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## THE EFFECT OF DIETARY SUPPLEMENTATION WITH $n-3$ POLYUNSATURATED FATTY ACIDS ON THE SYNTHESIS OF INTERLEUKIN-1 AND TUMOR NECROSIS FACTOR BY MONONUCLEAR CELLS

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**Abstract** We examined whether the synthesis of interleukin-1 or tumor necrosis factor, two cytokines with potent inflammatory activities, is influenced by dietary supplementation with  $n-3$  fatty acids.

Nine healthy volunteers added 18 g of fish-oil concentrate per day to their normal Western diet for six weeks. We used a radioimmunoassay to measure interleukin-1 (IL-1 $\beta$  and IL-1 $\alpha$ ) and tumor necrosis factor produced in vitro by stimulated peripheral-blood mononuclear cells. With endotoxin as a stimulus, the synthesis of IL-1 $\beta$  was suppressed from  $7.4 \pm 0.9$  ng per milliliter at base line to  $4.2 \pm 0.5$  ng per milliliter after six weeks of supplementation (43 percent decrease;  $P = 0.048$ ). Ten weeks after the end of  $n-3$  supplementation, we observed a further decrease to  $2.9 \pm 0.5$  ng per milliliter (61 percent decrease;

$P = 0.005$ ). The production of IL-1 $\alpha$  and tumor necrosis factor responded in a similar manner. Twenty weeks after the end of supplementation, the production of IL-1 $\beta$ , IL-1 $\alpha$ , and tumor necrosis factor had returned to the presupplement level. The decreased production of interleukin-1 and tumor necrosis factor was accompanied by a decreased ratio of arachidonic acid to eicosapentaenoic acid in the membrane phospholipids of mononuclear cells.

We conclude that the synthesis of IL-1 $\beta$ , IL-1 $\alpha$ , and tumor necrosis factor can be suppressed by dietary supplementation with long-chain  $n-3$  fatty acids. The reported antiinflammatory effect of these  $n-3$  fatty acids may be mediated in part by their inhibitory effect on the production of interleukin-1 and tumor necrosis factor. (N Engl J Med 1989; 320:265-71.)

**I**NTERLEUKIN-1 and tumor necrosis factor are polypeptide cytokines synthesized by monocytes and other cells in response to injury, as well as to infectious, inflammatory, or immunologic challenges.<sup>1-3</sup> These cytokines induce local inflammatory changes and mediate several systemic acute-phase responses.<sup>4,5</sup> Moreover, interleukin-1 and tumor necrosis factor often act synergistically — e.g., on vascular endothelium,<sup>6</sup> insulin production,<sup>7</sup> and the synthesis of metabolites of arachidonic acid.<sup>8</sup>

The  $n-3$  (also called omega-3) fatty acids are poly-

unsaturated fatty acids in which the last double bond is located between the third and fourth carbon atom from the methyl end of the fatty acid chain. These fatty acids are scarce in a normal Western diet but make up an appreciable part of the fat intake in diets rich in cold-water fish and seal meat.<sup>9</sup> Epidemiologic studies in populations of coastal Eskimo,<sup>10,11</sup> Japanese,<sup>12</sup> and Dutch<sup>13</sup> subjects have shown that a high intake of  $n-3$  fatty acids correlates with a low incidence of cardiovascular and inflammatory diseases such as asthma and Type I diabetes mellitus.<sup>11</sup> The modification of the fatty acid composition of cell membranes and the formation of eicosanoids by dietary supplementation with  $n-3$  fatty acids have subsequently been shown in human beings.<sup>14,15</sup> In healthy volunteers<sup>16</sup> and in patients with asthma,<sup>17</sup>  $n-3$  dietary supplementation results in decreased neutrophil chemotaxis and inhibited generation of leukotriene B<sub>4</sub> by neutrophils and monocytes. In clinical studies, dietary supplementation with  $n-3$  fatty acids has led to improvements in patients with rheumatoid arthritis<sup>18</sup> and in patients with psoriasis.<sup>19</sup> Prospective studies in animals fed  $n-3$  fatty acids support these clinical observations.<sup>20-23</sup>

Since interleukin-1 and tumor necrosis factor are the principal polypeptide mediators of inflammation,

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reduced production of these cytokines may contribute to the amelioration of inflammatory symptoms in patients receiving n-3 supplementation. In this study we investigated the effects of dietary supplementation with n-3 fatty acids on the synthesis of the cytokines interleukin-1 and tumor necrosis factor in normal subjects.

## METHODS

### Subjects and Study Design

The study was approved by the Human Investigative Review Committee of the New England Medical Center Hospitals. Nine healthy male volunteers entered the study after giving informed consent (average age, 28 years; range, 21 to 39). The initial study group consisted of six volunteers, and a second group of three volunteers entered the study six months after the first group. Both groups were studied in the same fashion. Since the results were similar in the two groups, the data for the nine subjects are pooled in this report.

For six weeks, the subjects added 18 g of marine lipid concentrate (six capsules of MaxEPA three times per day, kindly provided by R.P. Scherer, Troy, Mich.) to their otherwise unchanged diets. By gas chromatography, the n-3 fatty acid content of 1 g of the lipid concentrate was shown to consist of 153 mg of eicosapentaenoic acid and 103 g of docosahexaenoic acid. The subjects were free of disease and refrained from taking any medications during the course of the study. Before supplementation, the subjects' mean caloric intake ranged from 2000 to 3000 kcal per day. Six to 10 percent of the calories (mean, 28 g) were highly unsaturated fatty acids, but less than 1 percent were n-3 fatty acids. During supplementation, the intake of highly unsaturated fatty acids increased from 28 to 34 g.

The production of interleukin-1 and tumor necrosis factor by stimulated-blood mononuclear cells in vitro was assessed during four phases of the study: before the start of n-3 supplementation, during the 6th week of supplementation, and during the 10th and 20th weeks after the end of supplementation (the latter two phases being washout periods). To ascertain reproducibility, blood samples were obtained on three different days, two to three days apart, during each of the four phases of the study. The mean of these three replicate determinations was calculated and used for further analysis. We were unable to make measurements during the 20th week of the washout period in one of the six subjects in the first group of volunteers.

