. The decrease in the tender joint count at this time in patients consuming fish oil was significant compared with the tender joint count in patients consuming corn oil ($P = 0.043$).

Changes induced by dietary oil supplementation: evaluations from baseline to maximum duration diclofenac (week 18 or week 22). In patients ingesting fish oil, significant improvements from baseline were observed after the maximum duration of diclofenac at weeks 18 or 22 in the physician's and patient's global evaluation of disease activity (-0.33 ± 0.13 ; $P = 0.017$ and -0.38 ± 0.17 ; $P = 0.036$, respectively), physician's evaluation of pain (-0.38 ± 0.12 ; $P = 0.004$), duration of morning stiffness (-67.7 ± 23.3 minutes; $P = 0.008$), and the number of tender joints (-5.3 ± 0.835 ; $P < 0.0001$). The decrease in diastolic blood pressure in patients taking fish oil showed a trend toward significance (-5.4 ± 2.7 mm Hg; $P = 0.06$).

None of the changes in the patients receiving corn oil achieved significance during this time, although there was a trend toward a decrease in the number of swollen joints (-1.3 ± 0.68 ; $P = 0.06$). During this period, none of the changes from baseline in the fish oil group achieved significance when compared with the corn oil group.

Results by Paulus and OMERACT criteria. When analyzed by the Paulus criteria (21), there were no significant changes in disease activity between the fish oil and corn oil groups for any of the time periods evaluated ($P > 0.20$). By the OMERACT criteria (22) for the outcome measure, physician's global assessment, there were significantly more responders from baseline to the maximum duration of diclofenac in the fish oil group than in the corn oil group (7 responders of 20 patients taking fish oil; 1 responder of 21 patients taking corn oil; $P = 0.02$). For the same time period, for the tender joint count, there were more responders in the fish oil group than in the corn oil group, but the difference did not reach statistical significance (14 responders of 20 patients taking fish oil; 10 responders of 21 patients taking corn oil; $P = 0.146$). By OMERACT criteria, there were no significant between-group differences ($P > 0.20$) for any of the variables for either of the remaining time intervals analyzed.

Change in IL-1 β from baseline to maximum duration of diclofenac. A significant decrease from baseline was observed in IL-1 β levels in patients

receiving fish oil at this time (-7.7 ± 3.1 ; $P = 0.026$). None of the other within-group changes in cytokine levels from baseline achieved significance. None of the changes from baseline were significant when patients taking fish oil were compared with patients taking corn oil.

Change in cytokine levels from maximum duration of diclofenac to maximum duration of fish oil. We compared the change in cytokine levels between the maximum duration of diclofenac and 8 weeks later, which was the maximum duration of fish oil in patients in this group. None of the changes were significant within or among groups.

Change in cytokine levels from baseline to maximum duration of fish oil. From the baseline evaluation to the maximum duration of fish oil at week 26 or 30, there was a significant increase in $\text{TNF}\alpha$ levels in the patients taking fish oil (45.1 ± 13.6 ; $P = 0.013$) and in those taking corn oil (65.8 ± 27.5 ; $P = 0.038$). None of the other within-group changes from baseline to this time were significant. No significant changes in cytokines were observed when patients taking fish oil were compared with those taking corn oil at this time.

We also examined the effects of discontinuing diclofenac on the production of cytokines in all study patients combined, and found no significant differences between weeks 18 or 22 and weeks 26 or 30.

Analysis of the 3-day food diaries revealed a consistent pattern of nutrient intake throughout the study in both study groups (data not shown). Pill counts showed a 93% overall compliance rate in patients consuming fish oil and 88% in those taking corn oil supplements.

DISCUSSION

In the present investigation, we were interested in expanding the observations of the effects of fish oil to include an examination of the effects of $\omega 3$ fatty acids on the production of other cytokines in patients with RA. We also used a higher dose of $\omega 3$ supplements than any previously reported. The high-potency capsules enabled us to give a person weighing 75 kg a total daily dose of 9.75 gm of $\omega 3$ supplements at our study dosage of 130 mg/kg/day. Since dose-dependent effects of $\omega 3$ supplements have previously been reported in hypertension (23) as well as in RA (5), we were interested in whether the higher dose used here would result in further clinical benefit. In addition, by substituting a visually identical placebo diclofenac for the active drug, both patients and investigators could

remain blinded; this would allow us to assess whether background dietary manipulation would allow patients to successfully discontinue this class of medication.

Our results confirm that fish oil dietary supplementation results in significant improvement in tender joint counts and other clinical parameters of disease activity from baseline activity. However, none of the improvements in the patients receiving fish oil achieved significance at the time of the maximum duration of diclofenac therapy (at 18 or 22 weeks) compared with patients receiving corn oil. During this time interval, patients receiving corn oil also exhibited many improvements which did not achieve statistical significance. In addition, the magnitude of the improvement from baseline that we observed in patients taking high-dose fish oil was indistinguishable from those previously reported in patients consuming total doses of $\omega 3$ fatty acids that ranged from 3 to 6 gm/day (5,11). We cannot therefore recommend further investigations with the doses we used, which resulted in the daily ingestion of 9 gm of $\omega 3$ supplements in a person weighing 70 kg.

Improvements from baseline in patients with RA who take fish oil often do not achieve statistical significance compared with other dietary fatty acid interventions. This may be because the biologic effect are not powerful enough or because of either a placebo effect or real biologic effects induced by the so-called "placebo fatty acids." We have previously wrestled with the issue of an ideal control fatty acid to compare with fish oil (5) and in this investigation, chose corn oil, having used olive oil in 2 previous studies (5,11). It is not unlikely that there are some mono- or polyunsaturated fatty acids that have potentially significant immunologic effects (24-27). We believe that the issue of the ideal placebo dietary intervention to compare with fish oil has not yet been settled.

After switching from active diclofenac to diclofenac placebo, it was apparent that patients in both the fish oil and the corn oil groups exhibited significant flares when examined 4 weeks after discontinuation of this NSAID (Table 2). Yet, none of these flares remained significant in either group at the time of the evaluation 8 weeks after stopping active diclofenac. This could be because 5 patients in each group dropped out of the study at the time of their first visit after discontinuing active diclofenac (4 weeks after diclofenac was discontinued), leaving in the study only those patients who were better able to tolerate the discontinuation of this NSAID.

The patients' clinical status after discontinuing

diclofenac and while receiving fish oil and corn oil was examined in several ways. We examined their clinical status while off diclofenac after the maximum duration of fish oil exposure (week 26 or 30) and compared this with their baseline status while receiving diclofenac. We believe it is meaningful that the improvement in the number of tender joints was significant in the patients remaining on the fish oil supplementation regimen at this time when compared both with their baseline status and with the patients receiving corn oil supplementation during the same period. The patients' status after stopping diclofenac was also compared with their status after stopping their previous NSAID at the time of the screening visit. Most evaluations showed that the character of the flare was worse at the screening visit, when patients were not consuming dietary fatty acid supplements (data not presented).

Other investigators have reported on whether dietary supplements of fish oil can affect NSAID requirements in patients with RA (15,16,18,28). We believe that our data support the previous observations that selected individuals with RA may discontinue NSAID therapy while consuming ω 3 supplements.

We also observed reductions in blood pressure that were consistently greater in patients taking fish oil than in those taking corn oil. The reduction in diastolic pressure achieved significance 8 weeks after stopping diclofenac in patients who continued to receive fish oil supplementation. There are well-described effects of dietary supplementation with ω 3 fatty acids on the vascular system (29), which have been documented in patients with primary Raynaud's phenomenon (30) as well as hypertension (24,31).

We were unable to demonstrate an inhibitory effect of dietary fish oil supplementation on the serum concentrations of IL-2, IL-6, IL-8, or TNF α . We confirmed our previous observation that fish oil supplementation inhibits the production of IL-1 β (5), which others have also reported in patients with RA (19). Meydani et al (32) also reported an inhibitory effect of fish oil on the production of TNF α and IL-6, although they used an in vitro system of mitogen-stimulated peripheral blood mononuclear cells derived from normal volunteer donors. We actually observed an increase in TNF α levels 8 weeks after diclofenac was discontinued in patients who continued to take fish oil and corn oil. The significance of this observation is presently unclear.

In summary, we have demonstrated that patients with active rheumatoid arthritis who consume high-dose fish oil supplements exhibit improvements

over baseline in multiple clinical parameters, improvements that are not seen in patients who consume corn oil supplements. Only the improvement in the tender joint count achieved significance ($P = 0.04$) compared with those taking corn oil; however, the magnitude of the changes did not differ from that found in previous investigations employing lower doses. Therefore, the actual mechanism(s) of the improvements observed remains imperfectly defined. The benefits are associated with a significant decrease in IL-1 β . Although some patients in either dietary supplement group exhibited significant worsening of clinical parameters after stopping diclofenac, the flare was not associated with significant changes in serum cytokine concentrations. Although patients taking high-dose fish oil exhibited significantly fewer tender joints 8 weeks after stopping diclofenac than they did at baseline while taking the drug, and this effect was significant compared with the group taking corn oil, we were nevertheless unable to demonstrate a clinically important NSAID-sparing effect of fish oil immediately after discontinuation of diclofenac. Our results suggest a possible modest NSAID-sparing effect of fish oil dietary supplements, which should be further explored in well-designed clinical trials.

ACKNOWLEDGMENTS

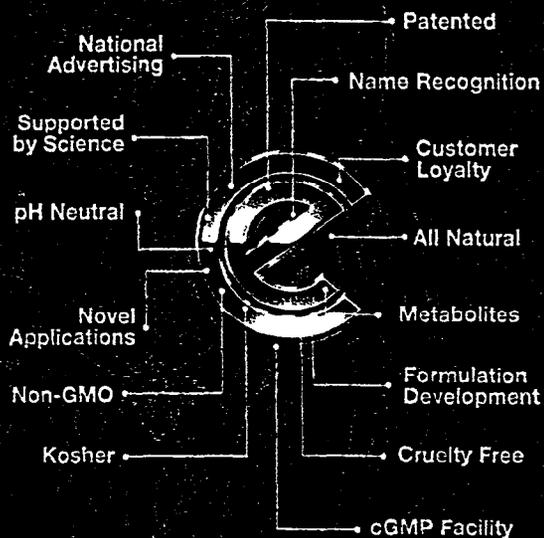
The authors would like to acknowledge the assistance of Drs. Robert A. Lew and Elizabeth Wright with the statistical analyses.

REFERENCES

1. Weber PC: Membrane phospholipid modification by dietary omega-3 fatty acids: effects on eicosanoid formation and cell function. In, *Biological Membranes: Aberrations in Membrane Structure and Function*. Edited by ML Karnovsky. New York, Alan R. Liss, 1988
2. Eaton SB, Donner M: Paleolithic nutrition: a consideration of its nature and current implications. *N Engl J Med* 312:283-289, 1985
3. Lee TH, Hoover RL, Williams JD, Sperling RI, Ravalese JRM, Spar BW, Robinson DR, Corey EJ, Lewis RA, Austen KF: Effect of dietary enrichment with eicosapentaenoic and docosahexaenoic acids on in vitro neutrophil and monocyte leukotriene generation and neutrophil function. *N Engl J Med* 312:1217-1224, 1985
4. Endres S, Ghorbani R, Kelley VE, Georgilis K, Lonnemann G, van der Meer JWM, Cannon JG, Rogers TS, Klampner MS, Weber PC, Schaefer EJ, Wolff SM, Dinarello CA: The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. *N Engl J Med* 320:265-271, 1989
5. Kremer JM, Lawrence DA, Jubiz W, DiGiacomo R, Rynes R, Bartholomew LE, Sherman M: Dietary fish oil and olive oil

- supplementation in patients with rheumatoid arthritis: clinical and immunologic effects. *Arthritis Rheum* 33:810-820, 1990
6. Robinson DR, Tateno S, Balkrishna P, Hirai A: Lipid mediators of inflammatory and immune reactions. *J Parenter Enteral Nutr* 12:375-425, 1988
 7. Prickett JD, Robinson DR, Steinberg AD: Dietary enrichment with the polyunsaturated fatty acid eicosapentaenoic acid prevents proteinuria and prolongs survival in NZB×NZW F1 mice. *J Clin Invest* 68:556, 1981
 8. Robinson DR, Prickett JD, Makoul GT, Steinberg AD, Colvin RB: Dietary fish oil reduces progression of established renal disease in (NZB × NZW)F₁ mice and delays renal disease in BXSb and MRL/l strains. *Arthritis Rheum* 29:539-546, 1986
 9. Prickett JD, Trentham DE, Robinson DR: Dietary fish oil augments the induction of arthritis in rats immunized with type II collagen. *J Immunol* 132:725-729, 1984
 10. Kremer JM, Bigaouette J, Michalek AU: Effects of manipulating dietary fatty acids on clinical manifestations of rheumatoid arthritis. *Lancet* 1:184-187, 1985
 11. Kremer JM, Jubiz W, Michalek A, Rynes RI, Bartholomew LE, Bigaouette J, Timchalk MA, Beeler D, Lininger L: Fish-oil fatty acid supplementation in active rheumatoid arthritis: a double-blinded, controlled crossover study. *Ann Intern Med* 106:498-503, 1987
 12. Sperling RI, Weinblatt M, Robin J-L, Ravalese J III, Hoover RL, House F, Coblyn JS, Fraser PA, Spur BW, Robinson DR, Lewis RA, Austen KF: Effects of dietary supplementation with marine fish oil on leukocyte lipid mediator generation and function in rheumatoid arthritis. *Arthritis Rheum* 30:988-997, 1987
 13. Cleland LG, French JK, Betts WH, Murphy GA, Elliott MJ: Clinical and biochemical effects of dietary fish oil supplements in rheumatoid arthritis. *J Rheumatol* 15:1471-1475, 1988
 14. Van der Tempel H, Tulleken JE, Limburg PC, Muskiet FAJ, van Rijswijk MH: Effects of fish oil supplementation in rheumatoid arthritis. *Ann Rheum Dis* 49:76-80, 1990
 15. Skoldstam L, Borjesson O, Kjallman A, Seiving B, Akesson B: Effect of six months of fish oil supplementation in stable rheumatoid arthritis: a double-blind, controlled study. *Scand J Rheumatol* 21:178-185, 1992
 16. Kjeldsen-Kragh J, Lund JA, Riise T, Finnager B, Haaland K, Finstad R, Mikkelsen K, Førre Ø: Dietary omega-3 fatty acid supplementation and naproxen treatment in patients with rheumatoid arthritis. *J Rheumatol* 19:1531-1536, 1992
 17. Nielsen GL, Faarvang KL, Thomsen BS, Teglbjaerg KL, Jensen LT, Hansen TM, Lervang HH, Schmidt EB, Dyerberg J, Ernst E: The effects of dietary supplementation with n-3 polyunsaturated fatty acids in patients with rheumatoid arthritis: a randomized, double blind trial. *Eur J Clin Invest* 22:687-691, 1992
 18. Belch JFF, Ansell D, Madhok R, Dowd AO, Sturrock RD: Effects of altering dietary essential fatty acids on requirements for non-steroidal anti-inflammatory drugs in patients with rheumatoid arthritis: a double blind placebo controlled study. *Ann Rheum Dis* 47:96-104, 1988
 19. Spersen GT, Grunnet N, Lervang HH, Nielsen GL, Thomsen BS, Faarvang KL, Dyerberg J, Ernst E: Decreased interleukin-1 beta levels in plasma from rheumatoid arthritis patients after dietary supplementation with n-3 polyunsaturated fatty acids. *Clin Rheumatol* 11:393-395, 1992
 20. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS, Medsger TA Jr, Mitchell DM, Neustadt DH, Pinals RS, Schaller JG, Sharp JT, Wilder RL, Hunder GG: The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 31:315-324, 1988
 21. Paulus HE, Egger MJ, Ward JR, Williams HJ, and the Cooperative Systematic Studies of Rheumatic Diseases Group: Analysis of improvement in individual rheumatoid arthritis patients treated with disease-modifying antirheumatic drugs, based on the findings in patients treated with placebo. *Arthritis Rheum* 33:477-484, 1990
 22. Boers M: OMERACT: conclusions of an international conference on Outcome Measures in Rheumatoid Arthritis Clinical Trials in Maastricht (abstract). *Arthritis Rheum* 35 (suppl 9): S202, 1992
 23. Knapp HR, FitzGerald GA: The antihypertensive effects of fish oil: a controlled study of polyunsaturated fatty acid supplementation in essential hypertension. *N Engl J Med* 320:1037-1043, 1989
 24. Traill KN, Wick G: Lipids and lymphocyte function. *Immunol Today* 5:3:70-75, 1984
 25. Erickson KL: Dietary fat modulation of immune response. *Int J Immunopharmacol* 8:6:529-543, 1986
 26. Johnston PV: Dietary fat, eicosanoids and immunity. *Adv Lipid Res* 21:103-141, 1985
 27. Payan DG, Wong MY, Chernov-Rogan T, Valone FH, Pickett WC, Blake VA, Gold WM, Goetzl EJ: Alterations in human leukocyte function induced by ingestion of eicosapentaenoic acid. *J Clin Immunol* 6:5:402-410, 1986
 28. Lau CS, Morley KD, Belch JFF: Effects of fish oil supplementation on non-steroidal anti-inflammatory drug requirement in patients with mild rheumatoid arthritis: a double-blind placebo controlled study. *Br J Rheumatol* 32:982-989, 1993
 29. Leaf A, Weber PC: Cardiovascular effects of omega-3 fatty acids. *N Engl J Med* 318:549-557, 1988
 30. DiGiacomo R, Kremer JM, Shah D: Fish oil dietary supplementation in patients with Raynaud's phenomenon. *Ann J Med* 86:151-157, 1989
 31. Appel LJ, Miller ER III, Seidler AJ, Whelton PK: Does supplementation of diet with fish oil reduce blood pressure? A meta-analysis of controlled clinical trials. *Arch Intern Med* 153:1429-1438, 1993
 32. Meydani SN, Lichtenstein AH, Cornwall S, Meydani M, Goldin BR, Rasmussen H, Dinarello CA, Schaefer EJ: Immunologic effects of National Cholesterol Education Panel step-2 diets with and without fish-derived N-3 fatty acid enrichment. *J Clin Invest* 92:105-113, 1993

Anatomy of a Strong Brand



Use the strength of the Ester-C® brand to differentiate your supplements, functional foods, beverages, and animal foods, treats and supplements. For samples and literature contact 520-445-8063 or info@intercal.com.

Inter-Cal CORPORATION *the intelligent use of nature™*

Inter-Cal Corporation, a Zila company ©2000.

See us at Supply Side East, Booth 1312 & 1314
Circle Reader Service #74

FATTY ACIDS

The Phospholipids: PC and PS

Phospholipids, nutrients in cell membranes that are essential for the body, are important "building blocks" for cells that form the lipid bilayer in each cell membrane. Two of the more popular phospholipids on the market are phosphatidylserine (PS) and phosphatidylcholine (PC). PC and PS support many cellular functions, including liver detoxification and homeostasis.

"PS has been getting a lot of attention lately," says Ivo Pischel, director of R&D and technical services at **Traco Labs Inc.** (Champaign, IL), which offers a line of phospholipid products. One reason for the heightened popularity of these supplements is that consumer magazines have referred to PS as a memory enhancer and breakthrough brain nutrient that offers nutritional support for brain function.

"Numerous clinical studies have shown that when PS is taken regularly as a dietary supplement, it can help maintain or improve memory, concentration, and learning," Pischel says. "As a result, PS is gaining in popularity among health-conscious consumers, especially those age 40 and over."

taste and odor that is a common turnoff for many would-be omega-3 consumers. The fact that OmegaTech's DHA does not come from fish oil also makes the product popular among vegetarians, according to Klacik. The versatility of the fermentation process has even allowed the company to extend its operations into the functional foods industry. For instance, OmegaTech DHA is used to produce the Gold Circle Farms brand of DHA-enriched eggs, which are created by adding algae to the chickens' feed.

Roche Vitamins Inc. currently supplies a line of specially deodorized fish oils and encapsulated powders. To avoid creating a product with undesirable flavors, the company performs a sensory rating on every batch of its ROPUFA

'30' n-3 food oil and ROPUFA '10' n-3 food powder.

"We use a GC-MS to detect the presence of certain molecules that might otherwise eventually contribute to fishy off-notes," Hnat explains.

Hnat adds that several food companies have been working on projects that involve Roche's omega-3 products. Projects that are currently



ROPUFA '10' n-3 food powder is part of Roche Vitamins Inc.'s line of specially deodorized fish oils and encapsulated powders. Photo courtesy of Roche Vitamins Inc.

CGMPs 101

The FDA is readying final CGMPs. In the meantime, there are ways to create CGMPs right now.

The establishment of the supplement CGMPs that were authorized under DSHEA would go a long way toward achieving uniformly high quality throughout the industry. Indeed, five different industry associations wrote to the Office of Management and Budget in November 2000 supporting the publication of FDA's proposed CGMPs before the end of the year. This, of course, did not happen. In lieu of final CGMPs, most manufacturers have been forced to choose from one of a few strategies.

The easiest option is do to nothing at all. It is an inexpensive albeit risky choice, since the industry cannot afford substandard product that further erodes consumer confidence. What's more, FDA is close to finalizing standards and may not give companies more than a year to comply. Since coming up to speed often takes more than a year, companies without any form of CGMPs risk not only a missed deadline, but also their future.

A more realistic option is to adopt the standards of a third-party certifier. This choice provides instant credibility in the market, but it can also be expensive. Also, when final CGMPs are published, changing to the new standard could increase the price further if the FDA program does not match the certifier's.

In 1999, the National Nutritional Foods Association (NNFA) inaugurated a voluntary certification program for its members, which involves a third-party audit of the entire manufacturing process from raw materials through packaging of the finished product. To date, 20 NNFA member companies have been certified, and about 70 more have registered, according to Phillip Harvey, NNFA's director of science and quality assurance. Harvey, who oversees the CGMP certification program, notes that in some

respects, the NNFA standards surpass those included in FDA's 1997 Advance Notice of Proposed Rulemaking.

Harvey acknowledges that the voluntary certification program's standards aren't as strict as the "pharma CGMPs." But he cautions against standards that are too strict, which would preclude the registration of manufacturers that may qualify under the voluntary program. "With the drug CGMPs, it's a process to get to that level," he says. "The program has to be achievable and effective. It has to be done in increments."

Still, some supplement manufacturers and many of their suppliers are already in compliance with FDA's pharmaceutical CGMPs. And even some who aren't already supplying the pharmaceutical market do believe that ultimately, the supplement CGMPs will resemble those for pharmaceuticals. "At some point, the nutritional industry is going to have to address these issues" with respect both to labeling regulations and CGMPs, says Clark Sayer, a consultant to Schaefer Technologies Inc. (Indianapolis, IN).

In Sayer's view, FDA's foray into supplement labeling—the February 2000 rule pertaining to structure-function claims—is the agency's beachhead in a larger campaign to bring the industry into regulatory compliance. "FDA will start with labeling, as they've already done," Sayer says. That ruling, now a year old, represents FDA's attempt to require manufacturers to prove their claims.

The agency's next move, Sayer says, will be to regulate packaging, including issues such as content, uniformity, and homogeneity. Finally, FDA will address the manufacturing process itself—via the CGMPs.

Sayer and others advise supplement manufacturers and their suppliers to

bring their operations into compliance with the pharmaceutical CGMPs even if they are not currently in the pharmaceutical business. "When FDA comes out with CGMPs, there is going to be a major fallout," Sayer says, adding that those following the pharmaceutical CGMPs "will be the ones that survive."

"The smaller manufacturer who can't be compliant is going to be gone," adds Jonathan Pinkus, president of Arizona Nutritional Supplements (Chandler, AZ). And, in order to be compliant, Pinkus says, the manufacturer is going to have to invest in an analytical lab, top-quality equipment, and the right staff.

In fact, some go so far as to recommend specifically seeking out facilities that are already in compliance with pharmaceutical CGMPs. "In choosing a supplier, I would recommend auditing their facility to ensure their CGMP compliance," says Michelle Zollner, marketing manager of Banner Pharmacaps (High Point, NC). "This entails touring the manufacturing plant and laboratories and the review of various documents." Zollner says this process includes, but is not limited to, reviewing FDA filings, standard operating procedures, CGMP documentation, training records, specifications, batch records, and validation reports.

Comments about the proposed CGMPs are currently under review, and the FDA has made passing the final regulations a priority. Although the agency has not set a date, there is speculation that the rules will be out this year. Now is the time to begin investigating how to put them in place.

The proposed CGMP standards can be found in their entirety on the FDA Web site, <http://vm.cfsan.fda.gov/~dms/supplmnt.html>. ♦

Phosphatidylcholine: A Superior Protectant Against Liver Damage

Parris M. Kidd, Ph.D.

Abstract

Phosphatidylcholine (PC) is one of the most important support nutrients for the liver. PC is a phospholipid, a large biological molecule that is a universal building block for cell membranes. A cell's membranes are its essence: they regulate the vast majority of the activities that make up life. Most liver metabolism occurs on cell membranes, which occupy about 33,000 square meters in the human. More than 2 decades of clinical trials indicate that PC protects the liver against damage from alcoholism, pharmaceuticals, pollutant substances, viruses, and other toxic influences, most of which operate by damaging cell membranes. The human liver is confronted with tens of thousands of exogenous substances. The metabolism of these xenobiotics can result in the liver's detoxicative enzymes producing reactive metabolites that attack the liver tissue. Dietary supplementation with PC (a minimum 800 mg daily, with meals) significantly speeds recovery of the liver. PC has also been shown to be effective against alcohol's liver toxicity in well-controlled studies on baboons. PC has other qualities that enhance its usefulness as a dietary supplement. PC is safe, and is a safer means for dietary choline repletion than choline itself. PC is fully compatible with pharmaceuticals, and with other nutrients. PC is also highly bioavailable (about 90% of the administered amount is absorbed over 24 hours), and PC is an excellent emulsifier that enhances the bioavailability of nutrients with which it is co-administered. PC's diverse benefits and proven safety indicate that it is a premier liver nutrient. (Alt Med Rev 1996;1(4):258-274)

Introduction

Phosphatidylcholine (PC) is a phospholipid nutrient that is a major building block for all known cells.¹ PC is the most abundant constituent of cell membranes, the thin and delicate yet dynamic surfaces on which cells carry out most of their activities (Figure. 1). The "workhorse" parenchymal cells that make up the liver are especially reliant on their membranes,² and it has been estimated that the human liver as a whole encapsulates some 33,000 square meters of cell membrane.³ The liver's wide range of functions, as well as its capacity for ongoing renewal, hinge on its ability to make new cell membranes, which are on average 65% PC. Decades of basic and clinical research on this nutrient indicate that it is critical for optimal liver function.

