

Long-chain polyunsaturated fatty acids in children with attention-deficit hyperactivity disorder^{1,2}

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ABSTRACT Attention-deficit hyperactivity disorder (ADHD) is the diagnosis used to describe children who are inattentive, impulsive, and hyperactive. ADHD is a widespread condition that is of public health concern. In most children with ADHD the cause is unknown, but is thought to be biological and multifactorial. Several previous studies indicated that some physical symptoms reported in ADHD are similar to symptoms observed in essential fatty acid (EFA) deficiency in animals and humans deprived of EFAs. We reported previously that a subgroup of ADHD subjects reporting many symptoms indicative of EFA deficiency (L-ADHD) had significantly lower proportions of plasma arachidonic acid and docosahexaenoic acid than did ADHD subjects with few such symptoms or control subjects. In another study using contrast analysis of the plasma polar lipid data, subjects with lower compositions of total n-3 fatty acids had significantly more behavioral problems, temper tantrums, and learning, health, and sleep problems than did those with high proportions of n-3 fatty acids. The reasons for the lower proportions of long-chain polyunsaturated fatty acids (LCPUFAs) in these children are not clear; however, factors involving fatty acid intake, conversion of EFAs to LCPUFA products, and enhanced metabolism are discussed. The relation between LCPUFA status and the behavior problems that the children exhibited is also unclear. We are currently testing this relation in a double-blind, placebo-controlled intervention in a population of children with clinically diagnosed ADHD who exhibit symptoms of EFA deficiency. *Am J Clin Nutr* 2000;71(suppl):327S-30S.

KEY WORDS Essential fatty acids, EFA, attention-deficit hyperactivity disorder, ADHD, thirst, dry skin, frequent urination, n-3 fatty acids, arachidonic acid, 20:4n-6, docosahexaenoic acid, 22:6n-3, children

INTRODUCTION

Children with attention-deficit hyperactivity disorder (ADHD) have problems paying attention, listening to instructions, and completing tasks; they also fidget and squirm, are hyperactive, blurt out answers, and interrupt others. These behaviors may severely affect school performance, family relations, and social interactions with peers. Roughly 20–25% of children with ADHD show one or more specific learning disabilities in math, reading, or spelling (1). Children with ADHD often have trouble performing academically and paying attention, and may be dis-

organized, have poor self-discipline, and have low self-esteem. A conservative estimate is that 3–5% of the school-age population has ADHD (2). Treatments for ADHD include behavior therapy and medications (3). Psychostimulant drugs and antidepressants are often used to calm children with ADHD, with an effectiveness rate of ≈75% (4). The advantages of using these medications include rapid response, ease of use, effectiveness, and relative safety. Disadvantages include possible side effects, including decreased appetite and growth, insomnia, increased irritability, and rebound hyperactivity when the drug wears off (5). However, these medications do not address the underlying causes of ADHD. Thus, studies to elucidate the potential contributors to the behavior problems in ADHD may lead to more effective treatment strategies for some children.

Linoleic acid (LA; 18:2n-6) and α-linolenic acid (18:3n-3) are essential fatty acids (EFAs) and must be consumed in the diet because humans and most other mammals lack the ability to synthesize them (6). These fatty acids are converted to a variety of longer, more highly polyunsaturated products that maintain cell membrane structure and are a source of material for the formation of localized hormones called eicosanoids, which are involved in almost every biologically significant process in the body (7). Additionally, n-3 fatty acids are specifically implicated in maintaining central nervous system function. Deficiency of n-6 fatty acids leads to impaired growth, dry and scaly skin, polydipsia, and polyuria, among other symptoms (8, 9). Deficiency of n-3 fatty acids in rats and monkeys is associated with behavioral, sensory, and neurologic dysfunction (10–12).