During the first six weeks of the study, when the second group of volunteers was taking the supplement, we also studied a control group of four healthy male volunteers who did not receive the supplement. In vitro production of interleukin-1 and of tumor necrosis factor was assessed twice, during week 1 and week 7. Blood sampling was performed on two different days during the first and seventh week, and the mean was calculated.

### Stimulation of Mononuclear Cells in Vitro

Venous blood was drawn into syringes pretreated with heparin (20 U per milliliter), and the mononuclear-cell fraction was obtained by Ficoll-Hypaque centrifugation. At the same time a sample of blood was obtained without heparin in order to prepare heat-inactivated (56°C, 40 minutes) serum. The mononuclear cells were washed twice in 0.15 M sodium chloride, slides were prepared by cytocentrifugation, and 100 cell-differential counts were performed in a blinded fashion on coded slides. RPMI-1640 culture medium (Whittaker M.A. Bioproducts, Walkersville, Md.), supplemented with 2 mM levo-glutamine, 100 U of penicillin per milliliter, and 100 µg of streptomycin per milliliter, was subjected to ultrafiltration to remove endotoxins, as described elsewhere.<sup>24</sup> The mononuclear cells were suspended at a concentration of  $5 \times 10^6$  cells per milliliter in RPMI medium with 2 percent heat-inactivated autologous serum, and 100 µl was divided into aliquots in 96-well flat-bottomed microtiter plates. An equal volume of either serum-free RPMI or RPMI containing various stimulants was added. The cells

were stimulated with endotoxin (lipopolysaccharide *Escherichia coli* 055:B5, Sigma, St. Louis) at 1 and 10 ng per milliliter, phytohemagglutinin (Difco, Detroit) at 3 µg per milliliter, or heat-killed *Staphylococcus epidermidis*, opsonized with 2.5 percent fresh autologous serum, at 20 bacteria per mononuclear cell. After incubation for 24 hours at 37°C in 5 percent carbon dioxide, the supernatants were removed from some wells and frozen at -70°C for the determination of prostaglandin E. The microtiter plates were frozen until the end of the study. At that time all the plates for each donor were simultaneously thawed and exposed to two more freeze-thaw cycles to complete cell lysis. The contents of triplicate wells, consisting of cell lysates and supernatants, were pooled and refrozen for determinations of interleukin-1 and tumor necrosis factor.

### Determinations of Interleukin-1 and Tumor Necrosis Factor

The total synthesis of cytokines (i.e., cell-associated cytokines plus secreted cytokines) was determined by radioimmunoassays for two forms of human interleukin-1 (IL-1β and IL-1α) and for tumor necrosis factor. Details on the specificity and sensitivity of each radioimmunoassay have been reported elsewhere.<sup>25-27</sup> Each radioimmunoassay was specific for the respective cytokine. For a given stimulus, samples from all four study phases for each donor were assayed in a single radioimmunoassay for each cytokine.

### Prostaglandin Determination

Prostaglandin E<sub>2</sub> content in mononuclear-cell supernatants was determined by direct competitive radioimmunoassay. Anti-prostaglandin E<sub>2</sub> serum, kindly provided by Dr. William Campbell (University of Texas Health Science Center at Dallas), has a cross-reactivity of 14 percent with prostaglandin E<sub>1</sub> and of 2.7 percent with prostaglandin E<sub>2α</sub>. Prostaglandin E<sub>2</sub> standard was provided by Dr. John Pike (Upjohn, Kalamazoo, Mich.). [<sup>3</sup>H]Prostaglandin E<sub>2</sub> was purchased from Amersham (Arlington Heights, Ill.). The assay does not distinguish between prostaglandin E<sub>2</sub> and prostaglandin E<sub>3</sub>. The assay sensitivity for prostaglandin E<sub>2</sub> is 50 pg per milliliter.

### Neutrophil Chemotaxis

Polymorphonuclear neutrophils were isolated from the pellet after Ficoll-Hypaque density-gradient centrifugation, and their chemotactic response to synthetic leukotriene B<sub>4</sub> (Sigma) was assayed with use of blind well chambers, as described elsewhere.<sup>28</sup> The migration distance of polymorphonuclear neutrophils was measured with the leading-front method. Each condition (buffer alone or with leukotriene B<sub>4</sub> added at 10<sup>-9</sup>, 10<sup>-8</sup>, 10<sup>-7</sup>, and 10<sup>-6</sup> M) was assayed in triplicate. Polymorphonuclear neutrophils from healthy subjects not receiving n-3 fatty acid supplementation were assayed in parallel (same-day control). The results are expressed as percent increases over spontaneous migration in buffer.

### Fatty Acid Analyses

For the measurement of plasma fatty acids, 18 ml of blood was drawn into EDTA-coated tubes and centrifuged for 20 minutes at 800×g. The plasma was removed and frozen at -70°C until analyzed. Lipids were extracted and methylated, as described elsewhere.<sup>29</sup> For the determination of the fatty acid composition of mononuclear cells, cells obtained on three different days during each of the four study phases were pooled, for a total of 4.5×10<sup>6</sup> mononuclear cells per subject. The lysates of these cells were centrifuged at 100,000×g and extracted, and the phospholipids were purified and transmethylated as described elsewhere.<sup>30</sup> The fatty acid methyl esters were analyzed by gas-liquid chromatography on an HP-5980 gas chromatograph.<sup>29,30</sup> The amounts of individual fatty acids were expressed as relative percentages, with total fatty acids set as 100 percent.

### Statistical Analysis

The results are expressed as means ±SEM. Two-tailed Student's t-tests for paired samples were performed on a Clinfo software system (Bolt, Beranek and Newman, Cambridge, Mass.) After Bonfer-

roni's adjustment for multiple t-tests in one group, P values less than 0.05 were considered to be significant.