In its programmed efforts to rid the body of potential toxins, the liver paradoxically generates toxins that can damage the liver liver tissue. This can happen because evolution has been tricked: manmade foreign substances activate the liver's natural enzyme detoxification pathways, but often the metabolites that the liver generates from them via such "bioactivation" are more toxic than the starting substrates. Whether their toxicity occurs directly or following bioactivation, virtually all of the agents that damage the liver do so by way of attack on the membrane systems of the parenchymal cells.

Membrane systems are central to the survival and specialized functioning of all cells. In order to carry out its metabolic responsibilities, the liver parenchymal cells are densely packed with membranes. Given this central role of membranes in the liver's functions, the demonstrated superiority of PC in supporting the liver against damage is thoroughly consistent with the known mechanisms of liver homeostasis, toxic liver damage, and the liver's recovery processes. Out of this comes a dramatic conclusion: PC is the single most important nutrient for the liver. (See Figure 2)

The Human Liver, the Detoxification Paradox, and PC

The liver is the body's main organ for disarming and disposing of toxins, yet is itself vulnerable to toxic attack. Such toxic attack is both endogenous (from toxins generated in the liver), and exogenous (due to toxins coming from the outside). Similar metabolic mechanisms are employed to deal with the toxins coming from either source, but due to the stressful influences of modern life, toxic overload is a constant possibility.

The healthy liver is the body's largest organ and is probably also its most metabolically versatile. The liver carries out hundreds, if not thousands, of sophisticated enzymatic reactions along numerous metabolic pathways. Enzymes residing within the membranes of the parenchymal cells produce biological molecules by synthesis from smaller molecules, by the modification of pre-existing metabolites or from newly-absorbed nutrients. The parenchymal cells also process hormones and many other metabolic waste products into water-soluble compounds for subsequent excretion. With the myriad of functions that it performs, the liver plays a pivotal role in maintaining homeostasis, i.e., health in all its aspects. But these routine liver functions do generate intrinsic, potentially toxic metabolites.

Normally the parenchymal cells are well equipped with protective antioxidant enzymes and with water-soluble antioxidants such as glutathione, cysteine, and taurine to neutralize endogenous toxic metabolic products. However, with the additional challenge posed to the liver's defenses by food-borne toxins and by the bioactivation products of xenobiotics, including lifestyle-related substances such as alcohol the liver's detoxification enzyme systems can be diverted to the compulsive generation of toxic metabolites that attack their maker. Last but not least, by being the first way-station for the blood draining the intestines (via the portal circulation), the liver tissue is directly exposed to preformed toxins that enter by the oral route.

It is highly doubtful that the human liver is evolutionarily equipped to cope with the tens of thousands of toxins generated by modern circumstances: pharmaceuticals, pollutants, and other toxins associated with a self-abusive lifestyle. As the liver becomes overburdened with such toxins, its stores of protective antioxidants are progressively depleted.⁴ Parenchymal cells die, and cell death spreads zonally. Left unchecked, necrotic and inflammatory damage comes to threaten whole regions of the liver.

Overall Clinical Benefits of PC for the Liver

A large number of controlled clinical trials, conducted mostly in Europe, have investigated PC for the management of liver damage coming from a variety of toxic insults. In a landmark study published in 1973, Wallnoefer and Hanusch in Germany followed 650 subjects with various degrees of liver damage for at least 5 years.⁶ This trial relied on biopsy, conducted in conjunction with blood analyses and clinical tests, to assess the scope and character of liver damage.⁷ The subjects received PC for periods that ranged from 4 weeks to several years. The distributions of subjects, listed in groups according to approximate degree of damage severity, was as follows: fatty degeneration, n=130; acute inflammation, n=157; persistent inflammation (subacute and chronic), n=41; chronic inflammation, n=122; chronic aggressive inflammation, n=70; advanced fibrotic damage, n=130. All subjects were begun on intravenous PC (950 mg*) along with oral PC (450-700 mg*), until blood parameters began to return to normal; they were then shifted to oral PC only.

All the groups of subjects in this study benefited from receiving PC. Of those with mild damage, more than half (51.1%) showed excellent improvement, and many subjects experienced reversal of their fatty degeneration. In the acute inflammation group, lab measures and biopsy indicated PC accelerated recovery by about 10 days. In the group with persistent inflammation, PC returned the enzyme parameters to normal after 30 days. In chronic aggressive inflammation, more than one-third (35.3%) experienced benefit and among those with advanced fibrotic damage, 17.5% benefited. In this last group with liver damage of the greatest severity, recovery was better when PC was given intravenously as well as by the oral route.

Notably, some of the subjects with persistent inflammatory damage included in this trial had failed to benefit from milk thistle extract ("silymarin") or steroid drugs, but benefited from PC. The investigators commented that for the best chance of success, the management of advanced liver damage should be continued for years rather than weeks or months; and that in their clinical experience PC proved to be the best single means for managing liver damage.

Sorrentino and collaborators (1982) studied 42 subjects with liver damage stemming from varied causes and exhibiting all degrees of severity.⁸ They divided the subjects into 2 groups of 21 each, then provided conventional management (diet, B vitamins) to one group. To the other group, they gave PC (1350 mg), fortified with B1, B2, B6, B12, and E. Blood samples and clinical assessments were taken after 1 month, then at 2 months (the end of the trial). The results were subjected to a customized best-fit, least squares statistical analysis. After the first month, the data on 7 of the 8 parameters were clearly in favor of PC (5 of the 7 were 95% significant), then at month 2 the eighth parameter-SGOT-also became significant in favor of PC. In suggesting that PC can benefit the various stages of liver damage, these findings are consistent with those of Wallnoefer and Hanusch⁶.

Clinical Assessment of PC In Alcoholic Liver Damage

Excessive alcohol consumption is still the single most common cause of toxic liver damage in Western societies. Alcohol damages the liver by various mechanisms.⁹ First, it increases oxidative stress: the ethyl alcohol molecule becomes metabolized by the liver cell to acetaldehyde, which is a reactive oxidant ("two-electron stealer"). Acetaldehyde combines with antioxidants, often into a molecular complex (an "adduct"), thereby draining the liver cells of their antioxidant power. Acetaldehyde also reacts with enzymes and other proteins and with DNA, damaging these and sometimes causing mutations. Membrane phospholipids and their associated fatty acids also can be damaged or destroyed by the highly reactive acetaldehyde, which can do as much damage as many free radicals (technically, one-electron stealers).

Being a weak polar solvent, alcohol has a dispersive/disruptive effect on the lipids that make up the matrix of cell membranes.⁹ Alcohol can literally dissolve PC and other phospholipids from the membrane, thereby inactivating the membrane proteins that depend on the lipids for activity and weakening the membrane to the point of rupture. By this means and through the acetaldehyde pathways, alcohol also attacks the mitochondria, the liver cell organelles that normally generate energy. By impairing mitochondrial function, chronic alcohol exposure robs the cell of precious energy resources needed for maintenance and for more sophisticated functions. As the cell becomes more energetically compromised, its death becomes inevitable.

Mitochondrial damage is the most likely toxic basis for the early clinical stage of alcoholic liver damage termed "fatty liver."^{9,10} The mitochondria are the organelles that normally burn fats (triglycerides) to make energy for the cell. When the mitochondrial membranes become destroyed by alcohol, the parenchymal cells can no longer adequately metabolize fats. Pools of triglycerides then become deposited within hepatocytes throughout the liver tissue. It is thought that as these fatty deposits grow, they can come to occlude the important functions of the cell and cause more severe functional damage.

Clinically, the fatty liver state represents a relatively mild degree of alcoholic damage to the liver, which can often be reversed through diligent personal commitment. However, if the individual continues to consume alcohol the fat-laden parenchymal cells can begin to die off in large numbers. An inflammatory situation then develops: in response to substances exuded from dying liver cells, immune cells migrate into the liver tissue from the circulation and attempt to "mop up" the debris. However, with the liver's energetics and antioxidant adaptability now compromised, the stage is set for the inflammatory process to get out of hand and usher in a chronic inflammatory state.⁹

If liver inflammation develops from alcohol toxicity and is not controlled, as with the continuation of alcohol consumption, cells in the liver called lipocytes are transformed and begin to produce collagen, which is the primary molecular basis for connective tissue deposition and fibrosis. At first the liver may adapt, accelerating its removal of collagen to keep pace with the rate of new deposition. If the liver's functional state cannot be improved, however, the rate of collagen removal eventually falls behind the rate of collagen deposition, and progressive collagen accumulation (fibrosis, scarring) begins to obscure ever-enlarging regions of the liver. Beyond this point, the liver's many functions become seriously compromised as it develops advanced, cirrhotic damage.¹⁰

Clinical trials conducted with PC against alcoholic liver damage have consistently produced favorable findings. Kruechel reported in 1979 on a double blind trial conducted in Germany on 40 male subjects who had fatty deposits in the liver resulting from alcohol intake, as verified by biopsy.¹¹ A majority of these subjects also likely had "Stage 2" inflammatory involvement, as indicated by abnormally-elevated serum iron, elevated immunoglobulin-A (IgA), and values of SGOT and SGPT 3-5 times higher than normal.

The subjects were taken off all pharmaceuticals and randomly divided into 2 groups of 20 each. One group received a placebo and the other, 1350 mg of fortified PC per day. Liver damage was monitored at days 14, 28, and 56 after beginning the treatment, based on the levels of SGGT, SGOT, SGPT, AP, LDH, Chol, TG, and BR. In addition LAP, immanoglobulins, platelets, reticulocytes, and the blood fatty acid spectrum were measured, but only at the beginning and at the end of the trial (day 56).

In this trial, measurable benefits from PC intake were apparent at the first time point 2 weeks after the start. At 4 weeks, most of the indicators of liver damage were clearly more improved for the PC group than for the placebo group. By 8 weeks, the trial's culmination, all the main parameters of liver function were significantly improved ($p < 0.05$). The parameters LAP and IgA-IgG-IgM, measured only at the end of the trial, also were significantly improved.

A blind clinical evaluation was conducted at the end of the trial, by a qualified investigator not informed of the randomization code. Of the PC group of 20 subjects, 6 were judged very good and 14 good. Of the placebo group, none was very good, 7

were good, 8 were moderate, and 5 showed no change. The differences were statistically highly significant in favor of the PC group. No side effects from the PC were observed. In this 2-month trial, PC definitely benefited subjects with alcoholic liver damage. It did not completely resolve the more severe inflammatory indicators, which perhaps could have been achieved had the trial gone for a longer period.

In Madrid in 1985, Schuller Perez and San Martin organized a double-blind trial.¹² They drew 20 subjects with alcohol-induced fatty liver deposits from a population and compared them with 20 matched control subjects. As in the Knuechel study just described, fortified PC was given at 1350 mg per day. The trial went for 12 weeks, and blood samples were taken at the beginning and at the end of this trial period. Initially the indicators SGGT, SGOT, SGPT, AP, and bilirubin all were higher in the PC group than in the controls, but by the trial's end they were significantly reduced and were lower than the controls. Alpha-2-globulin was also significantly increased ($p < 0.01$). Clinical assessment at the trial's end determined that in the PC group 3 subjects were good, 14 were average, while 3 had not improved. In the placebo group, 0 subjects were good, 9 were average, and 11 (more than half) had experienced no benefit. The authors concluded, "it is our view that the use of highly-unsaturated phosphatidylcholine for therapy of alcohol-dependent steatoses [fatty liver] is very productive."

The above two double-trials just summarized establish the benefits of PC as an oral nutritional supplement for the earliest clinically-characterized stage of liver damage from alcohol abuse - the presence of fatty deposits in the liver. These findings are consistent with those from Buchman and collaborators (1992), who gave PC double-blind to 15 subjects with fatty liver of non-alcoholic origin as part of an intravenous feeding regimen (TPN).¹³

The next and more serious stage of liver damage by alcohol is inflammation, which if left untreated can become life-threatening. In 1990, Panoz and collaborators reported on a double-blind trial conducted in England.¹⁴ The researchers divided 46 subjects with liver inflammation from alcohol abuse (verified by biopsy) into two groups. The PC group were placed on a high intake-about 4.6 grams daily of fortified PC, in contrast to the placebo group, and both groups were periodically assessed for 2 years. By the end of the trial there had been deaths in both groups, but a trend was seen toward increased survival in the PC group ($p = 0.086$, short of the $p < 0.05$ required for statistical significance). The group that seemed to benefit the most was the intermediate stage of severity (Pugh's B classification). Tolerance of the relatively high intake of PC was good.

The findings from these and other clinical trials conducted on human subjects with alcoholic liver damage are generally consistent with a large body of data from animal experiments.

The evolutionary strategy for normal liver "detoxification" seemingly is to make potentially problematic substances water-soluble, suitable for later excretion into the bile or the urine. Therefore the healthy liver attempts to first use the P450 enzyme complexes and related pathways, to put a charge on the molecule. It then attempts to conjugate this charged, more reactive "activated" metabolite with glucuronic acid or with glutathione or other antioxidants to render it water-soluble.⁴ If the first phase enzyme systems become induced, generating copious amounts of exceedingly reactive activated molecules, then the resources for conjugation can become insufficient. When this happens, activation can still proceed but conjugation fails, and the liver tissue becomes a sitting duck for oxidative attack by the activated metabolites. Alcohol and many xenobiotics can actually induce, i.e., turn on, the Phase 1 systems, thereby racking up the potential for the system to overproduce activated metabolites. This can explain why combined intakes of alcohol and/or drugs and/or pollutants or other xenobiotics can be severely threatening to the liver's integrity.^{4,9,10} In this scenario any agent that turns on Phase 1 of the detoxification system, can cause the system to concurrently convert excessive amounts of a second (or third) agent to reactive, oxidant metabolites.

The Baboon Model of Alcoholic Liver Damage

Animal studies have helped elucidate the means by which PC exerts its impressive clinical benefits against liver damage from many causes. In the case of alcohol, the most clinically relevant animal research to date has been the "baboon model" of alcoholism developed by Lieber and his colleagues at the Mount Sinai School of Medicine and the Bronx Veterans Affairs Medical Center in New York City, for more than 2 decades.^{10,15,16,48} Their findings constitute compelling evidence that dietary supplementation with PC is effective against alcoholic liver damage. In early experiments they fed alcohol to rats, and found that it impaired phospholipid synthesis in the rat liver. This partially accounts for fats accumulating in the liver cells ("fatty liver"), since PC and other phospholipids are needed to metabolize triglycerides. Then, for an "experimental model" closer to the human state, they turned to research on baboon primates (Figure. 3).

Lieber and his associates placed baboons on a daily regimen of alcohol intake. Over a period of years most of the baboons

developed features of alcoholic liver damage that closely resembled those seen in humans, making this a good "animal model" for human liver disease. The researchers also developed sophisticated methods for quantitating the tissue changes seen in liver biopsy samples, and refined biochemical analyses for use on small amounts of biopsy material.

Subsequently, using a blinded trial design, they set up two main groups of baboons, one of which received alcohol along with PC, the other receiving only alcohol.¹⁵ After running this primate trial for several years and decoding their results, Lieber's group found that the baboons fed alcohol with PC developed fatty liver and mild fibrosis, but did not progress to advanced liver damage for six years or longer. In contrast, the majority of baboons fed alcohol without PC progressed to advanced fibrosis ($p < 0.005$). While PC did not block the development of fatty liver in baboons that continued to receive alcohol, it dramatically slowed the progress to advanced disease.

Three of the baboons with fatty liver were subsequently taken off PC while continuing to be fed alcohol. These baboons rapidly progressed to extensive liver fibrosis (equivalent to advanced liver damage). From this study and a follow-up study using a similar design¹⁷, Lieber's group were able to firmly conclude that PC is an effective means for halting (not merely slowing) the progression from early-stage alcoholic liver damage into late-stage generalized fibrosis (cirrhosis). (Figure 3) PC is unique among both nutrients and drugs, as was pointed out in a supportive peer editorial,¹⁸ in its ability to halt the clinical progression of alcoholic liver damage.

Subsequent in vitro experiments by Lieber's group¹⁶ showed that the lipocytes, the liver cells that normally store moderate amounts of fats, under the influence of alcohol become transformed to collagen-producing cells (called "transitional cells"). In the intact, alcohol-treated liver these transitional cells intensify collagen production, but initially the liver keeps up by breaking down collagen faster (via increased collagenase enzyme activity). As alcohol damage progresses, the balance shifts: the liver's collagenase activity drops and continued collagen production by the transitional cells results in progressive collagen deposition and extensive fibrosis. This eventually deprives the liver of most of its function (the state of cirrhosis). It may well be that in the baboons fed PC along with alcohol, excessive collagen production was partially blocked by PC, and collagen breakdown was increased for a sustained period (also via increased collagenase). Ongoing dietary supplementation with PC seemingly restored normal collagen balance in the transitional cells, thereby blocking further fibrosis and protecting the baboons for several years and potentially longer.

These findings with primates strongly suggest that advanced liver damage in humans, clinically expressed as cirrhosis, may prove amenable to dietary PC. As a result of this research breakthrough by the Lieber group, excitement developed in the U.S. research community around the potential of PC to slow, to stabilize, and perhaps in some cases even to reverse, alcoholic liver damage. An editorial in the journal *Alcoholism: Clinical and Experimental Research* discussed PC as a possible "magic bullet" for this purpose.¹⁸ The Lieber baboon studies also established that choline does not have comparable benefits to PC for the liver. The small choline molecule is actually part of the headgroup of the large PC molecule, but when free choline was added to the baboon diet it proved toxic to the alcohol-damaged liver.⁴⁸

Benefits of PC Against Other Liver Toxins

Further clinical evidence indicates that PC supports liver cells against attack by a variety of toxic agents other than alcohol. The trials reported in this category are sparse because of the difficulties in assembling victims of toxic exposures. However, some clinical trials have been accomplished, and their findings indicate PC is also unique in its protection of the liver against toxins other than alcohol.

As discussed earlier, the liver is directly vulnerable to foreign substances ("xenobiotics") entering the body. Blood carrying newly-absorbed molecules proceeds directly to the liver from the intestines. Substances as diverse as drugs, whether legal or illegal; anesthetics; herbs, foods, and pollutants can be rendered more toxic after reaching the liver, due to bioactivation by the liver P450 and related enzyme pathways (see Figure 4). Almost all of these substances are liver toxins because of their conversion into reactive oxidants, which deplete the antioxidants and other Phase 2 conjugation resources. This unfortunate lack of discriminative activity by the liver underlies most of the notorious liver toxicity of pharmaceuticals. Excessive intake of substances from any xenobiotic category can predispose the liver to damage in response to otherwise-reasonable intakes of substances from other categories. A classic example is alcohol intake potentiating the metabolism of pharmaceuticals.

Drug Xenobiotics. Both prescription and over the counter pharmaceuticals can become activated to toxic metabolites in the liver.^{4,19} The most heavily consumed among these are the painkillers acetaminophen, aspirin (acetylsalicylic acid), ibuprofen, carbamazepine, indomethacin, phenylbutazone; the antibiotic tetracycline; the anti-arrhythmic drugs amiodarone, perhexiline,

and hexestrol; the blood pressure drug alpha-methyl dopa; the anticlotting medication sulfinpyrazone; the barbiturate phenobarbital; the chemotherapy drug methotrexate; the gout drug allopurinol; the anti-tuberculosis drug isoniazid (particularly in combination with rifampin); the CNS stimulant amineptine; the tricyclic antidepressant tianeptine; the anti-epileptics phenytoin and valproic acid; and the benzodiazepine sedative chlordiazepoxide. Anesthetics that are potentially toxic to the liver include halothane. Of the illicit drugs, cocaine has been extensively studied for its toxicity to the liver by bioactivation.

Marpaung and colleagues did a 1988 double-blind trial for which they assembled 101 tuberculous subjects who earlier had suffered liver damage from rifampin and 2 other anti-tuberculosis pharmaceuticals.²⁰ The PC group received 1350 mg of fortified PC daily, versus placebo for 3 months. Both groups showed good clinical improvement, but in the PC group SGOT and SGPT were significantly lower when compared with the group that received the placebo. Kuntz and collaborators had made a similar finding in 1979, by giving PC via the intravenous route.²¹

Long-term intakes of certain of the antiepileptic drugs, especially phenytoin, pose a high risk of liver damage. Hisanaga and collaborators (1980) in Japan followed 38 subjects who had received phenytoin and other antiepileptic drugs for an average of five years.²² A subgroup with the highest degree of damage (assessed by SGGT enzyme elevation), after being given PC orally for 6 months, experienced remarkable benefits.

Other, non-Pharmaceutical Xenobiotics. Chemicals produced by industry currently number at least sixty-five thousand. One of the chemical classes most toxic to the liver is the chlorinated and related halogenated hydrocarbons, of which carbon tetrachloride has been extensively researched as an experimental model. Included in this class is the dry cleaning solvent trichloroethylene, along with many commonly used herbicides and pesticides. In 1965 Kuntz and Neumann-Mangoldt documented an antidotal effect from PC against acute oral trichloroethylene poisoning.²³ Also, non-halogenated organic solvents, allyl alcohol, carbon disulfide, ethionine, and thioacetamide all are markedly liver-toxic, by mechanisms similar to those illustrated in Fig. 4. Numerous case histories have been published that document the benefits of PC in other types of xenobiotic toxicity.

Among plants that can be mistaken as foods, the deathcap mushroom (*Amanita phalloides*) carries toxins that are some of the most lethal agents known. Esslinger used PC, at first intravenously then also orally, to avert death in victims of deathcap poisoning.²⁴ In Esslinger's experience, PC worked against deathcap mushroom toxicity after milk thistle extract had failed to show benefit. He called PC "a valuable extension to therapy for this grave form of poisoning."

Natural plant toxins. In addition to the deathcap mushroom, aflatoxin from moldy peanuts is also one of the most toxic natural substances, and also becomes operative via bioactivation. Constituents of herbs also can be liver-toxic by bioactivation, the most notorious of these being the pyrrolizidine alkaloids found in comfrey and at least 59 other plants. **☞** **Radiation exposure.** Klemm and Pabst in 1964 gave PC to 161 subjects who had previously undergone radiation treatment.²⁵ Radiation scattered from the head-neck area tended to damage the liver, and PC afforded partial but clinically-meaningful protection against this occurrence.

Other toxic insults to the liver, such as from high galactosamine intake or partial hepatectomy (the surgical removal of liver tissue), and a variety of other sources, have proven amenable to improvement by PC in studies conducted with laboratory animals.

Controlled Trials with PC in Viral Liver Damage

A number of viruses can damage the liver, by precipitating widespread inflammatory breakdown which is further complicated by overactivation of the immune system (autoimmune complications). Once successfully installed in the liver parenchyma, such viruses can become chronic and very hard to dislodge. Liver viruses (here simply called LV) can wreak havoc with the liver's functions. Medical weapons for eliminating LV from the liver, or for ameliorating their progressive damage, have been limited. Controlled clinical trials have unequivocally established PC as safe and reliable nutritional support for the liver against the damage initiated by LV.

Muetting and collaborators in 1972 gave 16 subjects with chronic, aggressive LV a relatively high intake of PC (2,050 mg per day) for an average 8 months.²⁶ A number of clinical parameters improved, including measures of the liver's detoxification pathways that metabolize amino acids and phenols, and the authors concluded that PC was having a "normalizing" effect on the liver as a whole. From their large open study reported in 1973, over the course of which some subjects received PC for up

to 5 years. Wallnoefer and Hanusch noted a success rate for chronic, aggressive LV infection of 35.3 percent.⁷

Hirayama, Yano and collaborators conducted a double-blind trial in Japan in 1978, using 124 subjects with various LV.^{27,28} They gave PC (1350 mg per day) to a group of 58 subjects and placebo to 66 subjects, for twelve weeks. The PC group experienced significant reductions in SGOT and SGPT levels when compared with the placebo group; those with higher enzyme values to begin with appeared to benefit the most. A subsequent blinded biopsy assessment after 6 months confirmed that in the PC subjects, the liver parenchymal tissue had partially recovered from its earlier damage; focal necrosis/cell death was lessened in the PC group, and these subjects showed signs of liver regeneration.

In 1981, Kosina and collaborators conducted a sophisticated trial in Czechoslovakia that compared PC against drugs for the management of viral-related liver inflammation. They recruited 80 subjects with presumed acute LV infection (viruses hepatitis A and hepatitis B), and divided them into four groups of 20 subjects each.²⁹ The first 2 groups were drawn from subjects whose bilirubin levels were low (below 250 micromoles per liter) and were judged "moderately serious." Subjects in Group I were administered fortified PC (1350 mg) along with the "standard treatment" that involved diet, rest, vitamins, and glucose; Group II received the standard treatment only. Groups III and IV were judged "serious," with bilirubin levels above 250 micromoles per liter. Group III received fortified PC and 580 mg daily of the immunosuppressive drug prednisone (a drug option for the suspected immune system overactivation from LV); Group IV received prednisone plus the standard treatment.

PC had a clearly favorable effect in this trial. Concerning the resolution of viral damage, both Group I subjects (less severe) and Group III (more severe) had their liver tests return to normal markedly faster than the corresponding groups that did not receive PC. Subjects who did not receive PC were more likely to relapse (10% in the less severe, 25% in the severe), while no relapses occurred in the PC groups. Upset stomach, jaundice, and liver swelling, as well as the lab tests, all resolved faster in the groups treated with PC. There was a trend towards lower occurrence of the hepatitis B surface antigen (HBsAg) in the PC groups as treatment progressed.

Jenkins and collaborators at King's College, London did a double-blind trial in 1982 on 30 subjects with progressing liver damage from chronic LV (hepatitis B virus, negative for HBsAg), as verified by biopsy.³⁰ They randomly divided the subjects into two groups of 15 each, kept them on the standard immunosuppressive therapy (prednisolone or azathioprine), then gave one group PC (2,300 mg per day) and the other placebo, for 1 year. At the end of this period, the group given PC had no clinical changes, while the placebo (control) group had worsened. Biopsies revealed significant improvement of the liver structure in the PC group, versus no improvement for the controls. More of the PC subjects reported improved well-being than did the controls (62% versus 43%). In 3 of the 15 subjects given PC the viral infection was judged to be inactive at the end of the trial, while no subjects were judged inactive from the placebo group. Thus in this small controlled trial, PC halted and partly reversed chronic LV damage, improved overall well-being, and "turned off" the virus in as many as 20% of the subjects.

In 1985, Visco and collaborators assembled 60 subjects who were positive for hepatitis B virus (assessed as presence of HBsAg) and who had acute LV liver damage, and divided them into two groups.³¹ Within 10 days from the onset of jaundice, on a double-blind basis the subjects were started on either fortified PC (1350 mg) or placebo capsules. Lab tests were conducted frequently, and immune evaluations and clinical exams were done at 30, 90, and 180 days (6 months, end of trial).