FATTY ACID STATUS OF CHILDREN WITH ADHD

Several studies have focused on essential fatty acid metabolism in children with ADHD. Children with hyperactivity have been reported to be more thirsty than normal children and have symptoms of eczema, asthma, and other allergies (13). In a cross-sectional study in 6–12-y-old boys recruited from central Indiana, we showed that 53 subjects with ADHD had significantly lower proportions of key fatty acids in the plasma polar lipids

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[arachidonic acid (AA; 20:4n-6), eicosapentaenoic acid (20:5n-3), and docosahexaenoic acid (DHA; 22:6n-3)] and in red blood cell total lipids (20:4n-6 and 22:4n-6) than did 43 control subjects (14). This finding agrees with a previous report (13). However, a further finding was that a subgroup of 21 subjects with ADHD who exhibited a greater frequency of EFA deficiency symptoms (thirst, frequent urination, and dry hair) (40% of the sample) had significantly lower plasma proportions of AA and 22:6n-3 than did the other 32 subjects with ADHD (60% of the sample), who had few symptoms of EFA deficiency (Table 1). Moreover, the plasma fatty acid composition in the subjects with few symptoms was not significantly different from those in the control sample. This is important because previous studies that tested the role of fatty acid supplementation in treating ADHD did not report selection on the basis of EFA status or frequency of symptoms (15, 16).

n-3 AND n-6 FATTY ACIDS AND BEHAVIOR PROBLEMS

The relation between the type of fatty acid, n-3 or n-6, and behavioral symptoms has not been studied in children with ADHD. We found, by further analysis of the data for all of the subjects in our cross-sectional study, an inverse relation between total plasma n-3 fatty acid proportions and behavioral assessment scores (Conners' Parent Rating Scale) and teacher scores of academic abilities (17). This was not the case for n-6 fatty acids. However, low plasma proportions of both types of fatty acids were associated with a higher frequency of symptoms indicative of EFA deficiency. These results support a relation between n-3 fatty acid status and behavior in children that parallels the relation observed in rats (10) and monkeys (11). Previous intervention studies have used a source of n-6 fatty acids (evening primrose oil) to treat behavior problems and reported variable and unsuccessful results (15, 16, 18). However, because n-3 fatty acids appear to be the fatty acids important for brain and visual function (19, 20) and n-6 fatty acid supplementation does not improve n-3 fatty acid status, evening primrose oil supplementation alone would not be expected to improve behavior.

POTENTIAL CAUSATIVE FACTORS FOR THE LOWER COMPOSITION OF LCPUFAS IN SOME CHILDREN WITH ADHD

One would not expect to find that a single cause or even a handful of factors could explain why ADHD appears to be so rampant in our society. Because it is accepted that both genetic and environmental factors play a role in ADHD, many other factors—both intrinsic and extrinsic—could influence an individual's fatty acid status. Applying this argument to the subpopulation of children with ADHD who exhibit frequent symptoms of EFA deficiency and low LCPUFA status (L-ADHD), we considered several factors that might contribute to the lower proportion of AA and DHA observed in the plasma phospholipids of these children. Although many other potential explanations are possible, we considered 1) intake-related factors such as marginal consumption of EFA, 2) inefficient conversion to LCPUFA from EFA, and 3) enhanced metabolism of LCPUFAs.

Fatty acid intake

Many studies have related protein or total energy deficit with behavior or cognitive problems. A deficiency of specific

TABLE 1

Plasma phospholipid fatty acid composition of control subjects and subjects with attention-deficit hyperactivity disorder reporting few (ADHD group) or many (L-ADHD) symptoms indicative of essential fatty acid deficiency¹

Fatty acid	Control (n = 43)	ADHD (n = 32)	L-ADHD (n = 21)
	<i>% of total fatty acids</i>		
16:0	24.10 ± 1.60	23.60 ± 1.10	23.10 ± 1.70
18:0	14.80 ± 1.00	14.60 ± 0.90	14.40 ± 1.20
18:1-9	11.10 ± 1.40	11.60 ± 1.60	12.00 ± 1.60
n-6			
18:2n-6	22.50 ± 2.40	22.90 ± 2.40	23.30 ± 3.30
18:3n-6	0.07 ± 0.13	0.04 ± 0.09	0.20 ± 0.60
20:2n-6	0.51 ± 0.17	0.49 ± 0.19	0.50 ± 0.21
20:3n-6	3.00 ± 0.60	3.12 ± 0.69	3.16 ± 0.71
20:4n-6	11.00 ± 1.40 ^a	10.80 ± 1.40 ^a	9.60 ± 1.13 ^b
22:4n-6	0.65 ± 0.17	0.62 ± 0.23	0.57 ± 0.21
22:5n-6	0.57 ± 0.17	0.57 ± 0.20	0.49 ± 0.22
Total	37.80 ± 2.00	38.10 ± 2.30	37.30 ± 2.80
n-3			
18:3n-3	0.06 ± 0.09	0.03 ± 0.07	0.05 ± 0.10
20:5n-3	0.24 ± 0.21	0.17 ± 0.24	0.12 ± 0.16
22:5n-3	1.31 ± 0.25	1.26 ± 0.17	1.22 ± 0.32
22:6n-3	2.04 ± 0.58 ^a	1.92 ± 0.50 ^a	1.58 ± 0.28 ^b
Total	3.64 ± 0.66 ^a	3.38 ± 0.67 ^a	2.98 ± 0.36 ^b
n-6:n-3 ratio	10.66 ± 1.80 ^a	11.64 ± 2.20 ^a	12.70 ± 1.80 ^b
Precursor:metabolite ²	1.50 ± 0.29 ^a	1.54 ± 0.29 ^a	1.70 ± 0.37 ^b