**RESULTS**

**Plasma and Mononuclear-Cell Lipids**

The volunteers reported no untoward effects of ingesting the fish-oil concentrate. Compliance was confirmed by a significant increase in plasma levels of eicosapentaenoic acid, from  $0.4 \pm 0.2$  mg per deciliter at base line to  $17.3 \pm 0.7$  mg per deciliter during the sixth week of supplementation ( $P < 0.001$ ). Plasma eicosapentaenoic acid was still slightly increased ( $1.1 \pm 0.3$  mg per deciliter;  $P = 0.035$ ) 10 weeks after cessation of the supplement and returned to the presupplementation level ( $0.6 \pm 0.2$  mg per deciliter) within 20 weeks after cessation of the supplement. Triglyceride levels were reduced from the presupplementation level ( $1.03 \pm 0.02$  mmol per liter [ $91 \pm 20$  mg per deciliter]) to  $0.66 \pm 0.12$  mmol per liter ( $59 \pm 11$  mg per deciliter) after six weeks of n-3 supplementation ( $P < 0.040$ ). They returned to the presupplementation levels at 10 weeks ( $1.09 \pm 0.23$  mmol per liter [ $97 \pm 21$  mg per deciliter]) and 20 weeks ( $1.04 \pm 0.14$  mmol per liter [ $93 \pm 13$  mg per deciliter]) after cessation of the supplement. Cholesterol levels and leukocyte and platelet counts did not change significantly. These data confirm previous findings on the effects of similar doses of n-3 fatty acids on plasma levels of fatty acids<sup>30</sup> and on triglyceride levels.<sup>31</sup>

The composition of mononuclear-cell phospholipid fatty acids was determined in the mononuclear-cell membranes of five randomly chosen volunteers during the four study phases (Table 1). After six weeks of n-3 fatty acid supplementation, there was a significant increase in eicosapentaenoic acid, from 0.7 percent before the supplement to 3.8 percent ( $P = 0.02$ ), with a concomitant decrease of arachidonic acid, from 13.8 percent before the supplement to 8.6 percent ( $P = 0.02$ ). Ten weeks after the end of supplementation, there was still a trend to increased eicosa-

Table 1. Fatty Acid Composition of Mononuclear-Cell Membranes as a Percentage of Total Fatty Acid Content.\*

FATTY ACID	BEFORE n-3 SUPPLEMENT	AFTER n-3 SUPPLEMENT	TIME AFTER END OF n-3 SUPPLEMENT	
			10 WEEKS	20 WEEKS
			<i>mean percentage ± SEM</i>	
AA (n=6)	13.8±1.3	8.6±0.7†	9.5±1.8‡	13.8±2.6‡
EPA (n=3)	0.7±0.1	3.8±0.7§	1.0±0.3‡	0.6±0.1‡
DHA (n=3)	2.3±0.2	3.3±0.4‡	2.0±0.4‡	2.5±0.7‡
AA/EPA ratio	20.9±2.2	2.4±0.2¶	12.0±2.1	23.9±4.3‡

\*Mononuclear cells were obtained from five subjects receiving dietary supplementation of n-3 fatty acids. AA denotes arachidonic acid, EPA eicosapentaenoic acid, and DHA docosahexaenoic acid.

†Decreased from presupplement levels ( $P < 0.04$ ).

‡No significant change from presupplement levels.

§Increased from presupplement levels ( $P < 0.03$ ).

¶Decreased from presupplement levels ( $P < 0.003$ ).

||Decreased from presupplement levels ( $P < 0.03$ ).