By the 30-day mark, the group given PC was significantly more improved than the placebo group, with 50% being negative for HBsAg versus 25% for the controls ($p < 0.05$). PC improved the rate of clearance of virus antigen from the blood. The immune parameters were not significantly different, though liver enzyme tests showed trends favoring PC.

In 1990, Hantak and collaborators in Yugoslavia used PC to manage 24 subjects with LV (hepatitis B virus).³² All the subjects were chronically infected; they all had been virus carriers for at least 6 months. Seven had viral antigens (HBeAg) which indicated a relatively high degree of active infection. The other 17 subjects had no viral antigens and had antibodies to the virus (anti-HBeAg), indicating that they were in a stage of relative viral inactivity. All subjects received 900 mg of fortified PC per day. After 4 months, the less severely affected, antibody-positive subgroup showed statistically significant improvements in SGOT, SGPT, albumins, gamma-globulins, and other biochemical measures. The subgroup that began the study with active virus had statistically significant improvements in immune measures, suggestive of clinical benefit from PC. The effects of PC in this small and not well controlled trial were judged encouraging, and might have been more dramatic had the daily intake been as high as in other trials (a minimum 1350 mg of fortified PC, rather than the 900 mg that was given).

Controlled Trials with PC Against Severe Liver Damage

This category of liver damage is characterized by extensive fibrosis, which effectively stifles whole zones of the liver. Sometimes aggressive inflammatory changes are also present. This stage can be reached as a consequence of persistent alcohol intake, persistent viral infection, or the unchecked toxic effects of any of the many other agents that can damage the liver. Given the severity of the structural and functional damage to the liver at this stage, lesser benefits are to be expected from PC supplementation than at earlier stages. Yet still PC proved beneficial.

Fassati and collaborators in 1981 in a controlled trial conducted in Prague, Czechoslovakia, studied 61 subjects with moderately severe to severe functional breakdown of the liver.³³ The degree of advanced liver damage (extensive fibrosis, inflammation, elevated enzymes) was assessed by biopsy and by a wide range of blood biochemical tests. Thirty-four (34) subjects were given fortified PC (900 mg per day), and 27 subjects served as controls. The trial ran for 4 months, with each patient serving as their own control for statistical analysis.

Biochemical re-testing conducted at the end of the trial showed that except for the bilirubin values, all the other biochemical indicators were significantly improved ($p < 0.01$). These included the albumin/globulin ratio, albumin, bromsulfalein (BSP) clearance, SGPT, and SGOT. The number of subjects positive for HBsAg in the blood moved from 8 of 34 to 3 of 34 in the PC group; that of the controls moved from 7 of 27 to 6 of 27. The trend apparent in the PC group was not statistically significant due to the small numbers of HBsAg-positive subjects in both groups from the beginning of the trial. The investigators commented that fortified PC was the only intervention they were aware of that seemed to bring down viral antigen levels, and they urged further investigation of this possible benefit with larger groups of subjects.

In 1991, Ilic and Begic-Janev conducted a randomized, double-blind, placebo-controlled trial.³⁴ They recruited 50 subjects, all positive for HBsAg (hepatitis B virus antigen) who had extremely severe liver damage as verified by biopsy and immunologic testing. The test group was administered 1350 mg of fortified PC, and the control group received a placebo. Both groups were followed for 1 year, with periodic sampling for lab assessments, then at the end of the 12 months they were biopsied again.

After 12 months the subjects given PC had experienced considerably greater benefit, as assessed both from the structural biopsy findings and from the lab findings ($p < 0.001$). Among the PC group, 20 of 25 were judged good to moderately good, versus 6 of 25 being moderately improved in the placebo group. Six of the 25 in the PC group also lost the HBsAg viral antigen, versus only 3 of 25 for the placebo group. Such "seroconversion" indicated marked clinical improvement for these fortunate subjects. A number of cell-structural, biochemical, immunologic, and hematologic parameters were significantly improved in the PC group as compared with the placebo group. Improvement in the PC group continued well past the end of the trial.

As a rule, researchers working with such severely affected subjects obtained better results by maintaining the subjects on combined intravenous PC and oral supplementation until substantial improvement had begun.

Other trials with severe liver damage, though not controlled, are worthy of note. Wallnoefer and Hanusch in their pioneering study administered PC both intravenously and orally to 130 subjects with advanced, fibrotic liver damage.⁷ Once the clinical indicators began returning to normal, they switched to purely oral administration at relatively low intakes (450-700 mg), which was continued for months to years as necessary. PC produced benefits for 17.5% of these subjects, as confirmed from normalized enzyme levels and improved tissue structure on biopsy. Using a similar strategy, they achieved benefit for 35.3 percent of their subjects with chronic viral infection of a kind that was positive for viral antigen and has an aggressive tendency to progress to severe liver damage. Kuntz reported in 1989 on 10 subjects to whom he gave PC intravenously at 2,800 mg per day.³ Improvements were seen as early as the seventh day, and at the end of the 28-day trial period 3 subjects showed "dramatic, life-saving" improvement, 2 had "increasingly rapid improvement," 2 had gradual improvement, 2 had no change; and 1 of the 10 subjects had died.

Kalab and Cervinka worked with 30 subjects who had advanced liver damage for which pharmaceutical treatments had failed.³⁵ Orally administered fortified PC (1350 mg daily) produced clinical improvement after 6 months, with favorable effects on the usual enzyme indicators of liver damage. In summary, the experiences from the clinical trials discussed above concur with findings from others³⁶⁻⁴⁰ to paint a clear picture of PC as an effective and safe nutrient for liver damage of all degrees of severity.

PC Benefits the Liver Primarily Through Cell Membranes

The efficacy of PC in protecting the liver against toxic attack can be attributed to its important role in cell membranes. The membrane systems are among the cell constituents most vulnerable to toxic attack, and the diverse array of hepatotoxic substances operates through common pathways: free radical or other oxidative attack that depletes antioxidants, leading to oxidative overload and subsequent peroxidative damage to the cell's membranes.⁴ The ultimate consequence is the death of the cell.

The phospholipids of cell membranes are partially unsaturated, and by being packed tightly next to each other in the membrane they are highly vulnerable to oxidative attack from free radicals and other highly reactive, oxidant toxins. Under excessive or sustained attack, the membrane phospholipids become degraded ("peroxidized"), mainly through their fatty acid tails. As the phospholipids peroxidize, membrane continuity is interrupted. Holes begin to develop in the cell's outer membrane, resulting in loss of control over internal conditions. Enzymes and other larger bio-molecules begin to leak out, homeostasis fails, and the death of the cell becomes imminent.

Viral attack on the liver follows a model similar to chemical attack: viral invasion of the parenchymal cells initiates release of pro-inflammatory, oxidizing substances. Immune cells arrive in the area and begin releasing more oxidants via their "respiratory burst." These activities initiate cascades of peroxidative membrane damage to the liver cell membranes, and the damage spreads to neighboring zones within the tissue.

PC plays crucial roles in supporting the membrane-based structure and functions of the liver's parenchymal cells. When orally administered to experimental animals, in quantities usually equivalent to 1-3 grams per day for the human, PC had the following liver-protective effects:

1. Leakage of "indicator" enzymes from the liver tissue was lessened
2. Lipid peroxidation from free radical/oxidant insult was lessened
3. Membrane damage was slowed, membrane integrity was conserved
4. Cell death, fibrosis, and fatty infiltration of the liver tissue were diminished
5. Cell synthesis of RNA and protein increased, suggesting regeneration
6. Liver metabolism improved

This documented range of benefits from PC is consistent with its functions at the cell membrane. PC is required for the structural integrity of all the body's cell membrane systems, and is essential to their functionality.⁴¹⁻⁴⁵ PC is crucial both for the internal membranes to do their housekeeping and specialized functions, and for the cell's "master switch" Nits outer membrane. The outer membrane interfaces with both the external environment and the internal environment of the cell; PC supports the membrane receptors that "hear" these molecular messages and carry them across the membranes in both directions. This outer membrane is also the cells' reservoir for the eicosanoids and other phospholipid derivatives that act as outgoing vocabulary, speaking the language of that cell to others.

The accumulated findings from decades of research are that PC is an important protective nutrient for the liver, primarily through being a building block for cell membranes. PC is essential for the liver's baseline homeostatic housekeeping functions, for the liver's recovery following toxic damage, and not least to support the sophisticated liver metabolism that determines the individual's level of health and freedom from disease.

PC is highly bioavailable (about 90% of the administered amount is absorbed over 24 hours),⁴⁶ and PC represents a far more pleasant means for dietary choline repletion than choline itself. Lastly, even as the PC molecule is efficiently absorbed, it also is an excellent emulsifier that enhances the bioavailability of nutrients with which it is co-administered. Antioxidant nutrients and especially the flavonoids are likely to be better absorbed in combination with PC,⁴⁷ as are B vitamins, minerals, and numerous other nutrients.

Conclusion:

From the many controlled clinical studies conducted on thousands of human subjects to date, PC's confirmed clinical benefits include:

1. Successful improvement of specific indicators of liver damage
2. Faster functional and structural recovery of the liver tissue
3. Accelerated restoration of subjects' overall well-being

In the trials cited in this review, PC was very well tolerated at oral intakes that ranged up to 4.6 grams per day, and was found to be more effective the earlier it was administered. Subjects who are started on PC after their liver is already severely damaged are more likely to benefit from higher oral intakes of PC (up to or exceeding 4.6 grams per day). The most severe cases are likely to thrive with the help of intravenous PC, administered in combination with a high oral dose.

Lieber and colleagues' elegant studies with baboons as a primate model of alcoholic liver damage have established that PC can stave off steadily-worsening damage from chronic alcohol consumption; improvement from PC is far more likely if the subject's alcohol consumption is ceased. The small choline molecule is actually part of the headgroup of the large PC molecule, but when free choline was added to the baboon diet it proved toxic to the alcohol-damaged liver. Phosphatidylcholine is a highly bioavailable form of choline; it is also the most biologically significant and (for damaged livers, at least) the safest source of choline.

PC is undoubtedly a critically important nutrient for the liver, both because it is the primary cell membrane building block and because the liver is so functionally dependent on its estimated 33,000 square meters of membrane surface. Whether the liver has been damaged by alcohol, by other toxic chemicals, by pharmaceuticals, or by viruses, dietary supplementation with PC significantly speeds recovery. The clinical studies demonstrate that dietary PC in sufficient amounts revitalizes whole zones of cells in the recovering liver.

PC has other qualities that further enhance its remarkable usefulness as a dietary supplement. PC is well documented as safe to take, and seems fully compatible with pharmaceutical regimens and with other nutrients. The PC molecule enhances the bioavailability of nutrients with which it is co-administered, is highly bioavailable and represents a far better means for dietary choline repletion than choline itself.

The jury is still out on whether PC is truly a "magic bullet" for alcoholic liver disease, but its benefits against various severities of liver damage and its proven safety indicate that for the liver it is a nutrient of major importance.

References

1. Alberts B, Bray D, Lewis L, et al. *Molecular Biology of The Cell*. New York:Garland Publishing;1989.
2. Jones AL. Anatomy of the normal liver. In: Zakim D, Boyer TD, eds. *Hepatology: A Textbook of Liver Disease*. Philadelphia:WB Saunders; 1996:3-32.
3. Kuntz E. Pilot study with polyenylphosphatidylcholine in severe liver insufficiency. *Med Welt* 1989;40:1327-1329.
4. Kidd PM. Liver biotransformation of xenobiotic chemicals, foods, and drugs to free radical oxidants. In: Levine S, Kidd PM. *Antioxidant Adaptation: Its Role in Free Radical Pathology*. San Leandro, California:Biocurrents;1985: 221-281.
5. Fausto N. Hepatic regeneration. In: Zakim D, Boyer TD, eds. *Hepatology: A Textbook of Liver Disease*. Philadelphia:WB Saunders;1996:32-58.
6. Wallnoefer H, Hamusch M. "Essential" phospholipids in the treatment of hepatic disease. *Med Monatsschrift* 1973;27:131-136.

7. Friedman LS, Martin P, Munoz SJ. Liver function tests and the objective evaluation of the patient with liver disease. In: Zakim D, Boyer TD, eds. *Hepatology: A Textbook of Liver Disease*. Philadelphia:WB Saunders;1996:791-833.
8. Sorrentino F, Diene G, Corvaja E, et al. Use of polyunsaturated phosphatidylcholine (EPL) in association with vitamin B complex in liver therapy. *La Clinica Terapeutica* 1982;102:163-183.
9. Lieber CS. alcohol and the liver:1994 update. *Gastroenterology* 1994;106:1085-1105.
10. Lieber CS. Alcohol-induced liver disease. In: Maddrey WC, ed. *Gastroenterology and Hepatology: The Comprehensive Visual Reference*. Philadelphia: Current Medicine; 1996:9.1-9.21.
11. Knuechel F. Double blind study in patients with alcohol-toxic fatty liver. *Med Welt* 1979;30:411-416.
12. Schuller Perez A, San Martin F.G. Controlled study using multiply-unsaturated phosphatidylcholine in comparison with placebo in the case of alcoholic liver steatosis. *Med Welt* 1985;72:517-521.
13. Buchman AL, Dubin M, Jenden D, et al. Lecithin increases plasma free choline and decreases hepatic steatosis in long-term total parenteral nutrition patients. *Gastroenterology* 1992;102:1363-1370.
14. Panoz MZ, Polson R, Johnson R, et al. Activity of polyunsaturated phosphatidylcholine in HBsAg negative (autoimmune) chronic active hepatitis and in acute alcoholic hepatitis. In: Gundermann K, Schumacher R, eds. *50th Anniversary of Phospholipid Research (EPL) International Symposium*. Bingen/Rhein: wbn-Verlag;1990.
15. Lieber CS ; DeCarli LM ; Mak KM ; Attenuation of alcohol-induced hepatic fibrosis by polyunsaturated lecithin. *Hepatology* 1990;12:1390-1398.
16. Li J, Kim C, Leo MA, et al. Polyunsaturated lecithin prevents acetaldehyde-mediated hepatic collagen accumulation by stimulating collagenase activity in cultured lipocytes. *Hepatology* 1991;15:373-381.
17. Lieber CS, Robins SJ, Li J, et al. Phosphatidylcholine protects against fibrosis and cirrhosis in the baboon. *Gastroenterology* 1994;106:152-159.
18. Schenker S, Hoyumpa AM. Polyunsaturated lecithin and alcoholic liver disease: a magic bullet? *Alcoholism: Clin Exp Res* 1994;18:1286-1288.
19. Hoyumpa AM, Schenker S. Drugs and the liver. In: Maddrey WC, ed. *Gastroenterology and Hepatology: The Comprehensive Visual Reference*. Philadelphia: Current Medicine; 1996:6.1-6.22.
20. Marpaung B, Tarigan P, Zein LH, et al. Tuberkulostatische Kombinations-Ntherapie aus INH, RMP und EMB. *Therapiewoche* 1988;38:734-740.
21. Kuntz HD, Rausch V, Bammer E. Hepatotoxicity of rifampicin and the effect thereon of "essential" choline phospholipids. *Med Welt* 1978;29:452-454.
22. Hisanaga M, Utsumi S, Miyamoto S, et al. Abnormality of liver function in patients treated with antiepileptic drug and a trial of polyene phosphatidylcholine treatment for these patients. *Folia Psychiatr Neurol Japonica* 1980;34:318-319.
23. Kuntz E, Neumann-Mangold P. Acute peroral trichloroethylene poisoning. *Med Welt* 1965;16:2872-2874.
24. Esslinger F. Death cap mushroom poisoning: report of clinical experience. *Med Welt* 1966;19:1057-1063.
25. Klemm J, Pabst HW. Untersuchungen uber den Einfluss therapeutischer Teilkoerper-bestrahlungen auf die Leberfunktion und die Schutzwirkung essentieller Phospholipide. *Strahlentherapie* 1964;123:438-450. [In German, summary in English]
26. Muetting D, Dohn P, Reikowski J. Effect of high doses of essential phospholipids administered intravenously and perorally on metabolism of albumin and fats and on enzymatic activity of chronically ill liver patients. *Verhandlungen der*

Deutsche Gesellschaft für Innere Medizin 1972;17:1389-1392.

27. Hirayama C, Okamura M, Tanikawa K, et al. The clinical effect of polyene phosphatidylcholine in chronic hepatitis in a double-blind test. *Rinsho to kenkyu* 1978;55:194-198.
28. Yano M, Koga M, Shirahama S, et al. Blind assessment of liver biopsy findings in chronic hepatitis: drug efficacy trial of polyene phosphatidylcholine. *Shindan to chiryo* 1978;9:1783-1789.
29. Kosina F, Budka K, Kolouch Z, et al. Essential cholinephospholipids in the treatment of viral hepatitis. *Cas Lek Ces* 1981;120:957-960.
30. Jenkins PJ, Portmann BP, Eddleston ALWF, et al. Use of polyunsaturated phosphatidylcholine in HBsAg negative chronic active hepatitis: results of prospective double-blind controlled trial. *Liver* 1982;2:77-81.
31. Visco G. Polyunsaturated phosphatidylcholine associated with vitamin B complex in the treatment of acute viral hepatitis B. *La Clinica Terapeutica* 1985;114:183-188.
32. Hantak I, Boca M, Miculecky M, et al. Essential phospholipids in the treatment of chronic infection with the hepatitis B virus. *Vnitřní Lékarství* 1990;36:1164-1171.
33. Fassati P, Horesji J, Fassati M, et al. The effect of choline phospholipids on HBsAg and selected biochemistry tests in cirrhosis of the liver. *Cas Lek Ces* 1981;120:56-60.
34. Ilic V, Begic-Janev A. Therapy for HBsAg-positive chronically active hepatitis. Effect of "essential" phospholipids. *Med Welt* 1991;85:523-525.
35. Kalab M, Cervinka J. Essential phospholipids in the treatment of cirrhosis of the liver. *Cas Lek Ces* 1983;122:266-269. [In Slovak, with English summary]
36. Docker O. Therapy of chronic liver diseases and acute, life-threatening liver failure conditions. *Med Welt* 1960;20:1079-84.
37. Frosch B, Wagener H. Therapy of hepatitis epidemica. *Fortschritte Med* 1963;81:725-7.
38. Kautsch E. Comparison of therapy in acute hepatitis epidemica. *Med Klinische* 1965;60:1401-1410.
39. Rottini E, Bazzanella F, Marri DG, et al. Therapy of different types of liver insufficiency using "Essential" phospholipids. *Med Monatsschrift* 1963;17:28-30.
40. Wegner H. Essential phospholipids in the therapy of hepatitis infectiosa. *Munch Med Welt* 1965;5:227-231.
41. Cullis PR. Phospholipids and membrane transport. *Can J Biochem* 1980; 58, 1091-1095.
42. Hirata F, Axelrod J. Phospholipid methylation and biological signal transmission. *Science* 1980;209:1082-1083.
43. Spector AA, Yorek MA. Membrane lipid composition and cellular function. *J Lipid Res* 1985;26:1015-1035.
44. Shinitzky M. Membrane fluidity and receptor function. In: Kates M, Manson LA, eds. *Membrane Fluidity*. New York:Plenum Press; 1984.
45. Kidd PM. Cell membranes, endothelia, and atherosclerosis: the importance of dietary fatty acid balance. *Alternative Medicine Review* 1996; 1(3): 148-167.
46. Fox JM, et al. Pharmacokinetics of orally ingested phosphatidylcholine. In: Barbeau A, et al, eds. *Nutrition and the Brain*, Volume 5. New York: Raven Press; 1979:95-108.

47. Buzzelli G, Moscarella S, Giusti A, et al. A pilot study on the liver protective effect of soyoin-phosphatidylcholine complex (IdB 1016) in chronic active hepatitis. *Intl J Clin Pharmacol Ther Toxicol* 1993; 31:456-460.

48. Lieber CS, Leo MA, Mak KM, et al. Choline fails to prevent liver fibrosis in ethanol-fed baboons but causes toxicity. *Hepatology* 1985; 5:561-572.



[Return to Table of Contents](#)

August 2000

Astaxanthin

Continuing Education Module

Timothy J. Maher, Ph.D.

Sawyer Professor of Pharmaceutical Sciences

Dean, Research and Sponsored Programs

Professor of Pharmacology

Massachusetts College of Pharmacy and Health Sciences

This module was produced in association
with Massachusetts College of Pharmacy and Health Sciences

maphs

 **HEALTH PLUS INC.**
GATEWAY TO BETTER HEALTH... Naturally

LA HAYE

This module was made possible by an
unrestricted grant from Health Plus Inc.
and La Haye Laboratories, which
supports continuing education for the
natural products industry.

The module was peer reviewed by
pharmacists and other licensed
health care professionals.



This module is approved for two credits toward a New Hope Institute of Retailing Certificate of Completion in Natural Healing.



This module is approved for one contact hour (0.1 CEUs) of continuing pharmaceutical education credit. Massachusetts College of Pharmacy and Health Sciences is approved by the American Council on Pharmaceutical Education as a provider of continuing pharmaceutical education, ACPE #026-999-00-117-H01.



This module is approved for two contact hours of continuing education credit by American Health Science University/NINE. To be given credit, the CN[®] must submit a copy of the module and test to AHSU/NINE.

Continuing Education Module

by Timothy J. Maher, Ph.D.

Goal:

The goal of this module is to introduce the reader to the carotenoid astaxanthin and examine its antioxidant actions especially as it relates to potential therapeutic approaches in addressing cardiovascular disease, neurodegenerative disease, cancer, immune function status and visual health.

Objectives:

Following successful completion of this module, the participant will be able to:

- describe the unique antioxidant features of the carotenoid astaxanthin;
- list the sources in nature and the functions of astaxanthin in animals that produce and consume astaxanthin;
- explain findings of recent research that describe the effects of astaxanthin in cardiovascular disease, neurodegenerative disease, visual health, cancer and immune system function;
- describe the pharmacokinetics of astaxanthin and list its potential side effects.

Oxygen and Antioxidants

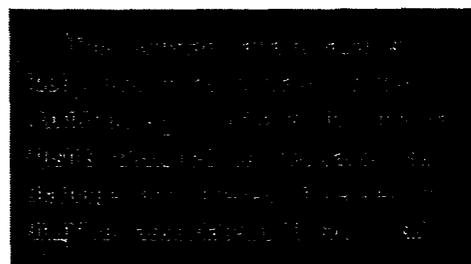
As aerobic organisms we depend completely on molecular oxygen for our existence; just a few minutes without oxygen usually results in irreversible damage or death. Our friendly atmosphere usually provides ample oxygen via the air we breathe, and even a relatively small decrease in the air's oxygen content has noticeable effects. For instance, when traveling from sea level to high altitudes where the oxygen content is slightly lower, most individuals will find tasks requiring even minimal exertion much more difficult. Even highly trained athletes will find performance in their events a greater challenge at high altitudes.

Although oxygen is absolutely critical for life, this molecule also has a dark side to its actions. Oxygen is found in a large number of harmful by-products that are constantly being produced and defended against by healthy organisms. These so-called reactive-oxygen species (ROS) contain reduced oxygen molecules including free radicals such as the superoxide, hydroxyl and peroxy radicals and non-radicals such as ozone, lipid peroxides,

hydrogen peroxide, and singlet oxygen.¹ Additionally, a number of nitrogen compounds containing oxygen, such as nitric oxide and peroxynitrite, also are extremely harmful. Many of these compounds are so highly energetic that they react almost instantly with many neighboring molecules such as proteins, DNA, RNA, carbohydrates and lipids. The consequence of such oxidative attack may include protein oxidation, DNA and RNA damage, and lipid peroxidation. Even small alterations to some of these basic molecules of life would be expected to have dire consequences.

This constant attack against the body, known as oxidative stress, is continuously countered by mechanisms designed to neutralize such damage and prevent diseases that might be associated with such insult (Table 1). While certain repair enzymes can sometimes reverse the damage produced by the ROS, the ability of antioxidants to neutralize the ROS prior to inducing damage is an extremely important defense mechanism that helps to support a healthy existence and most likely prevents disease.^{2,3} During the last

decade there has been a tremendous amount of interest in the roles ROS and oxidative stress might play in the development and progression of a number of neurodegenerative diseases including Alzheimer's, amyotrophic lateral sclerosis (Lou Gehrig's) and Parkinson's diseases, as well as macular degeneration. Many other diseases such as atherosclerosis, cataracts, cancer, cerebrovascular disor-



ders, multiple sclerosis, bacterial and viral meningitis, and epilepsy have also been suggested to involve an underlying oxidative insult.⁴ The rate at which many of the processes of aging occur have also been attributed by some to reactions involving oxidative stress. Because antioxidants are capable of scavenging harmful ROS and reducing overall oxidative

Table 1

Conditions Likely to be Associated with Oxidative Stress

- Aging
- Alzheimer's Disease
- Artherosclerosis
- Bacterial Meningitis
- Cancer
- Cataracts
- Cerebrovascular Diseases
- Epilepsy
- Lou Gehrig's Disease
(Amyotrophic Lateral Sclerosis)
- Macular Degeneration
- Parkinson's Disease
- Viral Meningitis

stress, it is not surprising that there has been much interest in investigating the use of such compounds to slow the progression of, and in some cases even prevent, a wide array of diseases.

Carotenoids and Astaxanthin

Of the antioxidants investigated for their potential health benefits, the carotenoids have received the most attention. These lipid-soluble pigments from plants, algae and some bacterial species, comprise well over 700 compounds that account for the beautiful red, orange and yellow colors observed in many of these lower species. While most higher animals are unable to endogenously synthesize these carotenoids, they do accumulate them via ingested foods. In many aquatic and land species the spectacular coloration observed results from the mixture of such carotenoids. Besides their obvious function of providing pigmentation for many species, the carotenoids also serve a multitude of important chemical functions such as their ability to absorb light, quench singlet oxygen, be oxidized and isomerize, bind to hydrophobic surfaces, and solubilize in organic media.⁵ However, not all carotenoids are equal, and the differences in activities of the individual carotenoids are thought to result from the uniqueness of their chemical structures.

While the most notable carotenoid is the vitamin A precursor, β -carotene, the carotenoid that has received much attention lately is astaxanthin (3,3'-dihydroxy- β - β -carotene-4,4'-dione).

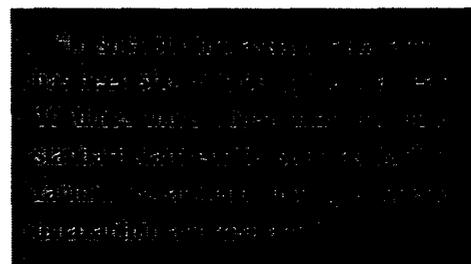
Chemically, astaxanthin is classified as a xanthophyll, which is a family of carotenoids with specific substitutions on the benzoid rings. While there are three possible isomers of astaxanthin, only one exists naturally (the S,S' configuration). Lower organisms synthesize astaxanthin beginning with acetyl CoA and proceeding through a number of important intermediates including phytoene, lycopene, β -carotene and canthaxanthin. Both astaxanthin and canthaxanthin are examples of conjugated keto-carotenoids, and both are further classified as xanthophylls. While β -carotene is a vitamin A precursor, astaxanthin cannot be converted to this vitamin and thus cannot support retinol-specific processes.