¹ $\bar{x} \pm SD$. Group means with different superscript letters are significantly different, $P < 0.05$ (ANOVA with Student Neuman Keuls post hoc test). Data from reference 14.

²Ratio of 18:2n-6 to the sum of 18:3n-6, 20:3n-6, 20:4n-6, 22:4n-6, and 22:5n-6.

m micronutrients (eg, iron, iodine, and vitamin A) has also been related to behavioral changes (21-24). These studies reported on populations at risk in developing countries and may have been confounded by other factors such as parental socioeconomic status, education, or intelligence. Environmental factors, such as general living conditions, repeated infections, and nutrient interactions, may also confound results. Poor quality of the total diet in these groups is probably more of a problem than are specific nutrient deficiencies. A diet low in energy, protein, or both would probably be low in specific long-chain fatty acids as well.

In rats, malnutrition in the prenatal or immediate postnatal period is sufficient to produce permanent alterations in brain structure, resulting in enduring changes in behavior (25, 26). Nutrient supplementation of human infants has shown beneficial results on cognitive function (23, 27). Studies have shown that children who had formerly received mother's milk scored better on tests of performance after 5 y than did those who had not (28). It has been suggested that malnutrition leaves the brain sufficiently intact to function under stable conditions, but enduring changes that may alter an individual's susceptibility to affective disorders become evident under stressful conditions (25, 26).

Although there is no lack of available nutrients in the Western world, a primary deficiency may occur because of insufficient nutrient intake. With the emphasis on low-fat diets for control of various chronic diseases, intake of foods containing long-chain fatty acids may be unnecessarily restricted. Also, intake may be affected because of food allergies or intolerance to certain foods,



resulting in a primary deficiency of long-chain fatty acids. Our original analysis of all 21 L-ADHD subjects did not indicate that primary EFA deficiency caused the observed low LCPUFA concentrations (14). Primary EFA deficiency is indicated by a low proportion of LA in both plasma phospholipids and red blood cells and a proportion of Mead acid (6) $>0.21\%$ of total fatty acids (29). Although occasional examples indicating borderline primary EFA deficiency could be found within the L-ADHD population, most of the children did not fit this profile.

Inefficient conversion of EFAs to LCPUFAs

A second possible cause for the low LCPUFA status of the L-ADHD group may be impaired conversion of the fatty acid precursors LA and α -linolenic acid to their longer and more highly unsaturated products. Possible sites of this inefficiency include the desaturase steps, the malonyl-CoA-dependent elongation steps, and the peroxisomal β -oxidation steps. Indirect evidence for such inefficiencies comes from studies in newborns and in individuals with inherited peroxisomal disorders, neural ceroid-lipofuscinosis, and retinitis pigmentosa (30). Evaluation of the blood fatty acid profiles of the L-ADHD population indicated that the inefficient conversion of LCPUFA precursors may have been a contributing factor to the low proportions of AA and DHA in the blood of these children. The ratio of precursors to metabolites for $n-6$ fatty acids was greater in the L-ADHD children than in both the ADHD children reporting few EFA deficiency symptoms and the control children (Table 1).