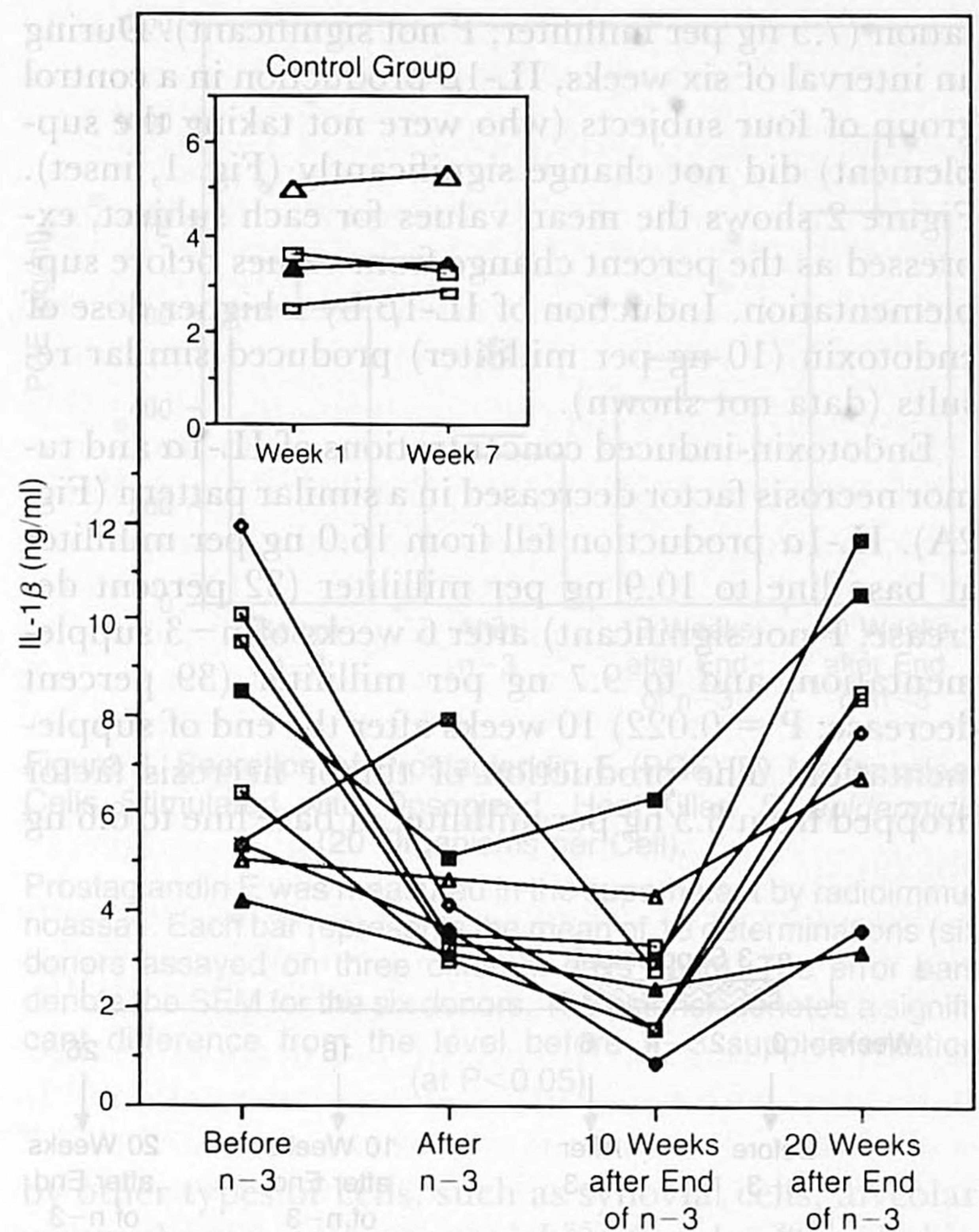


Figure 1. In Vitro Synthesis of IL-1β by Mononuclear Cells of Nine Subjects, Stimulated with Endotoxin (1 ng per Milliliter) during Dietary Supplementation.

Each point represents the average of three determinations on three different days, two to three days apart, in each subject. The inset shows the synthesis of IL-1β by mononuclear cells stimulated with the same dose of endotoxin in four control subjects without dietary supplementation, during an interval of seven weeks.

pentaenoic acid and decreased arachidonic acid concentrations, and the ratio of arachidonic acid to eicosapentaenoic acid remained significantly suppressed at a value of 12.0, as compared with 20.9 before supplementation ( $P = 0.02$ ) and 23.9 at week 20 after cessation of the supplement. Twenty weeks after the end of supplementation, both eicosapentaenoic acid and arachidonic acid had returned to the presupplementation levels.

**Effect of n-3 Fatty Acid Supplementation on the Production of Interleukin-1 and Tumor Necrosis Factor**

Figure 1 shows the individual values for the production of IL-1β at each phase of the study. The production of IL-1β in vitro was decreased after six weeks of n-3 supplementation. At that time, mononuclear cells stimulated by 1 ng of endotoxin per milliliter produced only 4.2 ng of IL-1β per milliliter, as compared with the presupplementation level of 7.4 ng per milliliter (43 percent decrease;  $P = 0.048$ ; Fig. 1). Ten weeks after the end of n-3 supplementation, IL-1β production had decreased further, to 2.9 ng per milliliter (61 percent decrease;  $P = 0.005$ ).

The production of IL-1β returned to the presupplementation level 20 weeks after the end of supplemen-

tation (7.5 ng per milliliter; *P* not significant). During an interval of six weeks, IL-1 $\beta$  production in a control group of four subjects (who were not taking the supplement) did not change significantly (Fig. 1, inset). Figure 2 shows the mean values for each subject, expressed as the percent change from values before supplementation. Induction of IL-1 $\beta$  by a higher dose of endotoxin (10 ng per milliliter) produced similar results (data not shown).

Endotoxin-induced concentrations of IL-1 $\alpha$  and tumor necrosis factor decreased in a similar pattern (Fig. 2A). IL-1 $\alpha$  production fell from 16.0 ng per milliliter at base line to 10.9 ng per milliliter (32 percent decrease; *P* not significant) after 6 weeks of n-3 supplementation, and to 9.7 ng per milliliter (39 percent decrease; *P* = 0.022) 10 weeks after the end of supplementation. The production of tumor necrosis factor dropped from 8.5 ng per milliliter at base line to 6.6 ng

Table 2. IL-1 $\beta$ , IL-1 $\alpha$ , and Tumor Necrosis Factor Induced in Mononuclear Cells by Heat-Killed *S. epidermidis*.\*

	BEFORE n-3 SUPPLEMENT	AFTER n-3 SUPPLEMENT	TIME AFTER END OF n-3 SUPPLEMENT	
			10 WEEKS	20 WEEKS
	<i>mean</i> $\pm$ <i>SEM</i>			
IL-1 $\beta$ (ng/ml)	31.8 $\pm$ 4.5	26.3 $\pm$ 5.1	25.2 $\pm$ 3.5	25.6 $\pm$ 2.6
IL-1 $\alpha$ (ng/ml)	5.8 $\pm$ 1.1	4.5 $\pm$ 1.0	5.9 $\pm$ 1.1	5.2 $\pm$ 0.8
TNF (ng/ml)	37.9 $\pm$ 3.4	32.1 $\pm$ 5.5	35.5 $\pm$ 3.9	32.5 $\pm$ 3.6

\*Mononuclear cells were obtained from nine subjects receiving dietary supplementation of n-3 fatty acids.

per milliliter (22 percent decrease; *P* not significant) after 6 weeks of the supplement and to 5.1 ng per milliliter (40 percent decrease; *P* = 0.008) 10 weeks after the end of supplementation. Twenty weeks after the end of supplementation, the production of these cytokines had also returned to the presupplementation levels.

The proportion of monocytes in the mononuclear-cell population (after Ficoll-Hypaque separation) was 19.1 $\pm$ 4.4 percent and did not change significantly after 6 weeks of the supplement or at 10 weeks after the end of supplementation.

The levels of interleukin-1 and tumor necrosis factor induced by the phagocytosis of heat-killed *S. epidermidis* were measured (Table 2). This phagocytic stimulus was four times more potent than the high dose of endotoxin (10 ng per milliliter) in inducing the production of IL-1 $\beta$  and tumor necrosis factor. These levels showed slight, insignificant decreases during week 6 of the n-3 supplement and at 10 weeks after the end of supplementation.