Astaxanthin provides the rich pink color observed in various aquatic species including the salmonids (e.g., salmon) and crustaceans (e.g., crabs, lobster, shrimp), and even some nonaquatic species such as the flamingo (whose diet includes some astaxanthin-producing organisms). The carotenoids found in phytoplankton, algae and plants normally participate in those organisms' photosynthetic processes by acting as secondary light-absorbing molecules. Salmonids and flamingos don't actually produce astaxanthin but instead obtain it from other animals they consume. The richest source of astaxanthin by far is the algae *Haemococcus pluvialis*, which is used commercially as a feed additive to provide color to "farm-raised" salmon and other fish (Table 2).

The astaxanthin that is contained in living lobsters is complexed with proteins called carotenoproteins that actually imparts a bluish-brown color to these animals. However, when the carotenoproteins are denatured, as occurs during the high temperatures associated with cooking, the astaxanthin is liberated and the bright red coloration results.⁶ Besides pro-

viding the coloration to such fish, and thus enhancing their economic value (e.g., few people would find white salmon attractive), some recent studies have indicated a "vitamin-like" role for astaxanthin in these fish.

Experimentally, the potency or capacity of an antioxidant to chemically neutralize or scavenge harmful oxygen- or nitrogen-reactive compounds can be determined routinely in the laboratory. One such assay measures the production of ROS-induced lipid peroxides in test tubes in the absence and in the presence of various concentrations of a suspected antioxidant. In such *in vitro* assays, astaxanthin has been shown to be typically at least 10 times more potent than the other standard carotenoids such as canthaxanthin, β -carotene, lutein, lycopene, tunaxanthin and zeaxanthin.^{7,8} When compared with α -tocopherol (vitamin E), astaxanthin's potency as an antioxidant ranges from approximately 80 times to as much as 550 times greater.^{9,10} Additionally, when tested against a wide array of ROS and nitrogen-reactive species, astaxanthin appears to be the most effective in scavenging this wide variety of harmful products. Astaxanthin is thought to be able to span the lipid/protein bilayer of



biological membranes, imparting a powerful antioxidant effect. Astaxanthin's combination of superior potency and versatility yield the ideal antioxidant. Therefore, it is not surprising to find that this carotenoid has been studied for its potential utility in a number of disease states in humans and disease models in animals.

Anti-cancer Activity and Astaxanthin

The anti-cancer activity of carotenoids and related compounds has been the focus of much attention since epidemiological reports of an association between low

Table 2

SOURCES AND CONTENT OF ASTAXANTHIN	
Source	Content (mg/kg)
Salmon	
Sockeye	26-37
Coho	9-21
Chum	3-8
Chinok	8-9
Atlantic	3-11
Red Seabream	2-14
Rainbow Trout	1-13
Krill	46-130
Krill Oil	727
Crayfish Meal	137
Yeast (<i>Præmia r.</i>)	30-800
Haematococcus pluvialis	10,000-30,000

levels of certain carotenoids and various types of cancers. Some of the cancers studied with respect to the carotenoid association include lung, esophageal, stomach, colon, rectal, prostate, breast, cervical, ovarian, endometrial, bladder and skin. For instance, while men with the lowest plasma β -carotene levels had an increased risk of prostate cancer, when supplemented with carotenoids (especially lycopene) their risk decreased by 36%. Carotenoids may be of benefit in the prevention or the amelioration of cancers via their ability to scavenge ROS, inhibit the growth of certain tumors, inhibit malignant transformation, enhance immune function, and upregulate certain genes (e.g., connexin 43). Astaxanthin, with its potent antioxidant activity and its beneficial immune actions, might be predicted to be especially active in a number of animal models of cancer.

The anti-cancer activities of astaxanthin, canthaxanthin and β -carotene against the growth of mammary tumors in young mice were determined.¹¹ For three weeks animals were fed either 0, 0.1, or 0.4 percent in the diet of the individual carotenoid prior to inoculation with a fixed amount of tumor cells. Astaxanthin dose-dependently inhibited growth of the tumor cells, and was the most effective of the various carotenoids tested.

with cancer involves the ability of this carotenoid to enhance membrane stabilization and promote the synthesis of the gene for the gap-junction protein, connexin-43. Alterations in this protein would be expected to beneficially influence cell-to-cell communication and increase the likelihood of the maintenance of cellular homeostasis and thus normal function.

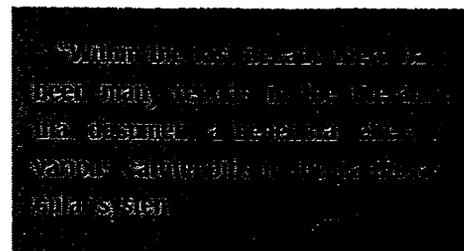
Cardiovascular Health

Within the last decade there have been many reports in the literature that document a beneficial effect of various carotenoids in the cardiovascular system. Much attention has been centered on the influence of these substances on the levels of cholesterol as contained in the form of various lipoproteins. Most studies have documented a positive correlation between levels of the atherogenic low-density lipoproteins (LDL) and the prevalence of diseases of the cardiovascular system (e.g., hypertension, angina pectoris, myocardial infarction). In contrast, it is generally recognized that an inverse correlation exists between high-density lipoproteins (HDL) and the incidence of cardiovascular health. A beneficial lipoprotein profile is one characterized by low levels of the cholesterol-rich LDL

Additionally, while canthaxanthin and β -carotene failed to alter lipid peroxidation activity in the tumors, astaxanthin was highly effective in this regard. Similar protective effects of astaxanthin were found in a mouse model of urinary bladder carcinogenesis.¹² Astaxanthin has also been reported to be effective against a number of other carcinogenic stimuli including aflatoxin, chloroform, viruses, and 4-nitroquinoline-1-oxide.^{13,14} A recent proposed mechanism for astaxanthin in influencing the pathways associated

("bad-cholesterol") with high levels of HDL ("good-cholesterol").

While there have been many studies in humans and in animals with the more common carotenoids and other antioxidants, few studies have been performed with astaxanthin. One study reported a significant increase in HDL levels when rats were treated with astaxanthin in the



diet.¹⁵ In this same study, administration of another carotenoid, β -carotene, was without effect on HDL. This study provides evidence of yet another difference in the activities of individual carotenoids. Obviously there is a need for well designed (e.g., double-blind, randomized) clinical studies in humans to determine if a similar beneficial effect on lipoprotein profiles will be realized.

Immune Function and Astaxanthin

Many studies have demonstrated the ability of astaxanthin to enhance antibody responses and augment humoral immune functions. In a recent study using mice treated with *H. pylori*, astaxanthin was found to significantly reduce bacterial load and gastric inflammation, while also being able to modulate cytokine release in splenocytes harvested from these treated animals.¹⁶ Interestingly, in this study astaxanthin was found to shift the observed T-lymphocyte response from a predominantly T-helper-1 (TH-1) cell response dominated by interferon- γ (INF- γ), to a mixed TH-1/TH-2 response with involvement of both INF- γ and interleukin-4 (IL-4). In this particular animal model, INF- γ is most likely involved with mediating the gastric damage and irritation observed in the gastrointestinal tract. On the other hand, the IL-4 is thought to be involved with the repair of the gastric mucosa. This was the first demonstration in the literature of a compound causing a shift from the usual

CHARACTERISTICS OF ASTAXANTHIN

- Extremely potent antioxidant
- Very versatile at scavenging various ROS
- Unique immune modulatory activity
- Effective against a number of cancer promoters
- Enhances gap junctions & cell communication
- Cardioprotective / lipoprotein - beneficial
- Easily enters the central nervous system
- Protects against photic-induced injury
- No toxicity reported to date

abundance of cytokines that normally mediate the damage associated with infection to one characterized by a greater amount of protective cytokines. Further studies comparing the utility of astaxanthin and β -carotene to enhance antibody responses in splenocytes in a T-cell dependent fashion, demonstrate that the former is effective while the latter is ineffective.¹⁷

Astaxanthin has been tested in a preliminary human study utilizing *H. pylori*-positive patients. When administered five times per day for three weeks (8 mg doses), astaxanthin significantly decreased gastritis in all subjects, even though they remained positive for *H. pylori*. Although there is just one preliminary human study reported thus far, based on the multitude of animal studies reported in the literature there has been much excitement regarding the potential utility of this versatile and potent carotenoid in the overall therapy of *H. pylori* infection in humans.

Visual Health

The carotenoids play an essential role in the physiological function and overall health of the eye in those animals that have vision. Most of the information regarding the role of carotenoids in the visual system has focused on β -carotene and its metabolic by-product vitamin A.



However, more information has recently appeared that documents the importance of the antioxidant role of a number of carotenoid and noncarotenoid compounds in the eye. In order for a particular antioxidant to function in the eye, that compound must traverse the blood-retinal barrier. The blood-retinal barrier is similar in its function and structure to the blood-brain barrier, about which we know much more. This specialized structure, which helps to prevent the unhindered passage of compounds into the central nervous system from the periphery, regulates which compounds will pass. Of

the carotenoids examined, astaxanthin appears to easily penetrate the central nervous system, a characteristic not typically seen with all carotenoids or antioxidants. Since the molecular weight of astaxanthin is under 600 daltons, and the molecule is very lipophilic, one would expect this compound to pass the blood-brain barrier with relative ease. Thus, for any antioxidant to produce a beneficial effect on visual health it is imperative that that compound be able to enter the central compartment of interest.

The eye is a complex structure that has been extensively studied from a chemical as well as a physical perspective. One of the most important structures that comes in contact with photic stimuli is the retina. Within the retina, in its most central portion, resides the macula, a dense accumulation of photoreceptor cells (e.g., rods and cones), which function to convert incoming light into nerve impulses. Our sharpest vision occurs at the very center of the macula in an area known as the fovea. This is where the highest concentration of photoreceptor cells are located. In addition to their role in participating in the visual process by absorbing light to produce images, the carotenoids also function to protect the retina from damage. The incoming light may contain highly energetic photons from blue and purple light, which can produce high levels of harmful ROS and cause damage to the photoreceptor cells if not adequately protected. The exposure to such energetic photons can cause lipid peroxidation due to the high concentration of polyunsaturated fatty acids in the macula's photoreceptive membranes, in addition to other photo-oxidative damage. Normally, the use of sun glasses can help to prevent some of this harmful exposure and minimize damage.

In some avian species (e.g., shore birds like the kingfisher) there is a very high density of astaxanthin found in the fovea, while in others (e.g., land-based birds) there appears to be very little, if any. This

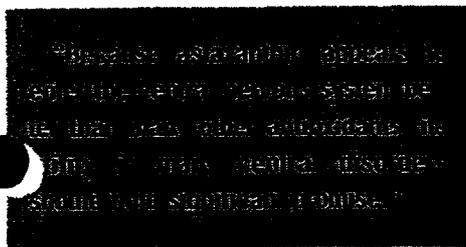
unique distribution of astaxanthin in the foveal cone oil droplets of such shore birds is believed to serve two functions: 1) to enhance their air-water interface visual acuity, and 2) to protect the retina from the harmful effects of glare off the water. These birds have been studied extensively and support the potential utility of antioxidants that do enter the eye as visual protective agents.

In preliminary animal experiments the protective effects of astaxanthin in preventing damage to the visual system has been reported. Following intraperitoneal administration of vehicle or astaxanthin (37.5 mg/kg), rats were exposed to 200 foot-candles of green-filtered fluorescent light at 490-580 nm for 24 hours. The thickness of the outer nuclear layer of the retina was then determined as a decrease in the thickness of this layer is associated with photic injury. While rats treated with vehicle had significant damage to the retina as evidenced by an approximate 35 percent decrease in the thickness of the outer nuclear layer, those treated with astaxanthin had only a 6 percent decrease in thickness. Another study demonstrated the ability of astaxanthin to prevent the depletion of rhodopsin levels in the retina following a similar photic insult.

Some diseases of the eye, especially involving the retina, may involve degeneration due to exposure to harmful photic stimuli. While genetic factors probably

play a significant role in a number of these disorders, the role of oxidative stress susceptibility is now thought to be an underlying mechanism in the damage observed. In humans macular degeneration is a general term used when describing a number of diseases of the retina. The most prevalent type is the "dry" type, which is characterized by the formation of small yellow deposits under the macula known as drusen. These drusen are eventually associated with a thinning and drying out of the macula, and a subsequent impairment of visual acuity.

In one human study there was reported to be an inverse relationship between the production of certain size drusen and the intake of provitamin A and dietary vitamin E.¹⁸ Because this study determined intake of these vitamins by using food frequency questionnaires, some of the findings were not robust enough to make clear recommendations regarding the role



of these particular antioxidants and visual health. For instance, this study failed to demonstrate a significant relation between intake of these antioxidants and the progression of maculopathy, probably also due to the small number of patients in this study who developed the late stages of maculopathy. In another study involving a review of the epidemiological literature on the association of nutritional antioxidants (vitamin C, vitamin E, carotenoids) and the progression of maculopathy, the authors concluded that such antioxidants are likely to delay the onset of age-related vision impairments.¹⁹ Much interest was expressed regarding the potential protective effects to be afforded by supplementation with the xanthophylls. However, not all xanthophylls might be appropriate for these types of studies, since a previous report²⁰ indicated the ability of canthaxanthin to concentrate in the eye and cause lens opacities. No such reports have appeared for astaxanthin, and important-

ly, the body does not appear to be able to convert canthaxanthin to astaxanthin. It is clear that there is a need for more carefully controlled, double-blind, large studies in the future to conclusively determine the effectiveness of astaxanthin and other antioxidants in promoting visual health.

Toxicity and Pharmacokinetics of Astaxanthin

To date there have been no reports of serious adverse effects associated with the administration of astaxanthin to animals. Attempts have been made to determine the lethal dose -50 but fail to find a dose that will adversely affect the animals. Even when the upper limit of 8 grams of astaxanthin per kilogram of animal body weight are administered for 10 days, no toxicity has been noted. There is a good likelihood that similar findings will be observed in humans. The doses of astaxanthin that have been suggested are on the order of 2-4 mg per day.

A recent report regarding the absorption and distribution of astaxanthin demonstrated that following oral administration of 100 mg to human volunteers, peak astaxanthin levels in plasma (1.24 mg/L) occurred at six hours. In this study, most of the astaxanthin was associated with the very low density lipoprotein, with some also associated with the HDL and LDL fractions. While this was an acute study, it will be important to study the distribution and metabolism of astaxanthin in chronic administration studies because this will more closely mimic the situation associated with the usual use of astaxanthin.

Summary

While not well known by many health-care providers at the present time, astaxanthin is a potent xanthophyll antioxidant that may have several advantages over other better-known carotenoids in a wide variety of disease states including neurodegenerative diseases, cancer, immune disorders, cardiovascular disease and visual health (Table 3). The ability of astaxanthin to scavenge a wide variety of free radicals and

other ROS, and its potency compared to other traditional, better-known antioxidants, makes it an attractive choice for future studies. Additionally, because astaxanthin appears to enter the central nervous system better than many other antioxidants, its utility in many central disorders should hold significant promise. To date there has been no toxicity reported with this potent antioxidant carotenoid.

REFERENCES

1. Acworth IN, McCabe DR, Maher TJ. The analysis of free radicals, their reaction products and antioxidants. In: Baskin S, Salem H, editors. Antioxidants, oxidants and free radicals. Washington DC: Taylor and Francis; 1997. p 23-77.
2. Tinkler JH, Bohm S, et al. Dietary carotenoids protect human cells from damage. *J Phytochem Phyto Biol* 1994;26:283-5.
3. Kurashige M, Okimasu E, et al. Inhibition of oxidative injury of biological membrane by astaxanthin. *Physiol Chem Phys Med NMR* 1990;22:27-38.
4. Beal MF. Oxidative damage in neurodegenerative diseases. *The Neuroscientist* 1997;3:21-7.
5. Krinsky N. The biological properties of carotenoids. *Pure Appl Chem* 1994;66:1003-6.
6. Weesie RJ. Resonance raman spectroscopy and quantum chemical modeling studies of protein-astaxanthin interactions in α -crustacyanin (major blue carotenoprotein complex in carapace of lobster homarus). *Biospectroscopy* 1999;5:358-70.
7. Miki W. Biological functions and activities of animal carotenoids. *Pure Appl Chem* 1991;63:141-61.
8. Palozza P, Krinsky NI. Astaxanthin and canthaxanthin are potent antioxidants in a membrane model. *Arch Biochem Biophys* 1992;297:291-5.
9. Shimidzu N, Goto M, Miki W. Carotenoids as singlet oxygen quenchers from marine organisms. *Fish Sci* 1996;62:134-7.
10. Di Mascio P, Murphy ME, Sies H. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Arch. Biochem. Biophys.* 1989;274:532-538
11. Chew BP, Park JS, et al. A comparison of the anticancer activities of dietary β -carotene, canthaxanthin and antaxanthin in mice in vivo. *Anticancer Res* 1999;19:1849-53.

Astaxanthin

Continuing Education Test Questions

Natural Healing Track

August 2000



Directions : Select your answer and check *one* best answer for each of the test questions. When you have answered all of the questions, please print or type all requested information and mail your completed test, along with processing fee, to the appropriate address listed below.

1. The highest concentration of astaxanthin based on weight is found in:
n a. flamingo meat.
n b. crustaceans such as lobsters.
n c. the algae *Haematococcus pluvialis*.
n d. sockeye salmon.
2. Reactive oxygen species (ROS):
n a. only react with lipids but not DNA and RNA.
n b. are only produced under anaerobic conditions.
n c. are slow to react.
n d. include hydrogen peroxide and hydroxyl radicals.
3. Astaxanthin:
n a. is classified as a carotenoid.
n b. is classified as a xanthophyll.
n c. has antioxidant properties.
n d. all of the above are correct.
4. Astaxanthin:
n a. can be synthesized by mammals.
n b. is used to provide color to farm-raised salmon.
n c. is a very water soluble compound.
n d. can be converted to vitamin A.
5. Which of the following has the greatest antioxidant capacity when tested *in vitro* in a lipid peroxidation assay?
n a. tocopherol (vitamin E.)
n b. β -carotene.
n c. lycopene.
n d. astaxanthin.
6. Excessive oxidative stress has been suggested to be linked to which of the following?
n a. aging
n b. cancer
n c. macular degeneration
n d. all of the above are correct
7. Astaxanthin treatment has been demonstrated to do which of the following?
n a. increase the bacterial load in animals inoculated with *H. pylori*
n b. decrease HDL lipoprotein levels
n c. increase LDL lipoprotein levels
n d. none of the above are correct
8. Carotenoids :
n a. may be useful in decreasing the incidence of prostate cancers.
n b. all cross the blood-brain barrier equally well.
n c. are all converted to vitamin A.
n d. are all equally safe for human consumption.
9. The responses in the immune system to astaxanthin treatment may include:
n a. a shifting from TH-1 responses (which are gastric irritation promoting) to TH-2 (which are gastric repairing).
n b. increased gastric inflammation.
n c. a thinning of the outer nuclear layer
n d. all of the above
10. Astaxanthin has been demonstrated to possess which of the following activities?
n a. anticarcinogenic
n b. protective against photic injury.
n c. cardioprotective.
n d. all of the above are correct.

For retailers:

n \$4 fee enclosed.*

Make check or money order payable to
New Hope Institute and mail to:

New Hope Institute of Retailing
1401 Pearl Street
Boulder, CO 80302

Each test with a score of 70% or higher is worth two credits toward your Certificate of Completion in Natural Healing.

*Please note change in processing fee.

Name: _____

Street address: _____

City/State/Zip: _____

Phone: _____

FAX: _____

Pharmacists should indicate state(s) in which CE credit is desired _____ Pharmacist License Number _____

For pharmacists:

n \$6 fee enclosed. Make check or money order payable to MCPHS and mail to:

Department of Continuing Education
Massachusetts College of Pharmacy and Health Sciences
179 Longwood Avenue
Boston, MA 02115

(Allow three weeks to process.)

This module is approved for 1 contact hour (.1 CEUs) of credit. MCPHS is approved by the American Council on Pharmaceutical Education as a provider of continuing pharmaceutical education.

ACPE #026-999-00-117-H01

Planned expiration date: August 2002

A statement of credit for 1 contact hour will be awarded upon achieving a passing grade of 70% or higher.

**Workshop on the Essentiality of and Dietary
Reference Intakes (DRIs) for
Omega-6 and Omega-3 Fatty Acids**

$\omega 6:\omega 3$

Program and Abstracts

The Cloisters

National Institutes of Health

Bethesda, Maryland, USA

April 7-9, 1999

Sponsored by:

National Institute on Alcohol Abuse and Alcoholism-NIH

Office of Dietary Supplements-NIH

The Center for Genetics, Nutrition and Health

International Society for the Study of Fatty Acids and Lipids

Astaxanthin

Continuing Education Test Questions

Natural Healing Track

August 2000



Directions : Select your answer and check *one* best answer for each of the test questions. When you have answered all of the questions, please print or type all requested information and mail your completed test, along with processing fee, to the appropriate address listed below.

1. The highest concentration of astaxanthin based on weight is found in:
 a. flamingo meat.
 b. crustaceans such as lobsters.
 c. the algae *Haematococcus pluvialis*.
 d. sockeye salmon.
2. Reactive oxygen species (ROS):
 a. only react with lipids but not DNA and RNA.
 b. are only produced under anaerobic conditions.
 c. are slow to react.
 d. include hydrogen peroxide and hydroxyl radicals.
3. Astaxanthin:
 a. is classified as a carotenoid.
 b. is classified as a xanthophyll.
 c. has antioxidant properties.
 d. all of the above are correct.
4. Astaxanthin:
 a. can be synthesized by mammals.
 b. is used to provide color to farm-raised salmon.
 c. is a very water soluble compound.
 d. can be converted to vitamin A.
5. Which of the following has the greatest antioxidant capacity when tested *in vitro* in a lipid peroxidation assay?
 a. tocopherol (vitamin E.)
 b. β -carotene.
 c. lycopene.
 d. astaxanthin.
6. Excessive oxidative stress has been suggested to be linked to which of the following?
 a. aging
 b. cancer
 c. macular degeneration
 d. all of the above are correct
7. Astaxanthin treatment has been demonstrated to do which of the following?
 a. increase the bacterial load in animals inoculated with *H. pylori*
 b. decrease HDL lipoprotein levels
 c. increase LDL lipoprotein levels
 d. none of the above are correct
8. Carotenoids :
 a. may be useful in decreasing the incidence of prostate cancers.
 b. all cross the blood-brain barrier equally well.
 c. are all converted to vitamin A.
 d. are all equally safe for human consumption.
9. The responses in the immune system to astaxanthin treatment may include:
 a. a shifting from TH-1 responses (which are gastric irritation promoting) to TH-2 (which are gastric repairing).
 b. increased gastric inflammation.
 c. a thinning of the outer nuclear layer
 d. all of the above
10. Astaxanthin has been demonstrated to possess which of the following activities?
 a. anticarcinogenic
 b. protective against photic injury.
 c. cardioprotective.
 d. all of the above are correct.

For retailers:

\$4 fee enclosed.*

Make check or money order payable to
New Hope Institute and mail to:

New Hope Institute of Retailing
1401 Pearl Street
Boulder, CO 80302

Each test with a score of 70% or higher is worth two credits toward your Certificate of Completion in Natural Healing.

*Please note change in processing fee.

Name: _____

Street address: _____

City/State/Zip: _____

Phone: _____ FAX: _____

Pharmacists should indicate state(s) in which CE credit is desired _____ Pharmacist License Number _____

For pharmacists:

\$6 fee enclosed. Make check or money order payable to MCPHS and mail to:

Department of Continuing Education
Massachusetts College of Pharmacy and Health Sciences
179 Longwood Avenue
Boston, MA 02115

(Allow three weeks to process.)

This module is approved for 1 contact hour (.1 CEUs) of credit. MCPHS is approved by the American Council on Pharmaceutical Education as a provider of continuing pharmaceutical education.

ACPE #026-999-00-117-H01

Planned expiration date: August 2002

A statement of credit for 1 contact hour will be awarded upon achieving a passing grade of 70% or higher.

National Institute of Child Health and Human Development

Workshop on the Essentiality of and Dietary Reference Intakes (DRIs) for Omega-6 and Omega-3 Fatty Acids, The Cloisters, National Institutes of Health, Bethesda, MD, USA

April 7-9, 1999

Background

Following the 3rd Congress of the International Society for the Study of Fatty Acids and Lipids (ISSFAL) in Lyon, France, June 1-5, 1998, the ISSFAL Board of Directors agreed to convene a workshop on the essentiality of and DRIs for omega-6 and omega-3 fatty acids. An international group of experts will present reviews and new data in a round table format with ample time left for discussion. The participants will include speakers and discussants from the National Institutes of Health, other government agencies, academia, industry, non-profit organizations, the World Health Organization, the Food and Agriculture Organization, and the Food and Nutrition Board.

Venue

The Workshop will be held in the Mary Woodard Lasker Center for Health Research & Education (The Cloisters, Building 60) at the National Institutes of Health in Bethesda, Maryland, USA.

Conference Secretariat

The Center for Genetics, Nutrition and Health, 2001 S Street, NW, Suite 530, Washington, D.C. 20009 USA, phone: (202) 462-5062, fax: (202) 462-5241, e-mail: cgnh@bellatlantic.net.

Hotel Accommodations

We have selected *The Bethesda Ramada* in Bethesda, Maryland, as our hotel [8400 Wisconsin Avenue, phone: (301) 654-1000], since it is within walking distance of the National Institutes of Health (NIH). Parking on the campus of the NIH is very limited. The *Medical Center* Metro stop (Red Line) is on the NIH campus.

Conference Cochairs

Artemis P. Simopoulos, M.D. (USA)

Norman Salem, Jr., Ph.D. (USA)

Alexander Leaf, M.D. (USA)

Sponsors

National Institute on Alcohol Abuse and Alcoholism-NIH

Office of Dietary Supplements-NIH

The Center for Genetics, Nutrition and Health

International Society for the Study of Fatty Acids and Lipids

National Institute of Child Health and Human Development

Cosponsors

BASF Corp., USA

BASF Health & Nutrition A/S

Bestfoods

ENRECO

F. Hoffmann-La Roche, Ltd.

Groupe Danone

Kraft Foods, Inc.

Martek Biosciences Corporation

Mead Johnson Nutritionals

Ocean Nutrition Canada, Ltd.

OmegaTech, Inc.

Pronova Biocare

Roche Vitamins, Inc.