Interestingly, the children with ADHD were breast-fed less often as infants than were the control children (14). The relation between this finding and the behavioral problems that these children exhibit is not clear. Newborns, both preterm and full term, are inefficient at converting precursors to LCPUFAs, and newborns and infants fed formula lacking LCPUFAs have lower proportions of red blood cell AA and DHA than do breast-fed infants (31, 32). Breast milk contains adequate LCPUFAs and several studies have shown that visual and brain functions mature faster in breast-fed than in bottle-fed infants (33–35). Delayed brain maturation is one factor implicated in the development of ADHD (36). LCPUFAs may have a role to play in this process. Several studies examined the relation between infant feeding and long-term cognitive development. Early childhood breast-feeding was reported to be associated with more advanced cognitive development in later childhood (37, 38). DHA is the hypothetical factor responsible for this cognitive development. Perhaps infant exposure to preformed DHA facilitates brain maturation and stimulates LCPUFA biosynthetic enzymes as well.


Enhanced metabolism

Another possible explanation for the lower blood LCPUFA compositions in the L-ADHD subjects is enhanced cellular metabolism of these fatty acids through nonenzymatic mechanisms. Enhanced nonenzymatic oxidation due to impaired cellular defense systems has been shown to lead to the depletion of many LCPUFAs in rat livers (39). Because this was the pattern indicated by many of the subjects in the L-ADHD group, we decided to explore this possibility as a contributing factor to the lower LCPUFA status in these children. Because oxidative stress can lead to utilization of endogenous cellular antioxidants such as α -tocopherol (40), we chose to measure

this fat-soluble vitamin in the L-ADHD and the ADHD subjects with normal fatty acid status and no symptoms of deficiency. This analysis was conducted on frozen plasma samples stored at -80°C by the method used in our laboratory (41). The plasma α - and γ -tocopherol concentrations for all the ADHD subjects tested, excluding those taking supplements, were not significantly different from those of the control subjects (data not shown). Similarly, no significant difference in mean (\pm SD) plasma α -tocopherol was noted between the L-ADHD group and the ADHD group reporting few symptoms of EFA deficiency [$5.1 \pm 6.7 \mu\text{mol/L}$ ($n = 11$) and $21.4 \pm 4.6 \mu\text{mol/L}$ ($n = 18$), respectively]. This was not the case for γ -tocopherol: children in the L-ADHD group had significantly higher plasma concentrations than children with ADHD reporting few symptoms of EFA deficiency (6.0 ± 2.9 and $4.1 \pm 1.2 \mu\text{mol/L}$, respectively, $P < 0.01$). Although this difference was statistically significant, its practical significance is questionable because α -tocopherol concentrations were not significantly different between groups.

CURRENT RESEARCH

As indicated previously, the differences in proportions of LCPUFAs between the L-ADHD and the control subjects were significant, but the values did not indicate clinical deficiency. Moreover, the relation between LCPUFA status and the behavioral problems that these children exhibit is not clear. We hypothesized that the lower proportions of blood AA, eicosapentaenoic acid, and DHA observed in the ADHD children may reflect a subclinical deficiency of these key fatty acids throughout the body. Because $n-3$ fatty acids, especially DHA, are present in large quantities in the retina of the eye and in certain regions of the brain, depletion of DHA from these regions may compromise sensory and brain function. A subclinical DHA deficiency may be responsible for the abnormal behavior of these children. Throughout the rest of the body, depletion of $n-3$ and $n-6$ LCPUFAs may be responsible for the outward symptoms of deficiency that these children report.

We recently completed a study to test the relation between the low proportion of fatty acid and abnormal behavior in these children (JR Burgess, L Stevens, W Zhang, et al, unpublished observations, 1999). The study involved testing a 2-part hypothesis that oral supplementation of specific fatty acids will increase the compositions of long-chain $n-6$ and $n-3$ fatty acids in the blood and presumably throughout the body and that increased proportions of fatty acids, especially brain DHA, will result in improved behavior. To test these hypotheses, we recruited subjects with ADHD who reported many symptoms of EFA deficiency and had lower proportions of EFAs (L-ADHD) than the control subjects, who showed no behavioral problems or signs of EFA deficiency. A supplement containing the specific LCPUFAs that were depleted in these children was used in a double-blind intervention study lasting 4 mo with a 2-group parallel (noncrossover) design. The subject number and duration were selected on the basis of the results of a report by Arnold et al (18) and the parallel design was chosen on the basis of observations made by Endres et al (42, 43) indicating that biochemical effects of $n-3$ supplementation linger for up to 5 mo after supplementation ceases. The answer to whether LCPUFA deficiency contributes to abnormal behavior in some children diagnosed with ADHD awaits the results of this and other intervention studies. 