To assess the possible role of altered interleukin-1 production in mediating the effect of n-3 fatty acids during an immunologic challenge, we also measured the induction of IL-1 $\beta$  and IL-1 $\alpha$  by phytohemagglutinin in the same cell population (Fig. 2B). After six weeks of n-3 supplementation, the production of IL-1 $\alpha$  had decreased significantly, from 14.0 to 12.0 ng per milliliter (14 percent decrease; *P* = 0.044). During the 10th week after the end of supplementation, however, both cytokines were significantly reduced: IL-1 $\beta$  by 53 percent (from 3.0 to 1.4 ng per milliliter; *P* = 0.003), and IL-1 $\alpha$  by 37 percent (from 14.2 to 9.0 ng per milliliter; *P* = 0.0001).

We studied a control group during the same interval that we studied the second group of volunteers in the first two phases of n-3 supplementation. Although endotoxin stimulation of the mononuclear cells from these control subjects produced lower amounts of IL-1 $\beta$  (Fig. 1, inset), the production of this cytokine at week 7 did not significantly change (102 $\pm$ 16 percent of the levels produced at week 1). The levels of IL-1 $\alpha$  and tumor necrosis factor were 116 $\pm$ 10 and 90 $\pm$ 6 percent, respectively, at week 7. At that time, cytokine production induced by *S. epidermidis* was 110 $\pm$ 8 percent of the levels at week 1 for IL-1 $\beta$ , 100 $\pm$ 13 percent for IL-1 $\alpha$ , and 111 $\pm$ 2 percent for tu-

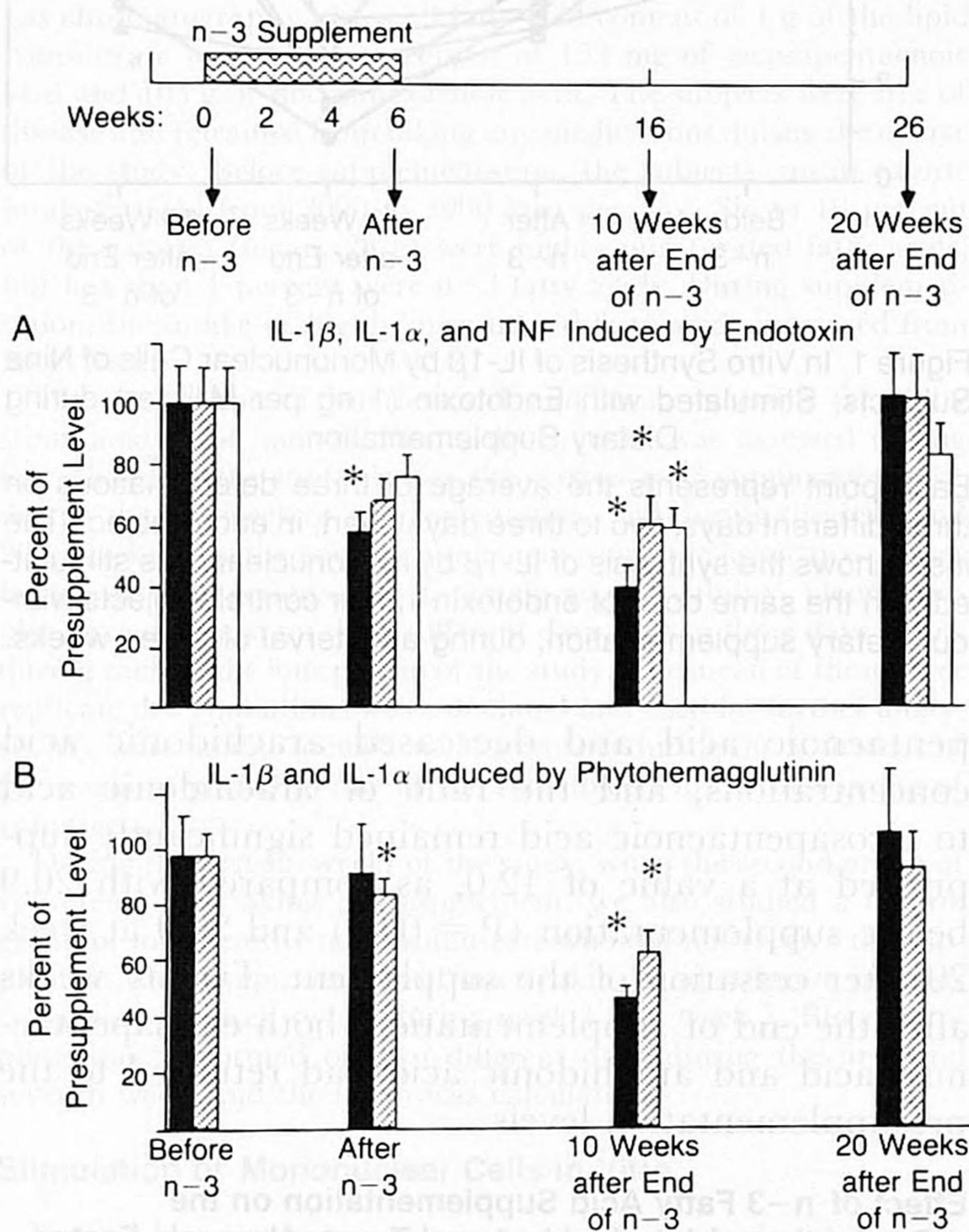


Figure 2. Synthesis of IL-1 $\beta$  (Solid Bars), IL-1 $\alpha$  (Hatched Bars), and Tumor Necrosis Factor (TNF; Open Bars) by Mononuclear Cells Stimulated with 1 ng of Endotoxin per Milliliter (Panel A) or 3  $\mu$ g of Phytohemagglutinin per Milliliter (Panel B).