**Workshop on the Essentiality of and Dietary Reference Intakes (DRIs) for Omega-6
and Omega-3 Fatty Acids**

National Institutes of Health, The Cloisters

April 7-9, 1999

WEDNESDAY, APRIL 7, 1999

Welcoming Remarks - Enoch Gordis, M.D., NIAAA-NIH

Why is this so? If their dietary absence was associated with more obvious clinical symptoms, there is no doubt there might have been an omega 3 champion. The rather subtle effects of the dietary absence (or low intakes) makes it hard to sell to the general public. For example, we know that effects of deficiency on the electroretinogram (ERG) amount to a loss of a- and b-wave amplitudes of say 30% with perhaps other more substantial losses in sub-components, however we cannot yet say what this might mean in terms of "vision" which is what the public relate to. Perhaps, we too often ignore the fact that in the EFA field there are substitute fatty acids which prevent complete absences of say 22 carbon PUFA in the retina (e.g. 22:5n-6 or 22:3n-9 substitute for 22:6n-3 in omega 3 and EFA deficiency, respectively). This argues for the importance of these types of PUFA in this tissue, however necessarily the availability of such substitutes reduces the physiological impact of a dietary deficiency. Perhaps, we should be looking for a tissue where it is possible to alter the DHA content without the substitute PUFA being present. Such a tissue is the guinea pig heart - with an ALA rich diet the level of DHA is less than 1% of the phospholipid fatty acids and it is only on the inclusion of DHA that the heart DHA level rises. Given the sound data showing the crucial role of omega 3 PUFA and DHA, in particular, on cardiac function in other species/situations, surely this tissue in this species might be a useful research tool.

In the early years, linoleic acid had a prominent role as an anti-cholesterol fatty acid, however since the 1970s the omega 3 PUFA have made a comeback in heart disease, vision and other diverse areas such as arthritis, bone development and neurological disorders. Where we currently stand is that we have much data on diet and the effect on tissue fatty acids, but relatively few data on exact intakes titrated against physiological function. This is especially true in the omega 3 and electroretinography field which was the first area where omega 3 PUFA (ALA!) were shown to have a specific physiological role. Furthermore, much of our research could be criticized because there are few studies where pure ALA has been used. It is surely no longer adequate to compare oil A (poor in ALA) with oil B (containing ALA) because of our ever increasing understanding of the potential actions of the many compounds found in the unsaponifiable fraction of

naturally-occurring oils. This highlights the need for pure ALA for research purposes.

Finally, we might be responsible for diluting the message of essentiality for the omega 3 PUFA because of the many arguments in house and in the public arena regarding the nutritional importance of ALA versus the long chain PUFA (EPA and DHA). I think it is instructive to recall that the research data show that ERG function is optimal in all animal models with dietary ALA and not dietary DHA and that the de Lorgeril and Singh data on secondary prevention indicate a role for ALA in the cardiac area.

An Evolutionary View of Dietary Recommendations

S. Boyd Eaton, M.D.

Emory University, Atlanta, Georgia, USA

Traditional Research - satisfactory for preventing classical deficiency

syndromes; less so for reducing chronic degenerative disease risk:

1. Clinical trials - generally focus on diagnosis or treatment, not prevention.

2. Mechanistic studies - limitless possible study subjects; limited funding, facilities, and investigators.
3. Epidemiology - conflicting results emphasized by media leading to public confusion and skepticism. Examples:
 - a. vitamin E, β carotene and lung cancer
 - b. fat and coronary heart disease
 - c. fiber and colon cancer
 - d. salt and overall mortality
 - e. calcium and osteoporosis
 - f. fat and breast cancer

Needed: Additional Approach - to focus future efforts, reconcile past investigative discrepancies, and provide solid theoretical basis for entire field. Viewing nutrition from the perspective of human evolutionary experience might achieve these ends.

Evolution and Nutrition

1. Basic Premise - current humans are genetic Stone Agers; cultural change since agriculture has exceeded capacity of genetic evolution to keep pace.
2. Essential goal - determine character of human nutrition during Stone Age experience.
3. Investigative approaches:
 - a. analysis of recent forager subsistence patterns
 - b. analysis of human skeletal remains - gross anatomy and radioisotopic
 - c. archaeological finds - animal remains, botanical residues, implements
 - d. nutritional analyses of game animals and wild plant foods - similar to those available before agriculture

4. Modeling

$$A(C^aX) + V(C^vX) = \text{daily energy intake}$$

A and V - mean energy content (kcal/g) of animal and vegetable foods

C^a and C^v - proportions of animal and vegetable foods, respectively

X - total number of grams of food required to provide daily energy

5. Previously reported results:

a. Protein: 30-35% total energy

b. Carbohydrate: 40-50%

c. Fat: 20-25%

Recently Revised Model Inputs

1. Hunter-gatherer subsistence patterns

a. old mean: 35% animal : 65% plant (by weight)

b. revised mean: 45% animal : 55% plant

2. Improved assessment of game nutritional properties

a. old view - based solely on muscle meats (i.e. "selected cuts")

b. new view - hunter-gatherers actually consume "total edible," hence fat content is 1.5 - 18%, not 1 - 5%.

New Estimates:

Mean macronutrient contribution (% total energy)

a. Protein 30-33%

b. Carbohydrate 31-34%

c. Fat 36%

General Fat Characteristics:

1. Energy contribution similar to Mediterranean (and current American)

pattern; unlike East Asian paradigm.

2. But character of fat is much different from U.S. pattern:

Claudio Galli, M.D., President, ISSFAL

Session I. Principles to be Considered in Determining Essentiality and DRIs

Cochairs: Artemis P. Simopoulos, M.D.

Harald S. Hansen, Ph.D., D.Sc.

9:00 - 9:30 a.m. *Criteria for Determining Essentiality and Standards for DRIs*

Vernon R. Young, Ph.D., D.Sc.

9:30 - 10:00 a.m. *Essentiality of Omega-3 Fatty Acids*

Arthur A. Spector, M.D.

10:00 - 10:30 a.m. *Defining the Omega-3 Status in Mammals*

Andrew J. Sinclair, Ph.D.

10:30 - 11:00 a.m. Coffee Break

11:00 - 11:30 a.m. *An Evolutionary View of Diet Recommendations*

S. Boyd Eaton, M.D.

11:30 - 12:30 p.m. Discussant and General Discussion

Harald S. Hansen, Ph.D., D.Sc.

12:30 - 2:00 p.m. Lunch

Session II: Essential Fatty Acids and Central Nervous System Function

Cochairs: Norman Salem, Jr., Ph.D.

William C. Heird, M.D.

2:00 - 2:30 p.m. *Evidence for the Essential Nature of DHA for the Human and Rat Nervous System*

Norman Salem, Jr., Ph.D.

2:30 - 3:00 p.m. *DHA Supplementation of Breastfeeding Mothers: Effects on Maternal Plasma and Milk Fatty Acids. Infant Plasma Fatty Acids. Infant Visual*

Function and Infant Neurodevelopmental Status

William C. Heird, M.D.

3:00 - 3:30 p.m. *Functional Basis for the Importance of Omega-3 Fatty Acids in Retinal and CNS Development*

Martha Neuringer, Ph.D.

3:30 - 4:00 p.m. *Long Chain Polyunsaturates and Human Visual Development*

Eileen Birch, Ph.D.

4:00 - 4:30 p.m. Coffee Break

4:30 - 5:00 p.m. *The Effects of DHA on Hostility*

Tomohito Hamazaki, M.D., Ph.D.

5:00 - 5:30 p.m. *Omega-3 Fatty Acids in Mood Disorders*

Andrew L. Stoll, M.D.

5:30 - 6:30 p.m. Discussants and General Discussion

Peter Willatts, Ph.D.

Joseph Hibbeln, M.D.

7:30 - 10:00 p.m. Dinner at the Bethesda Ramada

THURSDAY, APRIL 8, 1999

Session III. Cardiovascular Disease

Cochairs: Alexander Leaf, M.D.

Raffaele De Caterina, M.D., Ph.D.

9:00 - 10:00 a.m. *Polyunsaturated Fatty Acids and Cardiovascular Disease*

Alexander Leaf, M.D.

10:00 - 10:30 a.m. *n-3 Polynsaturated Fatty Acids Inhibit COX-2 Expression*

Raffaele De Caterina, M.D., Ph.D.

10:30 - 11:00 a.m. Coffee Break

11:00 - 11:30 a.m. *Alpha-Linolenic Acid in the Prevention of Cardiovascular Disease*

Serge Renaud, M.D.

11:30 - 12:00 p.m. *Omega-3 Long Chain PUFA and Triglyceride Lowering: Minimum Effective Intakes*

William S. Harris, Ph.D.

12:00 - 12:30 p.m. *Efficacy of n-3 PUFA and vitamin E in 11,324 post-MI patients:*

Results of GISSI-PREVENZIONE

Roberto Marchioli, M.D.

12:30 - 1:00 p.m. Discussant and General Discussion

William E. Lands, Ph.D.

1:00 - 2:00 p.m. Lunch

Session IV: Relationship of Essential Fatty Acids to Saturated, Monounsaturated, and Trans Fatty Acids

Cochairs: Claudio Galli, M.D.

Andrew J. Sinclair, Ph.D.

2:00 - 2:30 p.m. *Relationships Between Saturated, Monounsaturated, Polyunsaturated*

Fatty Acids: Dietary Data vs. Data from Plasma Fatty Acid and

Lipid Analyses

Claudio Galli, M.D.

Omega-3 Fatty Acids

Herbert D. Woolf, Ph.D.

9:52 - 10:04 a.m. *Essential Fatty Acids and the Products of the Groupe Danone for Human Nutrition*

Dominique Lanzmann-Petithory, M.D.

10:04 - 10:16 a.m. *Advantages and Disadvantages of the Use of Flax Seed as a Source of Omega-3*

Paul A. Stitt, Ph.D.

10:16 - 10:28 a.m. *Omega-3 LC-PUFA ñ from a Health Concept to Foods in the Shelves*

Reto Muggli, Ph.D.

10:28 - 10:40 a.m. *Infant Formulas with no DHA or ARA.. Are They Causing Harm?*

David J. Kyle, Ph.D.

10:40 - 11:00 a.m. Coffee Break

11:00 - 11:12 a.m. *Clinical Safety Studies of LCPUFA Supplementation of Premature and Term Infant Formulas*

James W. Hansen, M.D., Ph.D.

11:12 - 11:24 a.m. *Omega-3 Long Chain PUFA ñ Closing the Nutritional Gap*

Jacques Boudreau

11:24 - 11:36 a.m. *OmegaTech, Inc.*

William R. Barclay, Ph.D.

11:36 - 11:48 a.m. *Safety of Omega-3 Products Based on Fish Oil as Starting Material*

Bjorn Rene

11:48 - 12:00 p.m. Other

12:00 - 1:00 p.m. Discussants and General Discussion

2:30 - 3:00 p.m. *Nutritional and Metabolic Interrelationships Between Omega-3 Fatty Acids and Trans Fatty Acids*

Bruce J. Holub, Ph.D.

3:00 - 3:30 p.m. Coffee Break

3:30 - 4:00 p.m. *Choice of Monounsaturated, Trans and Omega-3 Fatty Acid-Rich Oils for the Prevention of Excessive Linoleic Acid Syndrome*

Harumi Okuyama, M.D.

4:00 - 5:00 p.m. Discussion

FRIDAY, APRIL 9, 1999

Session V. Dietary Recommendations and Omega-6:Omega-3 Ratio (LA, LNA, AA, EPA, DHA)

Cochairs: Peter R.C. Howe, Ph.D.

Bruce J. Holub, Ph.D.

9:00 - 9:20 a.m. *Intakes of Dietary Fatty Acid in the United States: Results from the USDA's 1994-1996 Continuing Survey of Food Intakes by Individuals*

Gary J. Nelson, Ph.D.

9:20 - 9:30 a.m. *World Health Organization/Pan American Health Organization (Status of EFA Worldwide)*

Manuel Peña, M.D.

9:30 - 9:40 a.m. *n-3 Fatty Acids: Food Supply, Food Composition and Food Consumption Data*

William D. Clay, Ph.D.

9:40 - 9:52 a.m. *BASF's Approach to Commercialization of Long Chain*

Bruce Holub, Ph.D.

Rebecca Costello, Ph.D.

1:00 - 2:00 p.m. Lunch

Session VII. Conclusions and Recommendations

Cochairs: Alexander Leaf, M.D.

Artemis P. Simopoulos, M.D.

2:00 - 5:00 p.m. *Roundtable Discussion*

ABSTRACTS

Wednesday, April 7, 1999

Session I. Principles to be Considered in Determining Essentiality and DRIs

Criteria for Determining Essentiality and Standards for DRIs

Vernon R Young, Ph.D., D.Sc.

Massachusetts Institute of Technology, Cambridge, MA 02139, USA

This introductory presentation to the workshop will begin with an initial, brief statement about the importance of knowledge on the quantitative needs for nutrients and the multiple uses of nutrient-based dietary reference values. From this introduction we will turn to (i) a consideration of the evolving conceptual and factual basis underlying the "essentiality" of nutrients and (ii) the definition and description of dietary reference intakes (DRIs). The latter include (following the structure proposed and applied recently by the US Food and Nutrition Board/Institute of Medicine/National Academy of Sciences):- Estimated Average Requirement (EAR); Recommended Dietary Allowance (RDA); Adequate Intake(AI) and Upper Tolerable Level (UL). The most useful DRI is the EAR, the reasons for which will be examined. Then a detailed discussion will follow with respect to the establishment of DRIs, including an emphasis on (a) the choice of the criterion (criteria) of nutrient adequacy chosen to establish a specific DRI and (b) the approach(es) that might be taken and data that are desirable to achieve this goal. The importance of seeking a congruence of evidence, where this is possible, in arriving at a DRI will be emphasized, by example. Finally some suggestions will be made with respect to the setting of DRIs for omega-3 and omega-6 fatty acids.

Essentiality of Omega-3 Fatty Acids

Arthur A. Speciani, M.D.

Department of Biochemistry, University of Iowa College of Medicine,

Iowa City, Iowa 52242, USA

There is a growing consensus that omega-3 fatty acids are essential nutrients for humans. Much of the evidence is based on physiological measurements such as neurological development and visual acuity. To better understand why this class of polyunsaturated fatty acids is required, we must

determine the biochemical basis for the essentiality. Of the eight fatty acids that comprise the

omega-3 metabolic pathway, the two that are most likely to have essential biochemical functions are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

EPA can be converted to prostaglandins, thromboxanes and lipoxygenase products. However, no

essential role for these EPA-metabolites has been reported, and it seems unlikely that the formation of these products is the reason that omega-3 fatty acids are essential. When elevated amounts of EPA are available, the incorporation of arachidonic acid (AA) into cell phospholipids and its conversion to eicosanoid mediators is reduced. Thus, EPA acts as a competitive inhibitor of AA, and this probably accounts for some of the beneficial effects of omega-3 fatty acids in the treatment of cardiovascular and inflammatory diseases. While the possibility that EPA is essential in order to modulate the effects of AA cannot be ruled out, the amounts ordinarily present in the plasma and tissues probably are too low to competitively inhibit the actions of AA. Therefore, modulation of AA metabolism is more likely to be a pharmacological effect of omega-3 fatty acid supplements rather than an essential physiological function.

The basis for considering DHA as the biochemically essential omega-3 component is much more

compelling. DHA is the most abundant omega-3 fatty acid in most tissues, and it is present in large amounts in the brain and retina. DHA is the omega-3 fatty acid required for normal development of the nervous system and optimum visual acuity. Furthermore, when an omega-3 fatty acid deficiency exists, the body compensates by replacing it with the corresponding fatty acid of the omega-6 series, omega-6 docosapentaenoic acid (DPAn-6). These findings strongly suggest that DHA has an essential biochemical function. The most likely possibility is a membrane structural effect involving the packing of phospholipid head groups or the interaction of the lipid domains with membrane proteins. The lipids that contain the highest percentages of DHA are ethanolamine plasmalogen, phosphatidylethanolamine and phosphatidylserine. Therefore, it is likely that the function of DHA involves the metabolism, trafficking or physical properties of these phospholipids. Other possibilities that must be considered include the conversion of DHA to a lipid mediator, binding of DHA to a nuclear receptor that regulates gene expression, or formation of a DHA-centered free radical.

A central question concerning the essentiality of omega-3 fatty acids is why DHA rather than the

corresponding member of the omega-6 series, DPAn-6, fulfills this purpose. The usual Western diet contains 10- to 20-times more omega-6 fatty acid, and the same metabolic pathway is utilized by both fatty acid classes. One possibility is that DHA is utilized more efficiently than DPAn-6.

However, studies with neural cells in culture indicate that there is no appreciable difference in the uptake, retention or incorporation into phospholipids of DHA as compared with DPAn-6. While more detailed measurements may reveal a functional difference between DHA and DPAn-6, no

such evidence is currently available. This suggests that DHA is utilized rather than DPAn-6 because it is more available to the tissues. Although the absolute amounts of these fatty acids in the plasma lipids are very small, there ordinarily is about five-times more DHA than DPAn-6. Furthermore, the main product formed by cultured astrocytes from omega-3 fatty acid precursors is DHA, whereas the main omega-6 product is AA. Astrocytes are the site where most of the polyunsaturated fatty acid precursors are elongated and desaturated in the brain. Thus, much more DHA than DPAn-6 appears to be available in the central nervous system.

These findings suggest the following hypothesis regarding the essentiality of omega-3 fatty acids.

Certain tissues, especially parts of the central nervous system, require a relatively large amount of a 22-carbon polyunsaturated fatty containing a 4,5-double bond for optimum function. The omega-6 metabolic pathway cannot satisfy this requirement because it operates primarily to produce AA for eicosanoid and inositol phospholipid synthesis. While some docosatetraenoic acid (22:4n-6) is made, it is primarily retroconverted to AA rather than proceeding down the pathway to form DPAn-6. Therefore, even though more omega-6 fatty acid precursors are available, the omega-6 pathway cannot produce enough DPAn-6 to satisfy tissue requirements. By contrast, the main product of the omega-3 pathway is DHA, not the 20-carbon intermediate. This fundamental

difference in the operation of the polyunsaturated fatty acid metabolic pathway is likely to be the biochemical reason why omega-3 fatty acids are essential.

(Supported by NIH grants HL49264 and CA66081)

Defining the omega 3 status in mammals

AJ Sinclair, Ph.D.

Department of Food Science, RMIT University, Melbourne, Victoria, Australia 3001

This talk examines the status of omega 3 polyunsaturated fatty acids (PUFA) as essential nutrients in mammals. The first issue to be addressed is the importance of having a surrogate champion which promotes the cause of a nutrient on a daily basis. The question, *what is your cholesterol level*, is a message which sustains the cholesterol-heart disease story. Clearly, despite the importance of anti-oxidants, fibre, folate and anti-platelet therapy in CHD, cholesterol has been a survivor. Do the omega 3 PUFA have such a champion? In other words, do we have the data to support the importance of these essential nutrients; the existence of the MRFIT data and the more recent secondary prevention data from France and India provide strong support for the essentiality of the omega 3 PUFA.

The history of the EFA reveals that the omega 3 PUFA were ignored by most for 40 years or more.

Paleolithic U.S.

% saturated less more

% C18 more less

% C14 + C16 less more

% monounsaturated more less

% polyunsaturated more less

% C20 + C22 more less

$\omega 6$: $\omega 3$ lower higher

New Inputs Alter Essential Fatty Acid Retrojections:

Paleolithic Current

1998 Estimate 1999 Estimate American

Total C20 + C22 3.01 g/d 5.79 g/d 0.80 g/d

AA : $\omega 3$ LCP 1.68 1.43 5.6

Overall $\omega 6$: $\omega 3$ 0.79 1.39 > 10.0

Wednesday, April 7, 1999

Session II. Essential Fatty Acids and Central Nervous System Function

Evidence for the Essential Nature of DHA in the Human and Rat Nervous System

Norman Salem Jr., Ph.D., Rebecca Greiner, Toru Moriguchi,

Jim Woods, Patricia Mena, and Ricardo Uauy

Laboratory of Membrane Biochemistry & Biophysics, NIAAA, National Institutes of Health, Rockville, MD, USA and the Institute of Nutrition and Food Technology, University of Chile, Santiago, Chile

A series of experiments were performed which demonstrate that diets that are low in n-3 fatty acids lead to low brain DHA and also lead to losses in nervous system function. Diets were constructed that varied only in the amount of alpha-linolenic acid intake derived from flax oil and adequate in linoleic

acid derived from safflower oil. No long chain (20C or more) polyunsaturates were present in these diets. Rats were raised for three generations on these diets and animals were tested at adulthood in the second and third generation. Brain and retinal DHA was markedly depressed in the second and third generations with increases in the long chain n-6 polyunsaturates, especially docosapentaenoate (22:5n6). Accompanying this "reciprocal replacement" of DHA were significant losses in performance on behavioral tasks related to learning and memory. The n-3 deficient rats acquired an olfactory discrimination task more slowly and made significantly more errors. This was significant as it extends the constellation of deficits described in n-3 deficiency to another sensory modality in addition to vision. In addition, n-3 deficient rats showed delayed escape latency in the Morris Water Maze task. This was more pronounced in the third generation where the DHA deficit was slightly greater relative to the second generation. Motor activity was not significantly different between groups. The swimming speed and distance traveled was greater for the n-3 deficient animals, yet they took a longer time to find the platform. In a subsequent memory test with the platform removed, the deficient animals made fewer crossings of the former position of the platform indicating that the n-3 adequate group better retained the memory of the position of the platform. This effect was particularly pronounced in the third generation. These experiments show that there are functional deficits associated with low brain DHA that may relate to sensory function, but it is more likely that they are due to losses in higher level functions related to information processing in the brain that are necessary for memory and learning.

In the second series of experiments, the focus was on the level of alpha-linolenate necessary to support nervous system DHA levels. An artificial rearing system was used to control the EFA content of rat pup diet from day 5-18 of life. At weaning ratios of linoleate to alpha-linolenate of 10:1 and even 1:1 did not produce the same level of brain DHA as a 1:12 ratio or that of dam-reared pups whose mothers were fed a diet containing 1.1% DHA as well as other LCPs, i.e., were well nourished. However, the 1:12 ratio led to a decrease in brain AA while the 10:1 ratio led to a slight increase over the dam-reared level. There was a similar picture in the retina, with the exception that even the extreme case of LA/LNA of 1:12 did not support the same level of retinal DHA as that of dam-reared animals. The high LNA diet (1:12) again led to a significant decrease in retinal AA. Thus it appears that increasing the level of alpha-linolenic acid in developing mammals is not an entirely adequate solution to the problem of supporting the neural DHA at a level comparable to that of a well nourished maternal reared individual. Raising the n-3 content to a 1:1 level does support a balanced EFA composition of the nervous system to a much greater extent than the 10:1 ratio, a ratio that is more typical of human infant formulas in North America.

The third issue to be addressed is the applicability of these studies to humans. Essential fatty acid metabolism was assessed *in vivo* in adults and in infants of various gestational ages and birth weights. A controlled trial in adults demonstrated conclusively that linoleic acid is converted to arachidonate and alpha-linolenate is converted to DHA. The rates of the n-6 metabolism appear faster than the n-3 conversions, in contrast to some previous findings. Increased levels of n-3 fatty acids associated with a fish-poultry based diet led to decreases in deuterium incorporation in DHA. Smoking and alcohol intake were associated with increased deuterium incorporation into DHA from linolenate. Infants are capable of LA to AA and LNA to DHA conversion *in vivo* within the first week of life even when born very prematurely (e.g., 1 kg BW). In fact, it was surprising that there was an inverse correlation of deuterium enrichment of DHA with gestational age. Although it is clear that premature and term infants express EFA metabolic activity, it must be understood that these are trace level studies; the metabolic activity towards DHA in particular is very limited and unlikely to be adequate to support rapid brain and organ DHA accretion during the first months of life.

Docosahexaenoic Acid (DHA) Supplementation of Breastfeeding Women: Effects on Maternal Plasma and Milk Fatty Acids, Infant Plasma Fatty Acids, Infant Visual and Neurodevelopmental Function and Indices of Maternal Depression

Craig L Jensen, Antolin M Llorente, Robert G Voigt, Thomas C Prager, J K Fraley,

Yali L Zou, Marcia C Berretta and William C Heird, M.D.

Children's Nutrition Research Center, Department of Pediatrics,

Baylor College of Medicine, Houston, Texas, USA

DHA, an important component of the structural lipids of brain and retina, is present in human milk but not in formulas currently available in the United States and it has been suggested that the better visual and cognitive development of breastfed infants is due, at least in part, to the presence of DHA in human milk. However, the DHA content of the milk of U.S. women, which is dependent on maternal plasma lipid DHA and, hence, intake of α -linolenic acid and/or DHA, is less than that of many other populations. Further, the DHA content of maternal plasma lipids decreases during lactation. Thus, it has been suggested that breastfeeding women and their infants might benefit from maternal DHA supplementation. Indeed, we and others have shown that maternal DHA supplementation prevents the usual decline in maternal plasma lipid DHA content and increases the DHA content of maternal plasma as well as that of milk and the recipient infants' plasma phospholipid. Based on these data, we hypothesized that maternal DHA supplementation also would result in better visual and neurodevelopmental status of the recipient infants and lessen the incidence of maternal depression which, in epidemiological studies appears to be higher in populations with low DHA intake.

To test these hypotheses, women were assigned randomly and blindly to receive either ~200 mg of DHA daily (n=80) or a placebo (n=65) for 120 days after delivery. Visual function of infants was assessed by transient visual evoked potentials (VEP) and visual acuity was measured by sweep VEP and the Teller Acuity Card Procedure at 4 and 8 months of age. Infant neurodevelopmental status at 12 months of age was assessed by the Clinical Adaptive Test/Clinical Linguistic and Auditory Milestone Scale (CAT/CLAMS) and the Gesell Gross Motor Developmental Quotient (GM DQ). Maternal depression was assessed by the Beck Depression Inventory (BDI), the Edinburgh Postnatal Depression Scale (EPDS) and the Structured Clinical Interview for Depression (SCID).

There were no differences at either 4 or 8 months in VEP latency, VEP amplitude, sweep VEP acuity or Teller acuity between groups whose mothers did or did not receive DHA. There also were no statistically significant differences in mean CAT (111.2 ± 11.0 vs. 107.3 ± 9.3) or CLAMS (101.5 ± 16.0 vs. 100.9 ± 13.9) scores of infants whose mothers did or did not receive DHA; however, the mean GM DQ of infants whose mothers received DHA was significantly greater than that of infants of mothers who did not (102.6 ± 13.3 vs. 95.2 ± 12.7 ; $p=0.03$). The incidence of postpartum depression as assessed by BDI, EPDS or SCID did not differ between groups and was lower than expected in both groups.