REFERENCES

1. Barkley RA. Attention-deficit hyperactivity disorder: a handbook for diagnosis and treatment. New York: Guilford Press, 1990.
2. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 4th ed. Washington, DC: American Psychiatric Association, 1994.
3. Hoza B, Vallano G, Pelham WE. Attention-deficit hyperactivity disorder. In: Ammerman RT, Hersen M, eds. Handbook of child behavior therapy in the psychiatric setting. New York: Wiley, 1995.
4. Swanson JM, McBurnett K, Wigal T, et al. Effect of stimulant medication on children with attention deficit disorder: a "review of reviews." *Except Child* 1993;60:154-61.
5. Ahmann PA, Waltonen SJ, Olson KA, Theye FW, Van Erem AJ, LePlant RJ. Placebo-controlled evaluation of Ritalin side effects. *Pediatrics* 1993;91:1101-6.
6. Holman RT. A long scaly tale—the study of essential fatty acid deficiency at the University of Minnesota. In: Sinclair A, Gibson R, eds. Essential fatty acids and eicosanoids: invited papers from the third international congress. Champaign, IL: AOCS Books 1992:3-17.
7. Smith WL. Prostanoid biosynthesis and mechanisms of action. *Am J Physiol* 1992;263:F181-91.
8. Burr GO, Burr MM. On the nature and role of the fatty acids essential in nutrition. *J Biol Chem* 1930;86:587-621.
9. Hansen AE, Haggard ME, Boelsche AN, Adam DJD, Wiese HF. Essential fatty acids in infant nutrition. III. Clinical manifestations of linoleic acid deficiency. *J Nutr* 1958;66:565-76.
10. Yehuda S, Carasso RL. Modulation of learning, pain thresholds, and thermoregulation in the rat by preparations of free purified alpha-linolenic acids: determination of the optimal n-3 to n-6 ratio. *Proc Natl Acad Sci U S A* 1993;90:10345-9.
11. Reisbick S, Neuringer M, Hasnain R, Connor WE. Home cage behavior of rhesus monkeys with long-term deficiency of omega-3 fatty acids. *Physiol Behav* 1994;55:231-9.
12. Enslin M, Milon H, Malnoe A. Effect of low intake of n-3 fatty acids during the development of brain phospholipid, fatty acid composition and exploratory behavior in rats. *Lipids* 1991;26:203-8.
13. Mitchell EA, Aman MG, Turbott SH, Manku M. Clinical characteristics and serum essential fatty acid levels in hyperactive children. *Clin Pediatr* 1987;26:406-11.
14. Stevens LJ, Zentall SS, Deck JL, et al. Essential fatty acid metabolism in boys with attention-deficit hyperactivity disorder. *Am J Clin Nutr* 1995;62:761-8.
15. Aman MG, Mitchell EA, Turbott SH. The effects of essential fatty acid supplementation by Efamol in hyperactive children. *J Abnorm Child Psychol* 1987;15:75-90.
16. Arnold LE, Kleykamp D, Votolato NA, Taylor WA, Kontras SB, Tobin K. Gamma-linolenic acid for attention-deficit hyperactivity disorder: placebo-controlled comparison to D-amphetamine. *Biol Psychiatry* 1989;25:222-8.
17. Stevens LJ, Zentall SS, Abate ML, Kuczek T, Burgess JR. Omega-3 fatty acids in boys with behavior, learning, and health problems. *Physiol Behav* 1996;59:915-20.
18. Arnold LE, Kleykamp D, Votolato N, Gibson RA, Horrocks L. Potential link between dietary intake of fatty acids and behavior: pilot exploration of serum lipids in attention-deficit hyperactivity disorder. *J Child Adolesc Psychopharmacol* 1994;4:171-82.
19. Connor WE, Neuringer M, Lin DS. Dietary effects on brain fatty acid composition: the reversibility of n-3 fatty acid deficiency and turnover of docosahexaenoic acid in the brain, erythrocytes, and plasma of rhesus monkeys. *J Lipid Res* 1990;31:237-47.
20. Wainwright PE, Huang YS, Coscina DV, Levesque S, McCutcheon D. Brain and behavioral effects of dietary n-3 deficiency in mice, a three generational study. *Dev Psychobiol* 1994;27:467-87.