The concentration of total cytokine (i.e., cell-associated plus secreted) was determined by radioimmunoassay. Concentrations are presented as percentages of presupplementation levels. With endotoxin stimulation, the concentrations were 7.4 ng of IL-1 $\beta$  per milliliter, 16.0 ng of IL-1 $\alpha$  per milliliter, and 8.5 ng of tumor necrosis factor per milliliter. With phytohemagglutinin stimulation, the concentrations were 3.0 ng of IL-1 $\beta$  per milliliter and 14.0 ng of IL-1 $\alpha$  per milliliter. Each bar represents the mean of 27 determinations (nine donors assayed on three days each). The error bars denote the SEM for the nine donors. An asterisk denotes a significant difference from the level before dietary n-3 supplementation (at *P* < 0.05).

mor necrosis factor. There was no significant change in the in vitro production of interleukin-1 and tumor necrosis factor in volunteers receiving no dietary supplementation during an interval of six weeks.

### Prostaglandin Synthesis

In the initial group of six volunteers, prostaglandin  $E_2$  release was measured in the supernatant of mononuclear cells stimulated by *S. epidermidis* (Fig. 3). Prostaglandin  $E_2$  release decreased from 612 pg per milliliter at base line to 302 pg per milliliter after 6 weeks of supplementation (51 percent decrease;  $P = 0.008$ ), to 423 pg per milliliter ( $P$  not significant) after 10 weeks of supplementation, and to 812 pg per milliliter ( $P$  not significant) after 20 weeks of supplementation. After cessation of the supplement there was no significant change in prostaglandin  $E_2$  release from the presupplement level.

### Neutrophil Chemotaxis

Random migration of polymorphonuclear neutrophils in both the study and control subjects was unchanged at all times (data not shown). The chemotactic responses of polymorphonuclear neutrophils after six weeks of n-3 supplementation were suppressed at all four concentrations of leukotriene  $B_4$ . Figure 4 shows that chemotaxis to  $10^{-7}$  M leukotriene  $B_4$ , which elicited the highest response, was  $83 \pm 8.2$  percent (mean of percent increase over random migration) before the n-3 supplement,  $62 \pm 5.0$  percent after 6 weeks of the supplement, and  $79 \pm 12.5$  percent 10 weeks after cessation of the supplement. The suppression of chemotaxis after six weeks of supplementation was significant as compared with the presupplement control ( $P = 0.038$ ) and with the control group assayed on the same day ( $94 \pm 11.9$  percent;  $P = 0.041$ ). This is in agreement with the findings of Lee et al.<sup>16</sup> on decreased neutrophil chemotaxis in healthy volunteers whose diet was supplemented with n-3 fatty acids.

### DISCUSSION

Our results show that dietary supplementation with n-3 fatty acids reduces the amount of inducible production of interleukin-1 and tumor necrosis factor by mononuclear cells in vitro. The reduced production of  $IL-1\beta$ ,  $IL-1\alpha$ , and tumor necrosis factor may contribute to the decreased inflammatory responses reported in patients receiving n-3 supplementation.<sup>18</sup>

We studied the production of interleukin-1 and tumor necrosis factor by total mononuclear cells rather than by enriched monocytes. Since monocytes, T cells,<sup>32</sup> B cells,<sup>33</sup> and large granular lymphocytes<sup>34</sup> synthesize interleukin-1 and tumor necrosis factor, we believe that the response of the whole cell population is biologically more relevant than that of enriched subpopulations. Although we studied peripheral mononuclear cells as an accessible source, it is probable that dietary n-3 fatty acids also decrease the production of interleukin-1 and tumor necrosis factor