We conclude that maternal DHA supplementation maintains or increases the DHA content of maternal plasma lipid and increases the DHA content of both maternal milk and the lipids of infant plasma. However, in this study, these positive effects of maternal DHA supplementation were not

accompanied by better visual function, visual-motor problem-solving ability or language development of the recipient infant and also did not affect the incidence of maternal depression. On the other hand, maternal DHA supplementation resulted in the recipient infants having somewhat better indices of motor development at 12 months of age. These data, therefore, do not support our hypothesis that maternal DHA supplementation improves visual and neurodevelopmental status of the recipient infant or lessens the incidence of maternal depression. They provide little support for the concept that breastfeeding mothers require supplemental DHA.

Functional Basis for the Importance of Omega-3 Fatty Acids

in Retinal and CNS Development

Martha Neuringer, Ph.D.

Department of Medicine and Ophthalmology,

Oregon Health Sciences University, Beaverton, Oregon, USA

Infants fed standard infant formulas lacking DHA have low blood and tissue levels of DHA compared with those receiving pre-formed DHA or human milk. Whether dietary intake of omega-3 fatty acids has a substantial impact on CNS levels depends on the infant's stage of development and prior nutritional status. Preterm infants, who are at an earlier stage of brain development and of DHA accretion, are at greater risk than term infants of failing to achieve normal DHA levels in the retina and nervous system. However, animal studies have shown that low tissue levels are rapidly corrected once a dietary supply becomes available and, once incorporated into neural tissue, DHA is tenaciously retained. Therefore CNS levels in older children or adults are unlikely to be altered significantly by low dietary intake of omega-3 fatty acids.

For the purpose of defining essentiality, the more important question is whether a difference in fatty acid status during development is related to functional deficits. The most consistent effects of omega-3 fatty acid deficiency and supplementation have been on measures of visual system function. In monkeys and in preterm human infants, diets low in DHA's precursor, alpha linolenic acid, lead to poorer development of both visual acuity and the electroretinogram, a measure of retinal physiology. Furthermore, supplementation with pre-formed dietary DHA has been associated with enhanced visual acuity development in most studies of preterm infants and in some, but not all, of term infants.

It is assumed that these effects are mediated by differences in the fatty acid composition, and particularly the DHA content, of retinal and neural membranes. However, the underlying mechanisms for these effects, and the critical site(s) for these effects within the nervous system, are not clearly understood. Changes in the electroretinogram, which specifically measures retinal function, are hypothesized to be the result of changes in the biophysical properties of photoreceptor outer segment membranes, the site for the absorption of photons and their transformation into neural signals. These membranes contain the body's highest levels of DHA. Differences in visual acuity development, on the other hand, may be due to changes within photoreceptor membranes, other elements within the retina, the central visual pathway, and/or the visual cortex. Possible mechanisms include alterations in the development of the fovea, changes in retinal sensitivity, or changes in the synaptic connectivity or activity of the visual cortex.

Studies of DHA supplementation in human infants have reported differences in visual acuity primarily during the first few postnatal months and in one major study at one year of age. The longer-term implications of these differences in infant acuity still are unclear, due to the lack of studies with more extended follow-up.

However, it is known that restriction of visual input during early development can lead to lasting effects on visual function, so it will be important to examine this issue more closely.

Differences in visual development are of interest not only in their own right, but also because they may reflect a more general effect on neural, and perhaps cortical, maturation. Studies reporting an advantage in intellectual development in breast-fed compared with formula-fed infants have prompted speculation that the DHA present in breast milk is a critical factor. However, the difference in DHA content is confounded with many other compositional differences, as well as socioeconomic and parenting factors which are known to strongly influence intellectual development.

In monkey studies and in randomized human clinical trials, differences have consistently been found in one aspect of cognitive development, visual attention. In monkey infants fed low levels of alpha linolenic acid, and preterm human infants fed formulas without DHA, the duration of fixations to visual stimuli are prolonged compared to infants with higher DHA status. Developmental psychologists have interpreted increased look duration as indicating slower speed of processing the stimulus and encoding it into memory. It is also possible that this effect reflects a specific difficulty in shifting or disengaging attention, an ability which develops during the first postnatal year, or a difference in the intensity of the infants' responses to visual stimuli. This effect appears to be independent of effects on visual acuity, as the two outcomes are not correlated in either monkey or human infants. Longer look durations are moderately correlated with poorer achievement in later tests of cognitive development, including IQ tests at school age. Thus, as with the effects on visual acuity, the implications of this difference for later development are unclear but worthy of further study.

Both animal and human studies of the effects of omega-3 fatty acid status on behavioral development have focussed on possible changes in cognition and learning. Other aspects of behavior generally have not been examined but are of equal interest. There are good rationales to hypothesize effects of omega-3 fatty acid status on, for example, sleep and temperament. Preliminary findings in rhesus monkeys indicate changes in both sleep and responsiveness to environmental stimuli. Changes in eicosanoids or in neurotransmitter metabolism provide plausible mechanisms for such effects.

The range of functional effects of omega-3 fatty acid deficiency and supplementation during development and their impact on later vision, cognition and behavior are not completely understood, nor are the relationship of these effects to the dose of dietary DHA and the age and duration of dietary intervention. These issues can only be resolved by longer-term studies with a range of dietary treatments and functional outcomes.

Long Chain Polyunsaturates and Human Visual Development

Eileen Birch, Ph.D.

Retina Foundation of the Southwest

University of Texas Southwestern Medical Center, Texas, USA

The Effects of DHA on Hostility

Tomohito Hamazaki, M.D., Ph.D.

Department of Clinical Application, Institute of Natural Medicine,

Toyama Medical and Pharmaceutical University, Toyama-shi, Toyama 930-0194, Japan

Numbers of studies have indicated an association between Type A behavior pattern (TABP) and CHD. TABP is characterized by aggression, hostility, excessive competitive drive, and time urgency. Because TABP is a vague and complex mixture of behavior patterns, many researchers began to investigate components of the TABP construct. Hostility is the most popular factor among them. Actually it better predicts important adult diseases than TABP.

We have been investigating the effects of DHA on extraggression (aggression against others, EA) of students using P-F study originally created by Rosenzweig. In P-F study testees are asked to give comments to frustrating pictures. Those comments are judged if they are aggressive against others (EA), self or nobody. EA contains three categories: obstacle-dominance, ego-defense

(extrapunitive) and need-persistence. Comments are judged as ego-defensive, if comments contain hostile words to others, or aggressive denial or rejection against others' reproach or accusation. Thus, we regarded ego-defense as hostility in the following studies.

According to our previous three-month double-blind study, hostility was enhanced by final exams in control students, whose average intakes of DHA were about 200 mg/d, whereas hostility was not enhanced in students who took DHA capsules (1.5-1.8 g DHA/d); in another study we found that if there was no stressor like exams, hostility was not changed significantly in either the control or the DHA group. We also found that DHA administration significantly enhanced the ratio of plasma epinephrine to norepinephrine in the DHA group compared with the control group during continuous psychological stress (final exams for two months). This DHA effect was mainly due to norepinephrine reduction in the DHA group.

Those studies above were all done with young adults. But people over 50 are more susceptible to stress-related adult diseases. Consequently, we decided to perform a similar study with older subjects to investigate the effects of DHA on hostility.

Method. Twenty-two males and 18 females of 50-60 yr of age volunteered for the present

double-blind study. They were all healthy, and one half of them were farmers from suburban farming villages in Nakornpathom, Thailand. They were randomly allocated either to the DHA group (11 males and 8 females) or to the control group (11 males and 10 females). Subjects in the DHA group took 10 DHA capsules/d containing 1.5 g DHA as a total for two months, and those in the control group took 10 control capsules/d, each capsule containing 280 mg of mixed plant oil (47 % olive oil, 25 % rapeseed oil, 25 % soybean oil and 3 % fish oil). At the start and the end of the study, volunteers took P-F study. Just before they took P-F study at the end of the study, they watched a provoking videotape for 20 min as stressor. The videotape contained many cruel scenes from the real crimes and disasters.

Results. EA was significantly decreased in the DHA group ($32 \pm 15\%$ to $25 \pm 11\%$, $M \pm SD$, $p < 0.02$), whereas not in the control group ($27 \pm 16\%$ to $23 \pm 10\%$). Inter-group difference was not significant by ANOVA. Hostility was significantly decreased in the DHA group ($17 \pm 8\%$ to $11 \pm 7\%$, $p < 0.05$), whereas not in the control group ($16 \pm 11\%$ to $12 \pm 8\%$). Although the inter-group difference was not significant by ANOVA, the ratio of increment in hostility in the DHA group (2 out of 19) was significantly ($p < 0.05$) lower than in the control group (8 out of 21).

Discussion. We provoked subjects of both groups by videotape, but extra aggression or hostility did not increase in either group. The place where PF study was performed (Silpakorn University,

Nakornpathom) was not familiar to most of the volunteers. Consequently, there might be effects of becoming accustomed to the test in a very unfamiliar place at the end of the study. Although the effects were marginal compared with the case of young adults with natural stressor, it is likely that DHA influenced hostility of people even in their fifties. Taken into account that hostility is a risk factor of adult diseases, enough amounts of DHA (up to 1.5 g/d) might be beneficial.

Omega-3 Fatty Acids in Mood Disorders

Andrew L. Stoll, M.D.

McLean Hospital, Belmont, Massachusetts, USA

Omega-3 fatty acids may inhibit neuronal signal transduction pathways in a manner similar to lithium and valproate, two effective treatments for bipolar disorder. To examine this pharmacological similarity more closely, a study was performed to examine whether omega-3 fatty acids also exhibit mood-stabilizing properties in bipolar disorder. This was a 4-month, double-blind, placebo-controlled study, comparing omega-3 fatty acids (9.6 g/d) vs. placebo (olive oil), in addition to usual treatment, in 30 patients with bipolar disorder.

The results of the study revealed strong mood stabilizing and antidepressant effects of the omega-3 fatty acids. A Kaplan-Meier survival analysis of the cohort revealed that the omega-3 fatty acid patient group had a significantly longer period of remission than the placebo group ($p = 0.002$; Mantel-Cox). In addition, for nearly every other outcome measure, the omega-3 fatty acid group performed better than the placebo group. Omega-3 fatty acids were well-tolerated and improved the short-term course of illness in this preliminary study of patients with bipolar disorder.

The omega-3 fatty acids offer some unique benefits, should they prove to be truly effective mood stabilizers. The advantages of the omega-3 fatty acids as mood stabilizers include the apparent acute efficacy in both the manic and depressive phases of bipolar disorder, their lack of toxicity, as well as high patient acceptance. In addition, omega-3 fatty acids confer some health benefits during chronic use, such as possible reduction in the risk of a fatal myocardial infarction. In addition, the omega-3 fatty acids have no documented adverse drug interactions, and appear to be safe (and possibly beneficial) in pregnancy and in children.

The disadvantages of the omega-3 fatty acids include their low potency, which results in a relatively large number of capsules per day. This may effect compliance. In addition, at the high doses used in the pilot study, several patients treated with either olive oil placebo or omega-3 fatty acids developed mild gastrointestinal distress, generally loose stools. This was completely abolished by lowering the dosage slightly or dividing the dosage into 3 or 4 separate portions. There is also the theoretical risk of increased bleeding during high-dose omega-3 fatty acid treatment. However, no change was observed in bleeding times during the controlled trial in bipolar disorder.

We have also treated more than 20 bipolar patients with open-label flaxseed oil. Flaxseed oil contains alpha-linolenic acid, a shorter chain omega-3 fatty acid. Measuring the clinical response to an open-label treatment is unavoidably subjective. However, the majority of the bipolar patients treated with flaxseed oil appeared to benefit. Many of these patients have described a distinct mood elevating effect from the flaxseed oil, and most have elected to remain on the flaxseed oil for the long-term. As with fish oil, the flaxseed oil was used adjunctively, in that the flaxseed oil was added to whatever mood stabilizing medication the patient was already receiving. The flaxseed oil was generally better tolerated than fish oil. However, whether causally related or not, we have observed several cases of hypomania in bipolar patients treated with flaxseed oil.

Our results support other data suggesting that the mechanism of action of mood stabilizers in bipolar disorder is the suppression of aberrant signal transduction and inhibition of kindling processes. This is consistent with a model of abnormal signal transduction in the pathophysiology of bipolar disorder. If further studies confirm their efficacy in bipolar disorder, omega-3 fatty acids may represent a new class of membrane-active psychotropic compounds, and may herald the advent of a new class of rationally designed mood stabilizing drugs.

Thursday, April 8, 1999

Session III. Cardiovascular Disease

Polyunsaturated Fatty Acids and Cardiovascular Disease

Alexander Leaf, M.D.

Departments of Medicine, Massachusetts General Hospital and

the Harvard Medical School, Boston, Massachusetts, USA

Coronary heart disease is the leading cause of death in the United States and in Western industrialized

countries. Many reports have appeared since the epidemiologic evidence of Bang and Dyerberg called attention to the low mortality from coronary heart diseases (CHD) among the Greenland Eskimos, which they attributed to potential antiatherosclerotic effects of the diet high in oil of marine vertebrates. Many studies have documented the effects of fish oils on a number of biochemical and physiologic factors that are believed to affect the atherosclerotic process. There are also a considerable number of experimental studies in animals which show a reduction in atherosclerosis when diets high in saturated fatty acids and cholesterol are supplemented with fish oils. Notably among these are the beneficial effects reported in swine and in nonhuman primates, but even in a nonhuman primate negative results have been reported.

What Bang and Dyerberg noted among the Greenland Eskimos has been largely confirmed among the Japanese. The Zutphen study by Kromhout and associates and the reanalysis of the Multiple Risk Factor Intervention Trial by Dolecek showed an inverse relation between fish intake and mortality from CHD, as have other studies. Dolecek analyzed the larger Multiple Risk Factor Intervention Trial dividing the 6000 subjects in the control group (Usual Care) for that Trial into quintals according to their mean ingestion of n-3 polyunsaturated fatty acids from 0 to 0.66 g daily and found significant inverse correlations between the ingestion of these fish oils and coronary heart disease, all cardiovascular diseases and all-cause mortality with the highest quintal having lowest mortality rates of some 40 to 50%. However, the rapid atherosclerosis-like processes that often cause restenosis following coronary angioplasty are not prevented by dietary fish oil supplements. There has been one prospective, randomized, placebo-controlled, secondary clinical trial which has reported a 29% reduction in all cause and cardiovascular mortality at 2 years follow-up in patients advised to eat oily fish 2 to 3 times per week compared with those not so advised. Another secondary, single blinded, clinical trial reported a remarkable reduction in all cause mortality at 27 months mean follow-up and recently again at almost 4 year follow-up in the same cohort of some 70% compared to controls. The Lyon Heart Study in which alpha-linolenic acid was considered the important dietary polyunsaturated fatty acid.

Reports by Charnock and McLennan have drawn attention to another aspect of coronary heart disease which the highly polyunsaturated fatty acids in fish oils seem to affect beneficially. They found that rats fed a diet high in a fish oil were protected from the fatal cardiac arrhythmias induced by experimental coronary artery ligation. We have confirmed their findings in dogs with Prof. George E. Billman, Ohio State University School of Medicine. We have then pursued the mechanism of the antiarrhythmic effect of the fish oil fatty acids. With isolated cultured heart cells we have produced arrhythmias with chemical agents which can cause fatal arrhythmias in humans. We have found in every instance that if we add the fish oil fatty acids to the fluid bathing the cells before we add the toxic agent, the arrhythmia is prevented. If we first induce the arrhythmia in the single cultured contracting heart cells and then add the fish oil fatty acids the arrhythmia is promptly stopped. This antiarrhythmic effect is due to stabilization of the excitability of every contracting cell in the heart. This in turn results from a modulating effect of the fatty acids on the ionic currents that initiate the heart beat. Further studies suggest strongly that the fatty acids interact with binding sites on the proteins of the ion channels thus affecting their conductivity to make the heart much less responsive to the electrical events that initiate fatal cardiac arrhythmias.

Once we had found that the polyunsaturated fatty acids modulate ion currents in an excitable tissue, the heart, we surmised that they must have a similar effect on all excitable tissues, since all utilize the same electrical communicating system and they do! We have reported that in the brain (hippocampal CA1 neurons) the voltage dependent Na^+ and the L-type Ca^{2+} currents are affected very much as are the same cardiac currents. One consequence of this action in the brain is that the

electrical threshold for inducing generalized seizure activity in the rat using the cortical stimulation model, is increased. So these fatty acids are anticonvulsants as well as antiarrhythmic agents. With the findings by some psychiatrists that these same fatty acids are apparently beneficial in the management of depression and bipolar behavioral disorders, the finding of an important effect of the fatty acids on the electrical activity of brain cells may have broader health implications than just to cardiovascular diseases. There remains much to be learned; we are probably just scratching the surface of the importance of polyunsaturated fatty acids to health and the prevention of diseases.

N-3 Polyunsaturated Fatty Acids Inhibit COX-2 Expression

*Raffaele De Caterina, M.D., Ph.D., , Aida Habib?, Laura Lubrano, Giuseppina Basta, Guido Lazzarini, Jacques Maclouf? and Babette Weksler**

*CNR Institute of Clinical Physiology, Pisa, Italy, *Cornell University Medical College,*

New York, USA and ?INSERM Unite 348, Hopital Lariboisiere, Paris, France

N-3 polyunsaturated fatty acids (n-3 FA), including docosahexaenoic acid (DHA) exert anti-inflammatory and anti-atherogenic properties, mostly ascribed to competition with arachidonic acid (AA) as substrate for cyclooxygenases and 5-lipoxygenase. A cytokine-inducible cyclooxygenase (COX-2) expressed at sites of inflammation permits high production of prostanoids and amplification of the inflammatory response. We previously showed that n-3 FA (particularly DHA) are inhibitors of cytokine-induced expression of adhesion molecules in vascular endothelial cells (EC). Since genes for adhesion molecules and COX-2 share consensus sequences for transcription factors and patterns of cytokine induction, we hypothesized that n-3 FA might be transcriptional regulators of COX-2 expression. We therefore measured changes in AA metabolism in cultured human saphenous vein EC following 48 h preincubation with 25 μ M DHA plus 24 h stimulation with IL-1 or LPS. We measured COX activity assessing 6-keto-PGF1a by RIA as a reflection of prostacyclin production. DHA decreased thrombin or AA-stimulated 6-keto-PGF1a to a greater extent in IL-1-stimulated EC than in the absence of IL-1, suggesting a greater inhibitory effect on COX-2 than on constitutively expressed COX-1. Inhibition of 6-keto-PGF1a production by DHA + the specific COX-2 inhibitor NS-398 was greater than inhibition by NS-398 alone, suggesting that DHA acted at a different level than on COX-2 enzymatic activity. Thus, COX-2 mRNA and protein expression were compared by Northern and Western analysis in control and DHA-treated EC stimulated with IL-1. DHA-treated EC showed a 50% inhibition of COX-2 expression at both mRNA and protein levels. Northern analysis of cells treated also with actinomycin D indicated that DHA exerted a transcriptional effect consistent with inhibition of NF- κ B as assessed by electrophoretic mobility shift assays. These results show that treatment of EC with DHA reduces COX-2 protein expression and enzyme activity by transcriptional regulation likely to involve NF- κ B activation, and offer a plausible alternative mechanism to many of the anti-inflammatory and anti-atherogenic effects of n-3 FA.

Alpha-Linolenic acid in the Prevention of Cardiovascular Diseases

S. Renaud, M.D.

INSERM, Unit 330, University Bordeaux 2, FRANCE

Cardiac mortality, especially sudden death, has been rarely prevented in dietary intervention trials to lower coronary heart disease (CHD). Only trials with an increased level of n-3 fatty acids (fish or fish oil) (DART Lancet 1989:2:757) have succeeded so far.

In Crete, cardiac death as shown by the seven country study is a rare event. In our duplication of the Cretan diet on 600 coronary patients (Lancet 1994:343:1454) cardiac death was reduced by 76 % and we did not observe any sudden death as compared to 8 in the control group with the prudent diet. Like the Crete population (Eur J Clin Nutr 1993:47:20), our subjects with the Cretan diet had a high level of oleic and alpha-linolenic acids in their plasma.

Studies have shown that arrhythmia of myocytes in culture, and ventricular fibrillation in dogs and rats are inhibited by n-3 fatty acids (Proc Natl Acad Sci USA 1997:94:4182). In rat reperfusion ventricular fibrillation was inhibited only by the alpha-linolenic acid rich canola oil but not by olive oil. (J Nutr 1995:125:1003)

In Crete it seems that it is through the consumption of walnuts, purslane and other greens as well as of snails, that a high intake of alpha-linolenic acid is achieved.

Recent prospective studies in USA (Harvard Public Health) and Europe (Euramic) indicate that the only fatty acid apparently inhibiting cardiac mortality in man is alpha-linolenic acid. Thus,

alpha-linolenic acid, in addition to regulating the level of prostaglandins and leukotrienes, may be the chief fatty acid protecting from the CHD clinical manifestations, cardiac death and coronary thrombosis.

Omega-3 Long Chain PUFA and Triglyceride Lowering: Minimum Effective Intakes

William S. Harris, Ph.D.

Saint Luke's Hospital, Kansas City, Missouri, USA

<u>Author</u>	<u>Year</u>	<u>Source</u>	<u>ω3 FA (g/d)</u>	<u>Δ Trig*</u>
Agren ¹	1996	Fish	1.05	-15% (PPL →)
Schaefer ²	1992	Fish	1.8	-7% (PPL →)
Silva ³	1996	Shrimp	0.81	-19%

Fahrer ⁴	1991	Fish	1.75	-19%
Jacques ⁵	1992	Fish	0.45	-8%
Gerhard ⁶	1991	Fish	1.95	-1%
Brown ⁷	1990	Fish	0.7	-7%
Ågren ⁸	1988	Fish	0.8	-16%
Fehily ⁹	1983	Fish	0.7	-7%
Brown ¹⁰	1991	Capsules	1.5	-25% (PPL →)
Oosthuizen ¹¹	1994	Capsules	1.6	-17%
Valdini ¹²	1990	Capsules	1.8	-16%
Gans ¹³	1990	Capsules	1.8	-33%
Beil ¹⁴	1991	Capsules	1.6	-20%
Radack ¹⁵	1990	Capsules	1.1	-10%
Roche ¹⁶	1996	Capsules	0.8	-21% (PPL →)
Demke ¹⁷	1988	Capsules	1.5	-24%
Schindler ¹⁸	1998	Capsules	1.1	-16-34% (depending on phenotype)
			(0.18 to 1.1 g/d)	
Saldeen ¹⁹	1998	Bread	0.3	-17%
Lovegrove ²⁰	1997	Multifoods	1.4	-4% (PPL →)
Sorensen ²¹	1998	Margarine	0.9	-12%

*Bold italic = statistically significant. PPL = postprandial lipemia; → = lower on ω3 FA

1. Agren JJ, Hamminen O, Julkunen A, et al: Fish diet, fish oil and docosahexaenoic acid rich oil lower fasting and postprandial plasma lipid levels. *Euro J Clin Nutr* 1996;50:765-771.

2. Schaefer EJ, Lichtenstein AH, Lamon-Fava S, et al: Effects of National Cholesterol Education Program Step 2 diets relatively high or relatively low in fish-derived fatty acids on plasma lipoproteins in middle-aged and elderly subjects. *Am J Clin Nutr* 1996;63:234-241.

3. De Oliveira e Silva ER, Seidman CE, Tian JJ, Hudgins LC, Sacks FM, Breslow JL: Effects of shrimp

consumption on plasma lipoproteins. *Am J Clin Nutr* 1996;64:712-717.

4. Fahrer H, Hoeflin F, Lauterburg BH, Peheim E, Levy A, Vischer TL: Diet and fatty acids: can fish substitute for fish oil? *Clin Exper Rheum* 1991;9:403-406.

5. Jacques H, Noreau L, Moorjani S: Effect on plasma lipoproteins and endogenous sex hormones of substituting lean white fish for other animal-protein sources in diets of postmenopausal women. *Am J Clin Nutr* 1992;55:896-901.

6. Gerhard GT, Patton BD, Lindquist SA, Wander RC: Comparison of three species of dietary fish: effects on serum concentrations of low-density-lipoprotein cholesterol and apolipoprotein in normotriglyceridemic subjects. *Am J Clin Nutr* 1991;54:334-339.

7. Brown AJ, Roberts DCK, Pritchard JE, Truswell AS: A mixed Australian fish diet and fish-oil supplementation: impact on the plasma lipid profile of healthy men. *Am J Clin Nutr* 1990;52:825-833.

8. Agren JJ, Hanninen O, Laitinen M, et al: Boreal Freshwater Fish Diet Modifies the Plasma Lipids and Prostanoids and Membrane Fatty Acids in Man. *Lipids* 1988;23:924-929.

9. Fehily AM, Burr ML, Phillips KM, Deadman NM: The effect of fatty fish on plasma lipid and lipoprotein concentrations. *Am J Clin Nutr* 1983;38:349-351.

10. Brown AJ, Roberts DCK: Moderate Fish Oil Intake Improves Lipemic Response to a Standard Fat Meal: A Study in 25 Healthy Men. *Arterioscler Thromb* 1991;11:457-466.

11. Oosthuizen W, Vorster HH, Jerling JC, et al: Both Fish Oil and Olive Oil Lowered Plasma Fibrinogen in Women with High Baseline Fibrinogen Levels. *Thromb Haemost* 1994;72:557-562.

12. Valdini AF, Glenn MA, Greenblatt L, Steinhardt S: Efficacy of Fish Oil Supplementation for Treatment of Moderate Elevation of Serum Cholesterol. *J Fam Practice* 1990;30:55-59.

13. Gans ROB, Bilo HJG, Weersink EGL, et al: Fish Oil Supplementation in Patients with Stable Claudication. *Am J Surg* 1990;160:490-495.

14. Beil UF, Terres W, Orgass M, Greten H: Dietary fish oil lowers lipoprotein(a) in primary hypertriglyceridemia. *Atherosclerosis* 1991;90:95-97.

15. Radack KL, Deck CC, Huster GA: n-3 Fatty acid effects on lipids, lipoproteins, and apolipoproteins at very low doses: results of a randomized controlled trial in hypertriglyceridemic subjects. *Am J Clin Nutr* 1990;51:599-605.

16. Roche HM, Gibney MJ: Postprandial triacylglycerolaemia: the effect of low-fat dietary treatment with and without fish oil supplementation. *Euro J Clin Nutr* 1996;50:617-624.

17. Demke DM, Peters GR, Linet OI, Metzler CM, Klott KA: Effects of a fish oil concentrate in patients with hypercholesterolemia. *Atherosclerosis* 1988;70:73-80.

18. Schindler OS, Rost R: Effect of low dose omega-3 fatty acid supplementation on plasma lipids and lipoproteins in patients with coronary sclerosis and dyslipoproteinemia. *E Ernahr* 1996;35:191-198.