21. Garcia SE, Kaiser LL, Dewey KG. Self-regulation of food intake among rural Mexican preschool children. *Eur J Clin Nutr* 1990;44:371-80.
22. McCullough AL, Kirksey A, Wachs TD, et al. Vitamin B-6 status of Egyptian mothers: relation to infant behavior and maternal-infant interactions. *Am J Clin Nutr* 1990;51:1067-74.
23. Pollitt E, Hathirat P, Kotchabhakdi N, Missel L, Vallysvu A. Iron deficiency and educational achievement in Thailand. *Am J Clin Nutr* 1989;50(suppl):687-96.
24. Schurch B. Malnutrition and behavioral development: the nutrition variable. *J Nutr* 1995;125:2255S-62S.
25. Levitsky DA, Strupp BJ. Malnutrition and the brain: changing concepts, changing concerns. *J Nutr* 1995;125:2212S-20S.
26. Strupp BJ, Levitsky DA. Enduring cognitive effects of early malnutrition: a theoretical reappraisal. *J Nutr* 1995;125:2221S-32S.
27. Grantham-McGregor SM. Assessments of the effects of nutrition on mental development and behavior in Jamaican studies. *Am J Clin Nutr* 1993;57(suppl):303S-9S.
28. Taylor B, Wadsworth J. Breast feeding and child development at five years. *Dev Med Child Neurol* 1984;26:73-80.
29. Siguel EN, Chee KM, Gong JX, Schaefer EJ. Criteria for essential fatty acid deficiency in plasma assessed by capillary column gas-liquid chromatography. *Clin Chem* 1987;33:1869-73.
30. Uauy R, Peirano P, Hoffman D, Mena P, Birch D, Birch E. Role of essential fatty acids in the function of the developing nervous system. *Lipids* 1996;31:S167-76.
31. Leaf AA, Leighfield MJ, Costeloe KL, Crawford MA. Factors affecting long-chain polyunsaturated fatty acid composition of plasma choline phosphoglycerides in preterm infants. *J Pediatr Gastroenterol Nutr* 1992;14:300-8.
32. Clark KJ, Makrides M, Neumann MA, Gibson RA. Determination of the optimal ratio of linoleic acid to alpha-linolenic acid in infant formulas. *J Pediatr* 1992;120:S151-8.
33. Birch DG, Birch EE, Hoffman DR, Uauy R. Dietary essential fatty acid supply and visual acuity development. *Invest Ophthalmol Vis Sci* 1992;33:3242-53.
34. Uauy R, Birch DG, Birch EE, Tyson JE, Hoffman DR. Effect of dietary omega-3 fatty acids on retinal function of very low birth weight neonates. *Pediatr Res* 1990;28:485-92.
35. Birch DG, Birch EE, Hoffman DR, Uauy RD. Retinal development in very-low-birth-weight infants fed diets differing in omega-3 fatty acids. *Invest Ophthalmol Vis Sci* 1992;33:2365-76.
36. Ucles P, Lorente S, Rosa F. Neurophysiological methods testing the psychoneural basis of attention deficit hyperactivity disorder. *Childs Nerv Syst* 1996;12:215-7.
37. Rodgers B. Feedings in infancy and later ability and attainment: a longitudinal study. *Dev Med Child Neurol* 1978;20:421-6.
38. Lanting CI, Fidler V, Huisman M, Touwen BC, Boersma ER. Neurological differences between 9-year-old children fed breast-milk or formula-milk as babies. *Lancet* 1994;334:1319-22.
39. Buttriss JL, Diplock AT. The alpha-tocopherol and phospholipid fatty acid content of rat liver subcellular membranes in vitamin E and selenium deficiency. *Biochim Biophys Acta* 1988;963:61-9.
40. Kehrer J. Free radicals as mediators of tissue injury and disease. *Crit Rev Toxicol* 1993;23:21-48.
41. Burgess JR, Kuo C-F. Increased calcium-independent phospholipase A₂ activity in vitamin E and selenium-deficient rat lung, liver, and spleen cytosol is time-dependent and reversible. *J Nutr Biochem* 1996;7:366-74.
42. Endres S, De Caterina R, Schmidt EB, Kristensen SD. n-3 Polyunsaturated fatty acids: update 1995. *Eur J Clin Invest* 1995;25:629-38.
43. Endres S, Ghorbani BS, Kelley VE, et al. 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* 1989;320:265-71.