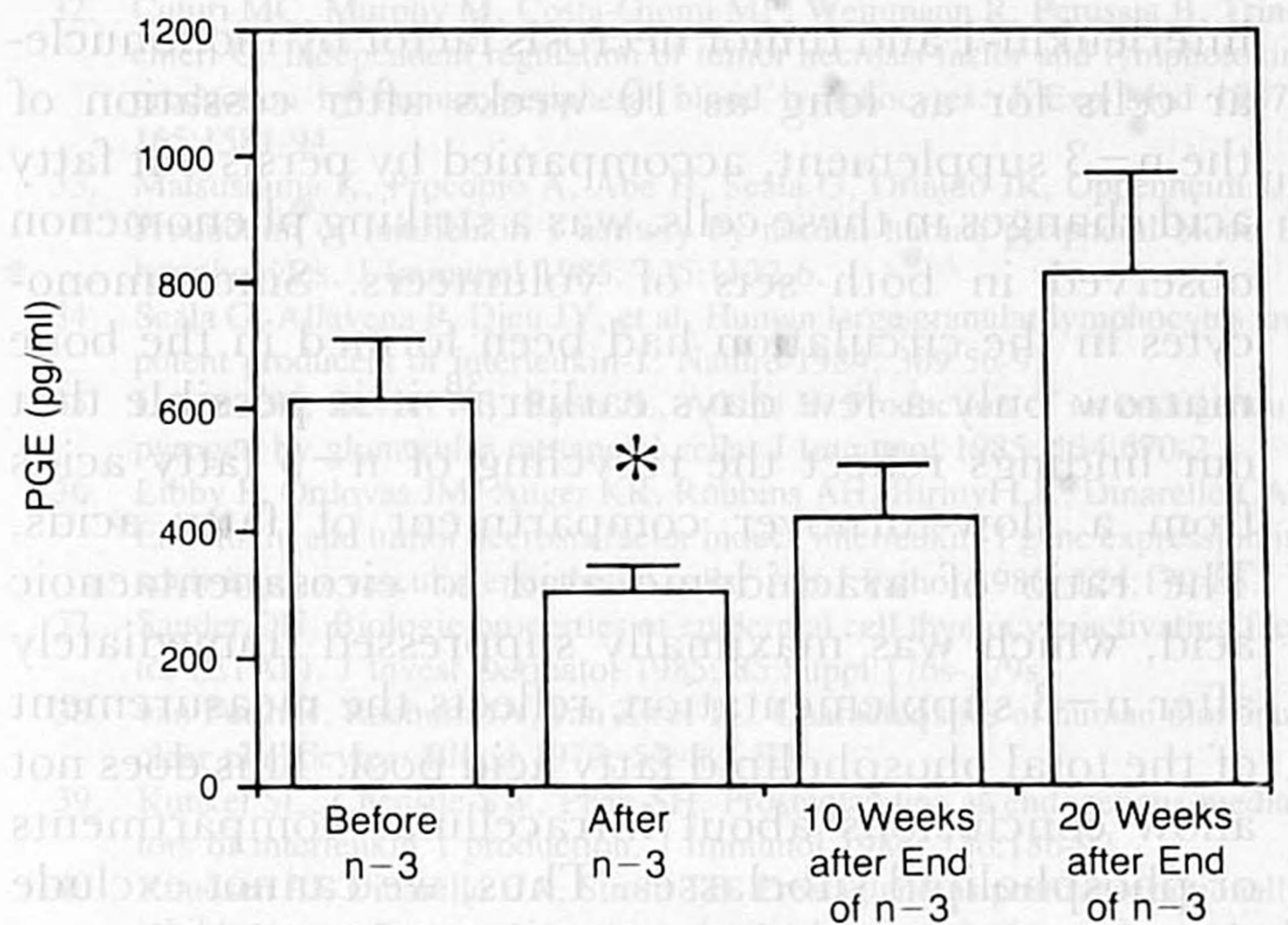


Figure 3. Secretion of Prostaglandin E (PGE) by Mononuclear Cells Stimulated with Opsonized, Heat-Killed *S. epidermidis* (20 Organisms per Cell).

Prostaglandin E was measured in the supernatant by radioimmunoassay. Each bar represents the mean of 18 determinations (six donors assayed on three different days each). The error bars denote the SEM for the six donors. The asterisk denotes a significant difference from the level before n-3 supplementation (at  $P < 0.05$ ).

by other types of cells, such as synovial cells, alveolar macrophages, and mesangial,<sup>35</sup> vascular,<sup>36</sup> and skin cells.<sup>37</sup> Since we measured total interleukin-1 and tumor necrosis factor (i.e., both cell-associated and secreted), our results demonstrate that n-3 fatty acids affect the synthesis rather than the release of these cytokines.

The persistent suppression of the production of

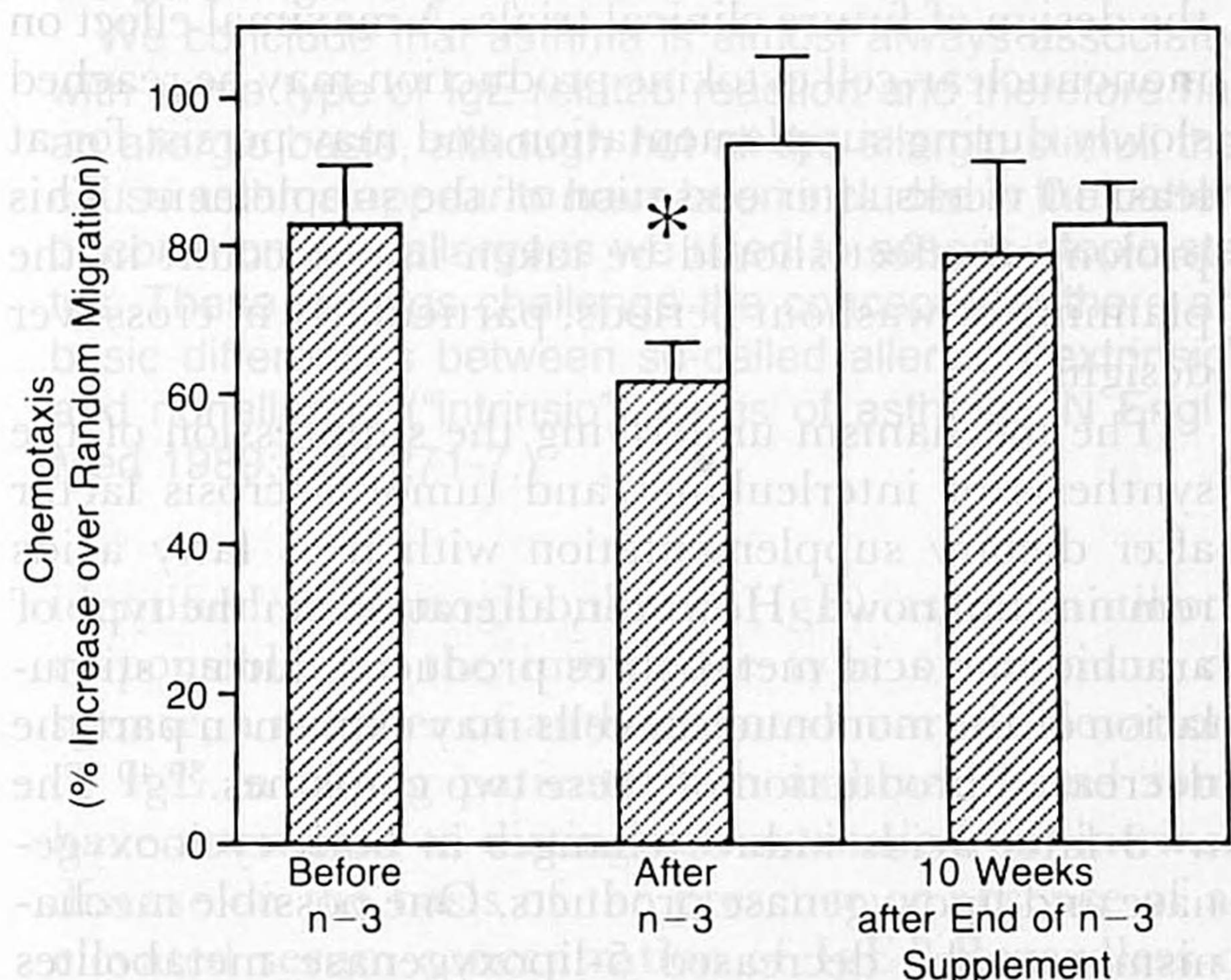


Figure 4. Chemotactic Response of Neutrophils to Leukotriene  $B_4$ .