19. Saldeen T, Wallin R, Marklinder I: Effects of a small dose of stable fish oil substituted for margarine in bread upon plasma phospholipid fatty acids and serum triglycerides. *Nutr Res* 1998;18:1483-1492.

20. Lovegrove JA, Brooks CN, Murphy MC, Gould BJ, Williams CM: Use of manufactured foods enriched with fish oils as a means of increasing long-chain n-3 polyunsaturated fatty acid intake. *Br J Nutr* 1997;78:223-236.

21. Sørensen NS, Marckmann P, Høy CE, van Duyvenvoorde W, Princen HMG: Effect of fish-oil-enriched margarine on plasma lipids, low-density-lipoprotein particle composition, size, and susceptibility to oxidation. *Am J Clin Nutr* 1998;68:235-241.

Efficacy of n-3 PUFA and vitamin E in 11,324 post-MI patients:

Results of GISSI-Prevenzione

*Roberto Marchioli, M.D. on behalf of GISSI-Prevenzione Investigators.**

Mario Negri Institute, S. Maria Imbaro (CH), Italy

The protective effects of fish oil supplements and vitamin E have been long debated.. Within Months of a myocardial infarction, 11.324 patients were randomized to an n-3 polyunsaturated fatty acid (PUFA) supplement (1g daily), a vitamin E supplement (300 mg daily), both, or neither. Baseline therapy included antiplatelet therapy in 90% of patients, beta blockers in 40%, and angiotensin converting enzyme inhibitors in 50%.

At 42 months follow up, patients who received n-3 PUFA had a significant 15% relative risk reduction in the combined rate of death plus nonfatal myocardial infarction and nonfatal stroke compared with those who did not receive n-3 PUFA (12.3% vs. 14.4% R= 0.001). By contrast, treatment with vitamin E caused a non significant 11% relative risk reduction in the combined endpoint. All of the beneficial effects of n-3 PUFA were due to 21% reduction in the risk of death.

There were no significant interactions between the two treatments. Both treatments were well tolerated. Gastrointestinal intolerance was the most commonly reported side effect.

* The GISSI-Group (Gruppo Italiano per lo Studio della Sopravvivenza nell'infarto) is jointly sponsored by the Associazione Nazionale Medici Cardiologi Ospedalieri (ANMCO) and by Istituto Mario Negri Consorzio Mario Negri Sud.

Thursday, April 9, 1999

Session IV. Relationship of Essential Fatty Acids to Saturated,

Monounsaturated, and Trans Fatty Acids

Relationships Between Saturated, Monounsaturated, Polyunsaturated Fatty Acids : Dietary Data vs. Data from Plasma Fatty Acid and Lipid Analyses

Claudio Galli, M.D.

Institute of Pharmacological Sciences, University of Milano, Milano, Italy

Background

Plasma levels of individual fatty acids (FA), of FA classes and of single intermediates in the metabolic series, are the result of diversified processes : intake with the diet, transport, uptake by cells and tissues, with the additional influence of metabolic processes (*de novo* synthesis or precursor - product conversion and retroconversion in the FA series , i.e. n-9, n-6, n-3).

Determinants of plasma fatty acids

The relationships between major FA classes (saturates, SAT, monounsaturates, MUFA and polyunsaturated fatty acids, PUFA) in plasma lipids and the intake / synthesis from precursors, are rather different. In fact SAT and MUFA are synthesized *de novo*, besides being provided by the diet, whereas PUFA are exclusively supplied by the intake. In addition, long chain PUFA (LCP) of the n-6 and n-3 series in plasma and tissues represent a combination of amounts produced through the endogenous conversion of the short chain polyunsaturated fatty acids (SCP), linoleic (LA) and α -linolenic (ALA) acids to the LCP, and amounts provided directly by the diet. While the first pathway, i.e. synthesis from SCP, is the only source of LCP in strict vegetarians, the combined processes (intake + endogenous synthesis) take place in omnivorous subjects. It is however difficult to evaluate the relative contributions of these two components, due to the limited quantitative data on these "minor" individual LCP in foods. The assessment of LCP synthesis from SCP in individual subjects represents also a difficult task.

Additional factors which contribute to determine the final FA profiles in plasma lipids are : a. The different rates and degrees of esterification of individual FA into lipid classes (phospholipids, PL; cholesterol esters; CE; triglycerides, TG), as it emerges also from *in vitro* studies with cultured cells. b. The positional selectivity in the incorporation of different FA classes into glycerol. SAT are almost exclusively incorporated into the 1-position in cell PL and into the sn-1 and 3 position of TG, whereas MUFA are predominantly and PUFA almost exclusively incorporated into the 2-position. The 1-position is metabolically stable, whereas continuous replacement of FA takes place in the 2-position, through hydrolysis and reacylation processes. FA which in the 2-position should therefore be more readily modulated by changes in the relative availability of MUFA and PUFA. This should in turn result in a significant impact of the relative dietary intakes of these FA classes on the relationships between MUFA and PUFA in plasma lipids. In contrast, the intake of SAT should minimally affect their relative levels in circulating lipids.

Evaluations of FA relationships in plasma

We have measured several relationships between FA in plasma lipids, with the aim to establish possible correlations which could be of help in elucidating the processes governing the final plasma FA profile.

FA distribution in plasma lipids

The distribution of individual FA in plasma lipid classes (PL, TG, CE) in humans varies appreciably even among FA of the same class or metabolic series, as it is for instance shown in Table 1. Of the total circulating AA, the greatest proportion is associated with PL, followed by CE, and minimal amounts are found in TG, whereas LA is mostly associated with CE, followed by PL and TG. Marked differences are found also in the distribution of DHA (mainly associated with PL) and EPA (largely associated with CE). These differences may affect the relative incorporation and exchanges of individual FA with cell lipids.

Table 1. Concentrations and % levels of individual FA in plasma lipid classes in 20 women.

 % distribution

FA μ g/ml PL TG CE

18:2 640 \pm 125 34 \pm 6 10 \pm 5 56 \pm 9

20:4 148 \pm 39 67 \pm 6 4 \pm 2 29 \pm 7

20:5 9.6 \pm 3.9 57 \pm 12 11 \pm 10 31 \pm 12

22:6 28.8 \pm 9.8 85 \pm 5 7 \pm 7 8 \pm 3

FA correlations

Evaluation of the product/precursors relationships within the n-6 and n-3 FA series in Tanzanian populations on low fat diets (7-12 en%), strict vegetarians (VD) and fish eaters (FD) ingesting relatively high amounts of AA and DHA (typical in tropical fish) revealed the following : in VD only good correlations are present in the n-6 pathway (from LA to AA and especially between DHGLA and AA), and between ALA and EPA, in the n-3 series. In FD, significant correlations are found only between LA and DHGLA in the n-6 series, and between EPA and DHA in the n-3. These findings will be discussed in the context of the contributions of the exogenous supply of preformed LCP (FD and omnivores, in general) vs that of the endogenous biosynthesis exclusively. The correlations in omnivorous Italian populations (>30 en % fat), are somewhat intermediate between those in the two Tanzanian populations.

Evaluation of the correlations between SAT, MUFA and PUFA in the three populations at study, revealed that : a. there is no correlation between SAT and MUFA, weak but significant negative correlations between PUFA and SAT (SAT vs PUFA : $y=42.6-0.24x$, $r=0.535$, $p < 0.001$ in Italians; $y = 48-0.38x$, $r=0.62$, $p<0.001$ in VD Tanzanians; $y= 46.2-0.29x$, $r=0.47$, $p<0.001$ in FD Tanzanians), very strong negative correlations between MUFA and PUFA (MUFA vs PUFA : $y=58.4-0.75x$,

$r=0.89$ in Italians; $y=53.8 - 0.71x$, $r=0.80$ in VD and $y=51.9 - 0.62x$, $r=0.79$ in FD). These data obtained in populations on diets with quantitatively and qualitatively very different fat contents, fit and are in agreement with the hypothesis that PUFA and MUFA compete for esterification, whereas this does not occur between SAT and MUFA. Additional relationships which will be discussed concern those between n-6 and n-3 levels. These, in plasma, at difference with the situation in cellular lipids, do not appear to be reciprocally modulated.

In a controlled clinical study with subjects on isocaloric diets (25 en% fat) with defined FA proportions (prudent diet, olive oil based diet and corn oil based diet) we have evaluated the relationships between dietary SAT, MUFA and PUFA as en% and the same FA classes as % of plasma FA. It appeared that differences in dietary SAT between 5 to 9.6 en% result in no difference in plasma SAT (% of total FA), whereas differences in dietary MUFA and PUFA result in proportional changes in the corresponding plasma FA.

Additional evaluations on the relationships between levels of FA classes as well as of individual FA, on one side, and plasma cholesterol and TG, on the other, reveal that correlations are present only with TG.

In conclusion, the observation of selected correlations among plasma FA, based on detailed analytical data, facilitates the interpretation of the dietary and metabolic relationships between FA and plasma lipids.

Nutritional and Metabolic Interrelationships Between Omega-3 Fatty Acids and Trans Fatty Acids

Bruce J. Holub, Ph.D.

University of Guelph, Guelph, Ontario, Canada N1G 2W1

Much attention on the health concerns of current intakes of trans fatty acids (TFA) via fast and processed foods has focused upon the potential for dietary TFA to significantly increase the LDL-cholesterol level while lowering HDL-cholesterol as well as increasing triglyceride and lipoprotein(a) levels in some studies. Epidemiological studies have indicated that TFA represent a major dietary risk factor for cardiovascular disease (CVD) in the North American population. A lesser focus has been placed on the potential for dietary TFA to interfere with the convertibility of linoleic acid and alpha-linolenic acid (α -LNA) to their longer-chain metabolic products. There is also evidence that TFAs may impair early growth in humans by impeding desaturation/elongation reactions. Recent data from Health Canada (Ratnayake and Chen) has indicated that the mean TFA intake (as trans 18:1) in Canadian adults represents 3.7% of total daily energy which is above recent estimated intakes of TFA for the US population (Allison et al, J. Am. Diet. Assoc., 1999). Young males in Canada (age 18-34 years) have a mean trans-18:1 intake of 12.5 g/day with intakes as high as 39 g/person/day. One of the richest sources of TFA in the Canadian food supply is breast milk from mothers who show a mean content of total TFA representing 7.2% of total fatty acids (and up to 17.2%). Furthermore, the total TFA: α -LNA ratio in Canadian breast milk is 6.2 to 1. These high ratios reflect the very high ratio of TFA:n-3 fatty acids in the diet of pregnant and lactating women. We have analyzed a wide variety of processed and fast foods in Canada (showing very high ratios of TFA:n-3 PUFA) as well as a wide variety of baby foods (cereals and biscuits) which, in many cases, show extremely high ratios

of TFA:n-3 fatty acids. Foods containing hydrogenated vegetable oils which greatly compromise the n-3 fatty acids intake while enhancing the TFA consumption, as well as the potential for TFA to interfere with the convertibility of α -LNA to docosahexaenoic acid (DHA), likely accounts for the lower DHA status in humans consuming higher intakes of processed and fast foods containing TFA. Mandatory food labeling in North America for TFA and omega-3 fatty acids is needed to allow consumers to reduce the consumption of the former while increasing the latter. Such regulatory changes can be expected to enhance the physiological DHA status and related human health parameters beginning at conception.

**Choice of n-3, Monounsaturated and Trans Fatty Acid-Enriched Oils
for the Prevention of Excessive Linoleic Acid Syndrome**

Harumi Okuyama, Ph.D.

Faculty of Pharmaceutical Sciences, Nagoya City University,

Mizuhoku, Nagoya 467-8603, Japan

Excessive linoleic acid intake and relative n-3 deficiency syndrome

Animal experiments and epidemiological studies have revealed that excessive intake of linoleic acid (LA, n-6) is a major risk factor for cancers of western type, allergic hyper-reactivity, coronary heart disease (CHD) and cerebrovascular disease (CVD) (1). Although epidemiological studies performed in the USA failed to reveal a positive correlation between LA intake and breast cancer mortality, this is probably because the proximate marker for breast cancer is the proportion of n-6 eicosanoid precursors in phospholipids, which is saturated both in the high and low LA intake groups in the USA. Empirical equations presented by Lands indicate that both increasing the intake of n-3 fatty acids and decreasing that of n-6 fatty acids are necessary for effectively decreasing the n-6 eicosanoid precursors in phospholipids and thereby decreasing cancer mortality. On the other hand, high n-6/n-3 ratio but not hypercholesterolemia has been proved clinically to be a major risk factor for thrombotic diseases. Over-production of inflammatory lipid mediators of n-6 series has been shown to be a major cause for the rapid increase in allergic hyper-reactive patients in Japan.

President's Summary 1997 from the Japan Society for Lipid Nutrition

After discussion through several annual meetings of the Japan Society for Lipid Nutrition, Presidents Summary 1997 was published (in Japanese) as a review article (J. Lipid Nutr. 6:5-42, 1997), in which 20% as total fat energy was recommended for those with moderate physical activity. For healthy populations, saturated plus monounsaturated : n-6 : n-3 = 2.5 : ≤ 0.8 : ≥ 0.2 (n-6/n-3 ≤ 4) was recommended. For the primary and secondary prevention of those diseases described above, an n-6/n-3 ratio of 2 was recommended. The latter value was based on: 1) even the n-6/n-3 ratio of Danes was 3 in a well known epidemiology of Greenland natives; 2) the ratio of current Japanese is 4 but the incidence of cancers of western type has been increasing rapidly, and the ratio of 4 or above cannot be recommended; 3) animal experiments have shown the effectiveness of decreasing n-6/n-3 ratio to below 2 for the suppression of carcinogenesis and metastasis; and 4) the safety of n-6/n-3 ratio of 1 has been established in animal experiments and in a retrospective study on hunters and gatherers' foods.

In order to meet the recommendations described above, vegetable oils with n-6/n-3 ratios of 2 or below and those with very low n-6 fatty acid contents (e.g., high-oleic type) are useful. However, there was another criterion to be considered; the presence of minor components which affect animal physiology seriously.

Survival time-shortening and renal injury induced by some vegetable oils and partially hydrogenated oils in SHRSP rats

Using soybean oil as a control, some oils were found to prolong the mean survival time of SHRSP rats by ca 10% (e.g., DHA-rich fish oil, perilla seed oil, flaxseed oil) while some others shortened it dose-dependently by ca 40% (double-low rapeseed oil, evening primrose oil, high-oleate safflower oil, high-oleate sunflower oil, olive oil and partially hydrogenated rapeseed and soybean oil). When the rapeseed oil was lipase-treated, the resulting free fatty acid fraction was almost free of such activity, indicating that the survival-time shortening activity is due to minor components other than fatty acids in these oils. Free fatty acid fraction from partially-hydrogenated soybean oil exhibited a survival time between those of the original oil and soybean oil. It should be emphasized that lard, sesame oil and high-linoleate safflower oil were relatively safe for the SHRSP rats.

Those oils with survival-time shortening activity were found to cause renal injury; lesions in blood vessels, accelerated proteinuria, decreased platelet count and elevated gene expression for TGF β , fibronectin and renin.

Choice of n-3, monounsaturated and trans fatty acid-enriched oils

In order to decrease the n-6/n-3 ratio of our current foods to 2 or below, the intake of high- α -linolenate oils such as perilla seed oil and flaxseed oil as well as seafood and vegetables should be increased. High-linoleate oils are inappropriate for human use as foods. For deep-frying and preservation purpose, high-oleate vegetable oils are useful but all the high-oleate vegetable oils and hydrogenated vegetable oils we have examined so far exhibited the survival time-shortening activity,

and I cannot recommend people to have these oils in large quantities. Instead, lard was safe for this animal model, and could be used in quantities not to induce obesity; animal fats as well as a high-LA vegetable oil intake caused insulin resistance in a NIDDM model of rats.

Reference

Okuyama, H., Kobayashi, T., and Watanabe, S. (1997) Dietary fatty acids ñ The n-6/n-3 balance and chronic, elderly diseases. Excess linoleic acid and relative n-3 deficiency syndrome seen in Japan. *Prog. Lipid Res.* 35:409-457.

Friday, April 9, 1999

Session V. Dietary Recommendations and Omega-6:Omega-3 Ratio

Intakes of Dietary Fatty Acid in the United States: Results from the USDA's 1994-1996

Continuing Survey of Food Intakes by Individuals

G.J. Nelson, Ph.D.

USDA, ARS, Western Human Nutrition Research Center, San Francisco, California 94129, USA

The USDA has been conducting biennial food intake surveys for many years. In the more recent surveys individual fatty acid intakes were estimated from food composition data accumulated and published in the USDA Handbook 8, "The Composition of Foods." In the compilation from the combined 1994 and 1996 surveys, the USDA has published data on 19

individual fatty acids in the US diet. Included are all the saturated fatty acids from C-4 to C-18, the monounsaturated fatty acids 16:1, 18:1, 20:1, and 22:1 and the polyunsaturated fatty acids 18:2, 18:3, 18:4, 20:4, 20:5, 22:5, and 22:6. The data is broken down by sex and age from less than 1 year to more than 70 and by percent of calories or grams per day. Although there are some age dependent trends in the consumption data, the majority of the population does not show significant differences between five and sixty years of age, and only slight differences due to sex. In the USA 11 percent of calories are consumed from saturated fat, 13 percent as monounsaturated fat, and 6 percent as polyunsaturated fat. Oleic acid is the preponderant monounsaturated fatty acid, and linoleic acid is the major polyunsaturated fatty acid. The USDA data for monounsaturated fatty acid presumably include trans isomers of monounsaturated fatty acids and are grouped together with 18:1 fatty acids. Current calculations using the best available estimated of trans fatty acid C-18 isomers in the foods consumed by the US population suggest that the actual consumption of trans configuration fatty acids is 3 percent and cis-monounsaturated fatty acids is about 10 percent. The saturated fatty acid category exhibits a broader distribution of fatty acids consumed than that observed for the unsaturated fatty acids; 12:0, 14:0, 16:0 and 18:0 all contribute significantly to the fat calorie intake in the US population. Palmitic acid accounts for 20 percent of fat calories and stearic acids about 9 percent. Of particular interest to this workshop is the intake of long-chain polyunsaturated fatty acids, especially those with twenty or more carbon atoms in the fatty acid chain. Unfortunately, the USDA data contain relatively little information on this topic. Due to the nature of survey information and the sparsity on information regarding long-chain polyunsaturated fatty acids in the food composition data from which the tables are prepared, no detailed view of the intake of omega-3 fatty acids can be made. The data do show that the US population consumes approximately 10 times the amount of omega-6 fatty acids as omega-3 (the ratio is 0.11 n3/n6),

but it is probable that the USDA data underestimated the omega-3 intake. In terms of grams per day the mean intake of linoleic acid plus arachidonic acid is 13.0 while the intake of α -linolenic acid plus docosahexaenoic acid is 1.5. The ratio of docosahexaenoic acid to arachidonic is, however, 1 (0.1 to 0.1 grams per day). As it is not possible to demonstrate an omega-3 fatty acid deficiency in the US population, the intake of 2 grams per day of omega-3 fatty acids must be at least the required daily intake when the intake of omega-6 is 20 grams per day. Of course, 13 grams per day of omega-6 fatty acids are likely to be considerably more than the required daily intake. Evidence from animals, and limited human data, suggests that the required daily intake is likely to be less than 5 grams per day (2 percent of calories). Whether the required daily intake of omega-3 fatty acids would be less if less omega-6 fatty acids were being consumed is unknown. If the total fat intake is reduced, it may be necessary to increase the intake of omega-3 fatty acids to avoid omega-3 fatty acid deficiency. Neither the USDA nor the federal Government have a recommendation for the DRI of polyunsaturated fatty acids presently. It is unlikely that a single amount could be recommended for all age ranges. The requirement for omega-3 fatty acids will probably be age dependent. Whether there is an absolute requirement for polyunsaturated fatty acids with twenty or more carbons in the chain remains to be determined.

World Health Organization/Pan American Health Organization

(Status of EFA Worldwide)

Manuel Peña, M.D.

Pan American Health Organization, Washington, DC, USA

The three main nutritional problems in the world are protein-energy malnutrition (PEM), micronutrient deficiencies (iron, vitamin A, iodine and folic acid) and overweight/obesity.

Stunting is the most common expression of PEM, but other forms of PEM are equally frequent among children below two years of age and child-bearing women.

Iron deficiency is the most widespread nutritional problem. Vitamin A and iodine deficiency showed a declining trend during the past years as a result of supplementation and fortification strategies, carried-out in countries.

Obesity is increasing worldwide at an alarming rate in both developed and developing countries. This situation is associated with rapid changes in dietary patterns and lifestyles.

Data from several countries show relatively high prevalence of obesity, particularly in women from poor urban areas. Furthermore, a sharp increase in morbidity and mortality rates due to nutrition-related non-communicable diseases has been reported.

One of the most important factors underlying this scenario among low socio-economic groups is the increase in energy intake associated with higher fat and refined carbohydrate consumption accompanied by a low iron, zinc, and folic acid intake.

Very little information exists on quality and composition of fats by low social-economic groups in the majority of countries.

Information in this area is of utmost importance to guide the selection and consumption of healthy diets as part of the Health Promotion strategy of PAHO/WHO.

n-3 Fatty Acids: Food Supply, Food Composition and Food Consumption Data

William D. Clay, Ph.D. and Barbara Burlingame

Nutrition Programmes Service Food and Nutrition Division,

Food and Agriculture Organization of the United Nations, Rome, Italy

Food supply data, food composition data, and food consumption data provide a fundamental basis for assessing the health and nutritional adequacy of individuals and populations. FAO has the UN mandate for these activities, and regularly produces Food Balance Sheets (FBS), which provide food supply data, including selected nutrient values. This paper will highlight estimates of available n-3 fatty acid containing foods from around the world. Most commonly, the nutrient data from FBS are expressed only in terms of energy, protein and fat. These international "default" nutrient values are being revised and the list of nutrients is now being expanded to include several micronutrients. In 1998, the cereals group was completed, and in 1999 the fish group will be revised. Under discussion is the possibility of including fatty acids and/or n-3 fatty acids as a special nutrient category for the fish group. Food composition activities in FAO come under the auspices of the FAO/UNU INFOODS project. We are providing assistance in all technical aspects of food composition. More and more frequently, fatty acids are included among the nutrients shortlisted by countries for inclusion in their national and regional food composition databases and tables. The inclusion of fatty acids is based on requests from the countries' users and potential users of food composition data, and

the countries' diet-related morbidity and mortality statistics. Commonly used laboratory instruments (gas chromatographs), well-defined analytical methodologies, and the availability of primary and secondary reference materials and standards, make analysis of n-3 fatty acids a routine activity for many laboratories. Data on n-3 fatty acids are now being generated, compiled and disseminated in many countries, including many developing countries. Sources and quantities of n-3 fatty acids will be presented. Food consumption data are routinely used, along with food supply data and food composition data, to establish food security at household, district and national levels. FAO prepares Nutrition Country profiles, which to date have not included assessment of n-3 fatty acids. However, now that acceptable quantities of high quality n-3 fatty acid data are becoming available from food composition laboratories, n-3 values can be incorporated into supply data, and food consumption studies will in the next few years be capable of reporting the n-3 fatty acid consumption in the assessments of food security.

BASF's Approach to Commercialization of Long Chain Omega-3 Fatty Acids

Herbert D. Woolf, Ph.D.

BASF Corporation, Mount Olive, New Jersey, USA

The goal to deliver omega-3 fish oils without adverse taste, odor and to prevent oxidative degradation has been a formulation objective. Utilizing spray-cooling technology for microencapsulating highly refined, deodorized and stabilized fish oils has proven to be successful in regard to producing powdered products that can be used to formulate fish oils into most all conventional food forms. Formulated food products, such as pastas, cereals, and even beverages, can be formulated with microencapsulated fish oils at levels of about 100 mg LCPUFAs per 100 gram product without detection of their inclusion.

The powdered microencapsulated product has been evaluated in clinical investigations to confirm its equivalency to the bioavailability of oils. Studies are being conducted to establish the product's use in formulated food form to deliver meaningful amounts of omega-3 fatty acids to pregnant women and ultimately to their breast fed infants.

BASF is enhancing its activities to educate and promote the incorporation of long chain omega-3 fatty acids to the food industry in a variety of ways: participating in the scientific community by funding studies and providing test materials, developing educational materials for health care providers as well as retailers and the consumer, promoting the use of omega-3s through advertisements and public relation activities, and developing a trademark to help draw attention to the incorporation of a unique food ingredient.

BASF will continue to support trade and professional associations working towards the establishment of Dietary Reference Intakes and health claim allowances.

Essential Fatty Acids and the Products of the Groupe Danone for Human Nutrition

Dominique Lanzmann-Petithory, M.D.

Groupe Danone, Centre Jean Theves, Athis Mons, FRANCE

Recent evaluations on the intake of fat in France show it to be in the range of 38 % of total calories : 50% of that fat is hidden in raw materials (27 % in meat and fish, 17 % in dairy products, 6 % in fruit and vegetables). The other 50% of the lipids consumed are added directly to the recipes; 30 % by the consumers themselves (butter, margarine, oil), 20 % by the food industries (meal, sausage, biscuits). Nevertheless, in recent years the intake of fat appears to be on the decline.

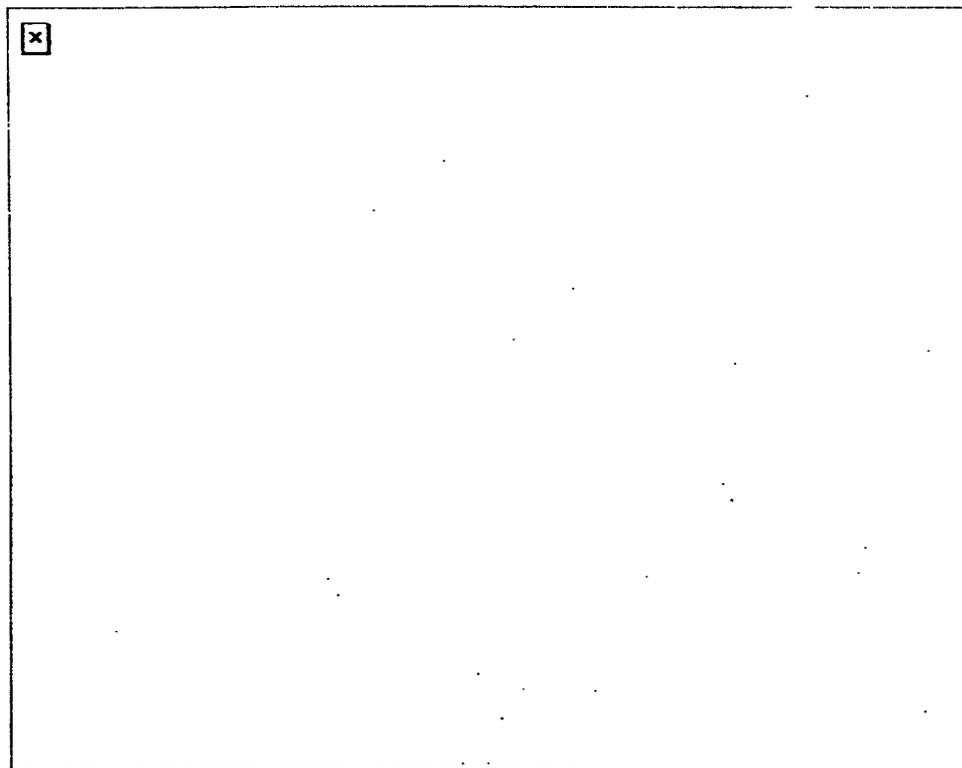
In an ongoing prospective study on 15,000 French people, it has been found that saturated fatty acids supply 17 % of calories, mono-unsaturated 14 % and polyunsaturated 6 % :

g/day	Men	Women
Total lipids	100.4	78.6
MUFA g/day	37.8	29.4
PUFA g/day	16.6	12.7
SFA g/day	44.8	35.6

Suvimax 1998

The ratio n-6/n-3 fatty acids is probably in the range of 15-20.

Over the last 30 years, recommendations have been changing in relation to the type of fatty acid to be included in the human diet and the total amount of fat suggested. The Danone group has been closely following this trend and has tried to adapt its products to the recent recommendations.



Trend in recommendations in fatty acids ratio : Danone approach

The present conclusion in France is that the population consumes too much saturated fat as well as too much n-6 polyunsaturated fatty acids.

Thus, the food industry has to change the fatty acid composition of its products to readjust the intake of fatty acids in the French population. Instead of using butter, tallow or different oils, canola can be utilized. Canola oil is a typical example of a fat containing a small amount of saturated fatty acids, and supplying both n-6 and n-3 polyunsaturated fatty acids in a proper ratio to counterbalance the fatty acid intake from meat, dairy products and other sources.

Thus, it seems fundamental that the experts in nutrition express clear recommendations in the field of fats and fatty acids, in relation to public health, since we, the food industry, will follow their recommendations.

Advantages and Disadvantages of the Use of Flax Seed as a Source of Omega-3

Paul A. Stitt,

Enreco, Inc. P. O. Box 730, Manitowoc, Wisconsin 54221, USA

Flax seed is presently being used worldwide as a source of Omega-3 in human and companion animals' diets. History of the use of flax seed as food for humans goes back 2,000 years. Flax seed has several distinct advantages and disadvantages as a source of Omega-3.

Disadvantages of flax seed include such factors as:

1. Presence of "Anti B-6" factor.

2. Presence of Cyanogenic Diglycosides.
3. Unstable after being ground.
4. Contains only short chain Omega-3.

Advantages of flax seed include such factors as:

1. High concentration of alpha-linolenic acid.
2. Presence of powerful anti-oxidants in some varieties.
3. Presence of high levels of soluble and insoluble fiber.
4. Presence of high levels of lignans that have anti-estrogenic properties.
5. FDA states, "no objection" as a food.
6. Desirable flavor in most foods.

Omega-3 LC-PUFA - from a Health Concept to Foods in the Shelves

R. Muggli,, Ph.D.,

F. Hoffmann-La Roche Ltd, CH-4070 Basel, Switzerland

Incorporating long-chain polyunsaturated fatty acids (LC-PUFA) into the diet, continues to be a topic of interest among food manufacturers. Nutritionists believe that addition of omega-3 LC-PUFA - eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) - to the diet would result in better nutrition and assist in chronic disease prevention. General scientific opinion appears to be that as little as 200-1000 mg of EPA/DHA may achieve this dietary goal.

The refining technology for marine oils has advanced to the degree that, with due care, careful handling and proper precautions, it is now possible to produce a variety of foodstuffs fortified with omega-3 LC-PUFA that taste as good as similar, unfortified products.

At the forefront of developments are infant formula and baby follow-on food in Europe and the Far East. In addition, breads, margarines (or other low-fat spreads), UHT milks, yogurts, fruit juices and beverages have started to enter the mainstream in Europe. Niche products such as soups, salad dressings, mayonnaise, ice tea drinks, cakes, biscuits and the restoration of omega-3 LC-PUFA to canned seafood and tuna are being launched.

Despite of this growing list, many food manufacturers are still reluctant to develop products fortified with omega-3 LC-PUFA due to the following non-technical barriers:

Recommendations: There are no officially recognized intake recommendations for omega-3 LC-PUFA. The food manufacturer has no standard of reference for the nutritional value or dietary fortification levels of omega-3 LC-PUFA.

Claims: No product label or health claims are permitted by the FDA and other regulatory authorities, which makes it extremely difficult to market a food with omega-3 LC-PUFA.

Safety: There is an unwarranted fear of allergenicity and the possible effects of omega-3 LC-PUFA on bleeding and insulin resistance. There are numerous reports that such adverse reactions do not occur even at the maximum dosages of omega-3 LC-PUFA which are considered to be health beneficial (1-2g).

Awareness: The awareness about the health benefits of omega-3 LC-PUFA is generally poor among consumers. The closing of this knowledge gap is made difficult by the bewildering number of and sometimes complicated names for the family of omega-3 LC-PUFA and its members e.g. PUFA, HUFA, n-3 LCP, omega-3 LC-PUFA, EPA, DHA, eicosapentaenoic acid, docosahexaenoic acid.

To make omega-3 LC-PUFA a standard food ingredient it is imperative that food industry suppliers, food manufacturers and professional organizations (such as ISSFAL) work hand in hand to remove these obstacles by providing authorities, health professionals and the public with truthful, scientifically valid information about the health benefits of omega-3 LC-PUFA.

Infant Formulas with no DHA or ARA. Are They Causing Harm?

David J. Kyle, Ph.D.

Martek Biosciences Corporation, Columbia, Maryland, USA

Over the past twenty years there have been a large number of retrospective studies comparing the neurological outcomes of breast-fed and formula-fed infants. A recent meta-analysis of the most relevant of these studies has indicated that there is a consistent 3-4 IQ point advantage to the breast-fed infants even after the contributions of all other confounding factors had been removed. Breast-fed babies, however, are getting many nutrients from the breast milk in addition to docosahexaenoic acid (DHA) and arachidonic acid (ARA) and many have argued that the contribution of DHA and ARA is

inconclusive. One observation, however, is very clear and consistent. Infants who are provided standard infant formula have significant deviations in their blood and brain biochemistry relative to the breast-fed babies. Full term infants fed unsupplemented formulas have a circulating DHA status (as indicated by red blood cell or plasma phospholipid DHA levels) of less than one-half that of the breast-fed infant. Furthermore, the brain DHA levels of formula-fed infants are about one-third lower than those of

breast-fed infants.

Since some groups have also argued that such changes in the blood and brain biochemistry in the formula-fed infant is irrelevant, it has been critically important to more fully understand the function of DHA in the tissues of the body. Recent studies have revealed that DHA has many critical functions in the normal development and metabolism of neuronal cells. These include, but are not limited to, the following: 1) the control of normal migration of neurones from the surface of the ventricles of the brain to the cortical plate during development; 2) the control of the normal resting potential of the neurone by regulation of sodium and calcium channels; 3) the regulation of the density of certain membrane proteins such as rhodopsin in the retina and, possibly, 4) the regulation of levels of certain neurotransmitters such as serotonin. With such key roles in normal neuronal development and function, it is quite plausible that abnormally low levels of this primary nutrient during the development of the brain may be one cause of the long term neurological detriments observed in formula-fed infants relative to breast-fed infants.

The final proof of the importance of DHA in early infant nutrition, however, comes not from demonstrating that the long term neurological outcome of formula-fed infants is poorer than breast-fed infants, or that this poor outcome is correlated with a DHA deficiency early in life, but from interventional studies which demonstrate that when the DHA deficiency is removed, the neurological outcomes revert to normal. There have been at least 24 well-controlled studies involving over 2,000 infants in the last 15 years (12 studies with term infants and 12 studies with pre-term infants) which have compared outcomes of standard formula-fed infants with DHA-supplemented formula-fed infants. In every study the DHA status of the infants was returned to normal (as defined by the DHA status of the breast-fed infants) when the formulas were supplemented with DHA. In all of these studies, except where fish oil was used as a source of DHA, the ARA levels were also normalized because of the use of supplemental ARA in the formulas. In several studies, precursors such as gamma-linolenic acid (GLA) or alpha-linolenic acid (ALA) were added to the formulas in an attempt to elevate ARA or DHA levels respectively. Even when added in significant excesses over what is found in breast milk however, these precursors did not elevate the DHA and ARA levels to those of the breast-fed infant. That is, the precursors do not adequately substitute for the preformed DHA and ARA provided in mother's milk. Of all the trials completed with DHA/ARA supplementation, single cell oils (SCO's) were used with the largest numbers of babies (45% with SCO's, 35% with egg yolk; and 20% with various fish oils).

Of the 24 DHA/ARA supplementation studies mentioned above, only 12 looked for functional outcomes differences (i.e., visual, neurological, or developmental assessments). Seven of those 12 studies reported statistically significant deficits in standard formula-fed babies compared to breast-fed babies (the gold standard). In all 7 cases, those deficits were normalized with the DHA/ARA supplementation. Of the remaining 5 studies, no statistically significant differences could be found between formula-fed and breast-fed babies using the test metrics employed in those studies and, therefore, no effect of DHA/ARA supplementation was observed.

The totality of these observations provide strong evidence that DHA is a critical nutritional requirement for the newborn infant and that an early deficiency of DHA could lead to long term neurological deficiencies. Given our present state of understanding, it is quite possible that the lack of availability of DHA and ARA-supplemented infant formulas in the United States and Canada today may be putting formula-fed newborn babies at risk. Since the only way that newborn babies in the United States and Canada can get DHA and ARA today is from their mother's milk, we must use our best efforts to encourage new mothers to nurse their babies for as long as possible to avoid potential long term neurological deficits to the child.

Clinical Safety Studies of LCPUFA Supplementation of Premature and Term Infant Formulas

Deborah A. Diersen-Schade, Ph.D., James W. Hansen, M.D., Ph.D.,

Kimberly L. Merkel, and Cheryl L. Harris

Mead Johnson Research Center, Evansville, Indiana, USA

Introduction: Many studies support a need for long chain polyunsaturated fatty acids (LCPUFA), and particularly docosahexaenoic acid (DHA, 22:6n-3), for optimal retinal and neural development in early infancy. Human milk contains LCPUFA, including DHA and arachidonic acid (ARA, 20:4n-6), but U.S. infant formulas do not. We have now completed two of the largest clinical trials of LCPUFA supplementation, one with very low birth weight infants and a second with healthy full term infants.

Premature Infant Study: DHA supplementation has been shown to enhance visual development of preterm infants, but some studies found decreased growth when DHA was provided without ARA. **Objectives:** (1) To establish the safety of feeding DHA and ARA from single cell oils to preterm infants and (2) to determine effects on visual acuity. **Design:** In a double-blind, controlled, multi-center trial, 194 preterm infants were randomized to preterm formulas differing only in fatty acid content: no DHA or ARA (control), 0.15 % (of energy) DHA, or 0.14 % DHA + 0.27 % ARA. Preterm formulas were fed for at least 28 days; all preterm infants then received unsupplemented term formula. Ninety breast-fed term infants were enrolled as a reference group. **Results:** Growth suppression was not seen in the DHA or DHA+ARA groups; in fact, post-hoc analyses indicated that weight gain of DHA+ARA infants was significantly enhanced compared to control. Weight of DHA+ARA infants was not different from breast-fed term infants at 48 and 57 wk postmenstrual age (PMA), but weight of control and DHA infants remained significantly less than breast-fed term infants through 57 wk PMA. There were no significant differences between preterm groups in incidence of serious adverse events, NEC/suspected NEC, or sepsis/suspected sepsis. Visual acuity determined by Teller Acuity Cards (TAC) at 48 and 57 wk PMA did not differ among preterm groups. **Conclusions:** Single cell oils are safe for use in preterm infant formulas to provide DHA and ARA at human milk levels. Providing DHA plus ARA enhances catch-up growth of premature infants; however, supplementation for 28 days did not affect TAC acuity 3 and 5 months later.

Term Infant Study: Studies of LCPUFA supplementation of formula-fed term infants have shown equivocal effects on visual and cognitive development, but several recent studies with typical human

milk levels of DHA have found beneficial effects. Because term formulas may be fed for a full year, the safety of LCPUFA supplementation over this time period must be established. **Objectives:** (1) To establish the safety of feeding DHA from single cell and fish oil sources, each in combination with ARA from single cell oil, to term infants to a year of age and (2) to evaluate effects of supplemented formula on visual acuity and mental and psychomotor development. **Design:** In a double-blind, multi-center trial, 383 term infants were randomized to formulas differing in fatty acid content: no LCPUFA (control), 0.15% (of energy) DHA and 0.3% ARA from single cell oils, or 0.15% DHA from fish oil and 0.3% ARA from single cell oil. **Results:** Weight gain from day 14 to days 60 or 120 was not significantly less in supplemented groups compared with the control. Furthermore, post-hoc analyses indicated that supplemented infants had larger growth rates than control infants from 14 to 60 and 120 days. No differences were observed in mean weight, length or head circumference at 180, 270, or 365 days; in formula acceptance and tolerance; or in incidence of serious adverse events. No differences were observed in visual acuity (TAC) at 120, 180, and 365 days or in Bayley MDI and PDI scores at 365 days, although Bayley scores were somewhat higher in supplemented groups than in the control. **Conclusions:** DHA from single cell oils and ARA from single cell oil are safe for use in term infant formulas when fed at human milk levels for a full year. Supplementation with DHA and ARA increased early growth of term infants, similar to our findings with preterm infants, but did not significantly affect TAC acuity or mental or psychomotor development.

Overall Conclusions: Our large clinical trials, along with numerous other clinical and toxicology studies, demonstrate the safety of adding typical human milk levels of DHA and ARA to both premature and term infant formulas over the time periods these formulas are typically fed. While our trials did not find significant benefits of LCPUFA supplementation for visual and cognitive development, we did find increased growth in both premature and term infants supplemented with DHA plus ARA. This increased growth may be particularly important with regards to enhancing catch-up growth of infants born prematurely.

Omega-3 Long Chain PUFA ñ Closing the Nutritional Gap

J. Boudreau

Ocean Nutrition Canada Ltd, 757 Bedford Highway, Bedford, Nova Scotia

Significant research shows that the populations of many industrialized nations, including the U.S., consume significantly lower levels of omega-3 long chain PUFA than science shows is required for maintaining good health. There needs to be a concerted effort by industry, government and the scientific community to ensure that this nutritional gap is eliminated.

Trends - The Time to Act is Now

There is significant momentum and steam building that highlights the need for a cooperative effort in ensuring that the populations benefit from the improved science and manufacturing capabilities now in place.

Some of the important trends taking shape include:

? The improved manufacturing capabilities that permit the fortification of good tasting, stable food products as seen in many parts of the world;

? The ability to manufacture highly concentrated oils that can be delivered as adjunctive therapies;

? The increasing awareness (54% - Applied Biometrics, October 1998) of consumers with respect to the health benefits of omega-3 that now needs to be converted into usage;

? Improved collaboration between industry and science;

? Improved science showing the benefits of increased consumption of omega-3 LC-PUFA.

Steps to Success

In order to ensure that consumers benefit from the science, it is going to be essential that officially recognized intake levels are set for omega-3 LC-PUFA. Omega-3 LC-PUFA will not gain mass-market acceptance or incorporation into standard food channels until the manufacturers have an officially recognized reference point and/or the ability to make an approved health claim.

Key steps to success:

? Establish officially recognized intake recommendations for omega-3 LC-PUFA that manufacturers can reference on the label;

? An FDA-approved health claim for omega-3 LC-PUFA with reference to cardiovascular health and triglyceride lowering;

? A better understanding of the correct omega-6 to omega-3 ratios and the upper and lower limits based on age and health status;

? The standardization of analytical methods to ensure consumers and industry are able to make true product comparisons against the science;

? Quality standards enforced to ensure that consumers are not exposed to substandard product with contaminants or oxidative problems.

To make omega-3 LC-PUFA a standard food ingredient, the time to act is now. We need to form partnerships between industry suppliers, food manufacturers, professional organizations and the government. The goal is to utilize the present market conditions in an effort to ensure that consumers are given the best opportunity at better nutrition through the proper balance and total consumption of omega-3 LC-PUFA.

Safety of Omega-3 Products Based on Fish Oil as Starting Material

Morten Bryhn, M.D., Ph.D. and Bjorn Rene

Pronova Biocare, Sandefjord, Norway

Pronova is the largest producer of omega-3 products in the world today with products ranging from pharmaceuticals via medium concentrated food supplements to refined crude fish oil.

Convincing monitoring of safety is only possible in controlled clinical studies, preferably by so-called Good Clinical Practice studies. The present database of patients in controlled studies on active treatment comprises more than 9000 individuals mainly in long-term studies of more than one year; 60% more than 3.5 years. This study population consists of patients with chronic diseases related to the cardiovascular and renal system but also diabetics with age ranging from early adolescence up to 70 years and more.

Today we have no report of serious adverse effects, whatsoever. Even if bleeding time has been prolonged with omega-3 products, there are no reports of serious bleeding events even in patients on concomitant medication with Aspirin or Warfarin. Adverse effects are seen in 10-20% of the patients in studies mainly originating from the GI tract. Eructation of fishy taste is the most common finding. Interestingly, the frequency of eructation is the same in the placebo group receiving corn oil as in the active treatment group indicating that eructation is a function of ingesting oil in general. Studies including diabetics with a total number of approximately 1500 patients have not shown derangement of diabetic control. Patients with chronic renal disease, renal failure and even transplanted patients on chronic cyclosporin medication have not shown any systemic adverse effects but rather an improvement of renal function. In studies on pregnant women there have been no bleeding complications and the amount of bleeding during labour has not been significantly different from controls.

The regulatory authorities in countries like the US and several EU countries have examined the safety file of the pharmaceutical, Omacor, and there have been no major objections. Omacor is a registered pharmaceutical in several EU countries and an application for an NDA in the US is planned for later this year. At the recent American College of Cardiology meeting in New Orleans, the results of GISSI Prevention were presented. This is a study including 11,324 post-MI patients comparing 1g of Omacor, vitamin E and the combination with a control group. All patients were optimally treated with aspirin, beta-blockers, statins, etc. The Omacor group but not vitamin E showed a 20% reduction of mortality, and treatment was very well tolerated. Conducted by the prestigious Mario Negri Institute of Milan, Italy, this study is the most important documentation of efficacy and safety for any omega-3 product in the world today.

An interesting adverse report from one patient on omega-3 treatment in Houston, USA was an "urge to swim". We take this more as a joke but we would like to use this metaphor claiming that products using fish oil as starting material, and therefore containing both EPA and DHA, are the state-of-art today and based on a natural dietary principle and accepted by regulatory authorities as safe during long term use. Pure DHA products are expensive and the DHA content will readily be retro-converted to EPA in humans to meet metabolic needs. Mechanistic studies on separate effects of EPA or DHA will have to be conducted in *in vitro* systems but the results will have only minor impact on therapy traditions using omega-3 products introduced today.

In conclusion, Pronova, as the world's largest producer of omega-3 products using fish oil as starting material, holds the largest database on safety as well as efficacy in patients and healthy individuals today. These products are regarded as safe when used either as pharmaceuticals, food supplements, or

in fortification of food products.

Workshop Participants

William R. Barclay, Ph.D.

OmegaTech, Inc.

Boulder, Colorado, USA

Mark A. Bieber, Ph.D.

Bestfoods North America

Somerset, New Jersey, USA

Eileen Birch, Ph.D.

Retina Foundation of the Southwest

University of Texas Southwestern Medical Center

Texas, USA

Jacques Boudreau

Ocean Nutrition Canada, Ltd.

Bedford, Nova Scotia, CANADA

William D. Clay

Food and Nutrition Division

Food and Agriculture Organization of the United Nations

Rome, ITALY

Rebecca Costello, Ph.D.

Office of Dietary Supplements

National Institutes of Health

Bethesda, Maryland, USA

Jerry M. Cott, Ph.D.

National Institute of Mental Health

National Institutes of Health

Bethesda, Maryland, USA

Linda Crafts

National Institute on Alcohol Abuse and Alcoholism-NIH

Bethesda, Maryland, USA

Raffaele De Caterina, M.D., Ph.D.

Lab for Thrombosis and Vascular Research

CNR Institute of Clinical Physiology

Pisa, ITALY

Deborah Diersen-Schade

Mead Johnson & Company

Evansville, Indiana, USA

S. Boyd Eaton, M.D.

Emory University

Atlanta, Georgia, USA

Claudio Galli, M.D.

Universita di Milano

Istituto Scienze Farmacologiche

Milano, ITALY

Klaus Gawrish, Ph.D.

National Institute on Alcohol Abuse and Alcoholism-NIH

Bethesda, Maryland, USA

Enoch Gordis, M.D.

National Institute on Alcohol Abuse and Alcoholism-NIH

Bethesda, Maryland, USA

Gilman Grave, M.D.

National Institute of Child Health and Human Development

Bethesda, Maryland, USA

Tomohito Hamazaki, M.D., Ph.D.

Research Institute of Wakan-Yaku

Toyama Medical & Pharmaceutical University, Toyama, JAPAN

Harald S. Hansen, Ph.D., D. Sc.

The Royal Danish School of Pharmacy

Institute of Biological Sciences

Copenhagen, DENMARK

James W. Hansen, M.D., Ph.D.

Mead Johnson & Company

Evansville, Indiana, USA

William S. Harris, Ph.D.

Mid America Heart Institute

Saint Luke's Hospital

Kansas City, Missouri, USA

William C. Heird, M.D.

Children's Nutrition Research Center

Baylor College of Medicine

Houston, Texas, USA

Joseph R. Hibbeln, M.D.

National Institute on Alcohol Abuse and Alcoholism-NIH

Bethesda, Maryland, USA

Bruce J. Holub, Ph.D.

Department of Nutritional Sciences

University of Guelph

Guelph, Ontario, CANADA

Peter R.C. Howe, Ph.D.

Department of Biomedical Science

University of Wollongong

Wollongong, New South Wales, AUSTRALIA

Peter J. Huth, Ph.D.

Kraft Foods

Glenview, Illinois, USA

Robert Katz, Ph.D.

Omega-3 Research Institute

Bethesda, Maryland, USA

Hee-Yong Kim, Ph.D.

National Institute on Alcohol Abuse and Alcoholism-NIH

Bethesda, Maryland, USA

David J. Kyle, Ph.D.

Martek Biosciences Corporation

Columbia, Maryland, USA

William E. Lands, Ph.D.

National Institute on Alcohol Abuse and Alcoholism-NIH

Rockville, Maryland, USA

Dominique Lanzmann-Petithory, M.D.

Groupe Danone

Centre Jean Theve

Athis Mons, FRANCE

Alexander Leaf, M.D.

Massachusetts General Hospital

Charlestown, Massachusetts, USA

Burt Litman, Ph.D.

National Institute on Alcohol Abuse and Alcoholism-NIH

Bethesda, Maryland, USA

Roberto Marchioli, M.D.

Clinical Pharmacology and Epidemiology

Consorzio Mario Negri Sud

Santa Maria Imbaro (CH) ITALY

Deanna McCarthy, R.D.

OmegaTech, Inc.

Boulder, Colorado, USA

Reto Muggli, Ph.D.

F. Hoffmann-La Roche Ltd

Basel, SWITZERLAND

Gary J. Nelson, Ph.D.

USDA, ARS

Western Nutrition Research Center

San Francisco, California, USA

Martha Neuringer, Ph.D.

Department of Medicine and Ophthalmology

Oregon Health Sciences University

Beaverton, Oregon, USA

Ian Newton, Ph.D.

Roche Vitamins Inc.

Parsippany, New Jersey, USA

Sandra Ohnesorg

BASF Health & Nutrition A/S

Ballerup, DENMARK

Harumi Okuyama, M.D.

Nagoya City University

Faculty of Pharmaceutical Sciences

Nagoya, JAPAN

Robert Orr

Ocean Nutrition Canada, Ltd.

Bedford, Nova Scotia, CANADA

Manuel Peña, M.D.

Pan American Health Organization

Pan American Sanitary Bureau

Washington, D.C., USA

Roshini Ponnampereuma

The Center for Genetics, Nutrition and Health

Washington, D.C., USA

Serge Renaud, M.D.

INSERM, Epidemiologie,

Sante Publique et Developpement

Bordeaux, FRANCE

Bjorn Rene, Ph.D.

Pronova Biocare, A.S.

Sandefjord, NORWAY

Ray Rice, Ph.D.

International Society for the Study of Fatty Acids and Lipids

Tiverton, Devon, UNITED KINGDOM

Peggy Roberts

The Center for Genetics, Nutrition and Health

Washington, D.C., USA

Norman Salem, Jr., Ph.D.

National Institute on Alcohol Abuse and Alcoholism-NIH

Rockville, Maryland, USA

Wayne Sander

● OmegaTech, Inc.

Boulder, Colorado, USA

Artemis P. Simopoulos, M.D.

The Center for Genetics, Nutrition and Health

Washington, D.C., USA

Andrew Sinclair, Ph.D.

Department of Food Science, RMIT

Melbourne, Victoria, AUSTRALIA

Arthur A. Spector, M.D.

Department of Biochemistry

The University of Iowa, College of Medicine

● Iowa City, Iowa, USA

Paul Stitt, Ph.D.

Essential Nutrient Research Company

Manitowoc, Wisconsin, USA

Andrew Lawrence Stoll, M.D.

McLean Hospital

Belmont, Massachusetts, USA

P. Willatts, Ph.D.

Department of Psychology

University of Dundee

Dundee, UNITED KINGDOM

● Herbert Woolf

BASF Corporation

Mount Olive, New Jersey, USA

Vernon R. Young, Ph.D., D. Sc.

Nutritional Biochemistry

School of Science

Massachusetts Institute of Technology

Cambridge, Massachusetts, USA

Index

Speakers Page

Birch, E 17

Boudreau, J 42

Clay, WD 33

De Caterina, R 21

Eaton, SB 11

Galli, C 25

Hamazaki, T 17

Hansen, JW 40

Harris, WS 23

Heird, WC 14

Holub, BJ 28

Kyle, DJ 39

Lanzmann-Petithory, D 34

Leaf, A 20

Marchioli, R 25

Muggli, R 37

Nelson, GJ 31

Neuringer, M 15

Okuyama, H 29

Peña, M 32

Renaud, S 22

Rene, B 43

Salem, NS 13

Sinclair, AJ 10

Spector, AA 8

Stitt, PA 36

Stoll, AL 19

Woolf, H 34

Young, VR 8