The migration of neutrophils in response to  $10^{-7}$  M leukotriene  $B_4$  is expressed as a percentage of increase over random migration. The bars represent the mean  $\pm$  SEM for nine subjects in the study group (hatched bars, n-3 supplement) and for five control subjects (open bars, no dietary supplement). Ten weeks after the end of supplementation, five of the nine subjects in the study group were studied. The asterisk denotes a significant difference from the level before n-3 supplement (at  $P < 0.05$ ).

interleukin-1 and tumor necrosis factor by mononuclear cells for as long as 10 weeks after cessation of the n-3 supplement, accompanied by persistent fatty acid changes in these cells, was a striking phenomenon observed in both sets of volunteers. Since monocytes in the circulation had been formed in the bone marrow only a few days earlier,<sup>38</sup> it is possible that our findings reflect the recycling of n-3 fatty acids from a slow-turnover compartment of fatty acids. The ratio of arachidonic acid to eicosapentaenoic acid, which was maximally suppressed immediately after n-3 supplementation, reflects the measurement of the total phospholipid fatty acid pool. This does not allow conclusions about intracellular compartments or phospholipid subclasses. Thus, we cannot exclude the altered fatty acid composition of a critical compartment at 10 weeks after the end of supplementation, when the suppression of cytokine production was maximal.

We observed functional changes in cytokine production as late as 10 weeks after n-3 supplementation was discontinued; other investigators have also observed the long persistence of biochemical changes associated with n-3 fatty acid supplementation.<sup>30</sup> In a clinical trial in patients with rheumatoid arthritis,<sup>18</sup> leukotriene B<sub>4</sub> generation in neutrophils remained below base-line levels for as long as 18 weeks after n-3 fatty acid supplementation was stopped. Concomitantly, in the crossover design of that study, the treatment group still had clinical improvement when it entered the placebo phase after a washout period of four weeks. The prolonged effects of dietary n-3 fatty acid supplementation on the production of interleukin-1 and tumor necrosis factor has implications for the design of future clinical trials. A maximal effect on mononuclear-cell cytokine production may be reached slowly during supplementation and may persist for at least 10 weeks after cessation of the supplement. This prolonged effect should be taken into account in the planning of washout periods, particularly in crossover designs.

The mechanism underlying the suppression of the synthesis of interleukin-1 and tumor necrosis factor after dietary supplementation with n-3 fatty acids remains unknown. However, alterations in the type of arachidonic acid metabolites produced during stimulation of the mononuclear cells may explain in part the decreased production of these two cytokines.<sup>39,40</sup> The n-3 fatty acids induce changes in both cyclooxygenase and lipoxygenase products. One possible mechanism may be decreased 5-lipoxygenase metabolites such as leukotriene B<sub>4</sub>.<sup>15,16</sup> Adding exogenous leukotriene B<sub>4</sub> to human mononuclear cells enhances endotoxin-stimulated production of interleukin-1.<sup>41</sup> Conversely, inhibitors of 5-lipoxygenase reduce the production of interleukin-1.<sup>42,43</sup> Supplementation with the same n-3 fatty acid preparation in the same dosage and during the same time as in the present study decreased monocyte leukotriene B<sub>4</sub> production by 58 percent in healthy volunteers.<sup>18</sup> Thus, a possible mechanism for decreased interleukin-1 production is

decreased synthesis of leukotriene B<sub>4</sub> and generation of the biologically less active metabolite leukotriene B<sub>5</sub> from eicosapentaenoic acid.

Although the clinical importance of our observations is uncertain, our findings may explain in part the antiinflammatory effects of n-3 fatty acids.<sup>18-21</sup> Whether other unsaturated fatty acids have similar effects remains to be determined.

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## ASSOCIATION OF ASTHMA WITH SERUM IgE LEVELS AND SKIN-TEST REACTIVITY TO ALLERGENS

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**Abstract** We investigated the association of self-reported asthma or allergic rhinitis with serum IgE levels and skin-test reactivity to allergens in 2657 subjects in a general-population study. Regardless of the subjects' status with respect to atopy or their age group, the prevalence of asthma was closely related to the serum IgE level standardized for age and sex ( $P < 0.0001$ ), and no asthma was present in the 177 subjects with the lowest IgE levels for their age and sex ( $> 1.46$  SD below the mean). The log odds ratio increased linearly with the serum IgE level after we controlled for possible confounders and the degree of reactivity to skin tests. In contrast, aller-

gic rhinitis appeared to be associated primarily with skin-test reactions to common aeroallergens, independently of the serum IgE level.

We conclude that asthma is almost always associated with some type of IgE-related reaction and therefore has an allergic basis, although not all the allergic stimuli that cause asthma appear to have been included in the battery of common aeroallergens we used to assess atopic status. These findings challenge the concept that there are basic differences between so-called allergic ("extrinsic") and nonallergic ("intrinsic") forms of asthma. (*N Engl J Med* 1989; 320:271-7.)

IT is generally accepted that there are both allergic and nonallergic forms of asthma, and the distinction is usually based on the presence or absence of allergy skin-test reactivity to one or more aeroallergens considered capable of inducing the disease. Since Rackemann introduced the terms in 1918,<sup>1</sup> asthma that can be shown to be related to hypersensitivity to a foreign substance has been described as "extrinsic"; otherwise, the disease has been classified as "intrinsic."

After Ishizaka<sup>2</sup> and Johansson<sup>3</sup> and their colleagues

identified immunoglobulin E (IgE) as the antibody responsible for the immediate type of immune response, a number of authors noted marked elevations of IgE in young patients with asthma,<sup>4,5</sup> and some have attempted to distinguish extrinsic from intrinsic disease on the basis of the presence or absence of an elevated serum concentration of IgE.<sup>6</sup> Regardless of the specific criteria, however, extrinsic asthma appears to be the predominant form of the disease among children and young adults, and intrinsic asthma is considered to be more common among older subjects.

These widely accepted concepts, based largely on clinical observations, fail to take into account the relations of both allergy skin-test reactivity (hereafter called atopy) and serum IgE levels to age in the general population. Both tend to decrease with advancing

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