Polyunsaturated fatty acids increase neutrophil adherence and integrin receptor expression

Edna J. Bates,* Antonio Ferrante,* Dianne P. Harvey,* and Alf Poulos†

*Department of Immunology and University of Adelaide Department of Paediatrics and †Department of Chemical Pathology, Women's and Children's Hospital, Adelaide, Australia

Abstract: Fish oils are abundant in polyunsaturated fatty acids of the n-3 series (in particular eicosapentaenoic, 20:5 and docosahexaenoic acid, 22:6). Such fatty acids are generally considered to be beneficial in the prevention of cardiac disease and to have anti-inflammatory properties. Neutrophil adherence is an essential early event in an acute inflammatory response, and we have demonstrated that both 20:5 and 22:6 stimulate adherence in vitro. Arachidonic acid (20:4, n-6) was also stimulatory. Significant stimulation of adherence was seen from 5 to 80 μM (nontoxic concentrations) 22:6, 20:5, or 20:4. At the lower fatty acid concentrations tested (≤40 μM) 20:5 was less active than 22:6 or 20:4 at stimulating adherence. Above 40 μM there was no difference in the ability of the three fatty acids to stimulate adherence. At the lower fatty acid concentrations tested (≤10 μM) 22:6 was less active than 20:4, whereas above 10 μM they were equally active. Immunofluorescent flow cytometric analysis of neutrophil integrin (adherence) receptors showed that the complement C3bi receptor (CD11b) was up-regulated by these fatty acids. There was no change in CD11a or CD11c. Saturated fatty acids of the same chain length were without effect on adherence or receptor expression. The findings suggest that these polyunsaturated fatty acids may, under certain conditions, be proinflammatory with respect to their acute effects on the interaction of neutrophils with microbes, endothelium, and other tissues. *J. Leukoc. Biol.* 53: 420–426; 1993.

Key Words: *adhesion • adherence receptors • granulocytes • n-3 and n-6 fatty acids*

INTRODUCTION

Fish oils contain an abundance of n-3 fatty acids derived from linolenic acid (18:3, n-3), in particular eicosapentaenoic (20:5, n-3) and docosahexaenoic (22:6, n-3) acids. The beneficial effect of fish diets in lowering the incidence of cardiovascular disease in certain human populations [1, 2] has been related to the presence of such n-3 fatty acids. In addition, n-3 fatty acids have anti-inflammatory effects and have been used in the treatment of rheumatoid arthritis [3]. These fatty acids may exert their anti-inflammatory and cardioprotective effects by interfering with prostaglandin and leukotriene metabolism in neutrophils and monocytes [4], as well as by inhibiting the production of cytokines [5]. However, we have shown that 20:5, 22:6 and 20:4 (arachidonic acid, n-6 derived from linoleic acid found in vegetable oils) are potent activators of the oxygen-dependent respiratory burst in human neutrophils [6, 7]. Such a stimulation of the respiratory burst would be expected to increase the inflammatory response.

Increased adherence of circulating neutrophils to vascular endothelium is an essential early event in an acute inflammatory response, preceding neutrophil migration through vessel walls and accumulation in tissues. The adhesion of neutrophils to endothelial cells results from interactions between adhesion molecules on both cell types. A major group of such molecules on the surface of neutrophils is the integrin family of adhesive glycoproteins (CD11/CD18 complex) consisting of a common β chain defined by the CD-18 antigen and three different α chains specified as CD11a (LFA-1), CD11b (Mac-1), and CD11c (p150-95) antigens [8]. We have examined the effects of both n-3 (20:5, 22:6) and n-6 (20:4) fatty acids on neutrophil adherence and compared them to the effects of saturated fatty acids of equivalent chain length and known stimulators of neutrophil adherence: the bacterial chemotactic peptide, *N*-formylmethionyl-leucyl-phenylalanine (fMLP) and the cytokine tumor necrosis factor α (TNF-α). The relative expression of neutrophil integrin receptors after such treatments has also been assessed.

MATERIALS AND METHODS

Preparation of neutrophils

Neutrophils were prepared from the heparinized blood of healthy volunteers by a rapid single-step method [9] in which blood was layered onto Ficoll-Hypaque medium of density 1.114 and centrifuged at 400*g* for 30–40 min at room temperature. Leukocytes resolved into two distinct bands, the lower containing neutrophils. The cells were washed three times in Hanks' balanced salt solution without phenol red (HBSS). Preparations were of 96–99% purity and were more than 99% viable as judged by their ability to exclude trypan blue.

Preparation of fatty acid micelles

Dipalmitoyl phosphatidylcholine (DPC) and free fatty acids were obtained from Sigma Chemical Company, St. Louis, MO. To overcome fatty acid insolubility in aqueous solution we prepared mixed DPC (400 μg) and fatty acid (100 μg) micelles in HBSS by sonication as previously described [6] and used these either neat or diluted up to fourfold. Control incubations contained micelles of DPC alone appropriately diluted.

Abbreviations: BSA, bovine serum albumin; DPC, dipalmitoyl phosphatidylcholine; FACS, fluorescence-activated cell sorter; fMLP, N-formylmethionyl-leucyl-phenylalanine; HBSS, Hanks' balanced salt solution; IgG, immunoglobulin G; LTB_4, leukotriene B_4; mAb, monoclonal antibody; PBS, phosphate-buffered saline; TNF-α, tumor necrosis factor α.

Reprint requests: Edna J. Bates, Department of Immunology, Women's and Children's Hospital, 72 King William Road, North Adelaide, S.A. 5006, Australia.

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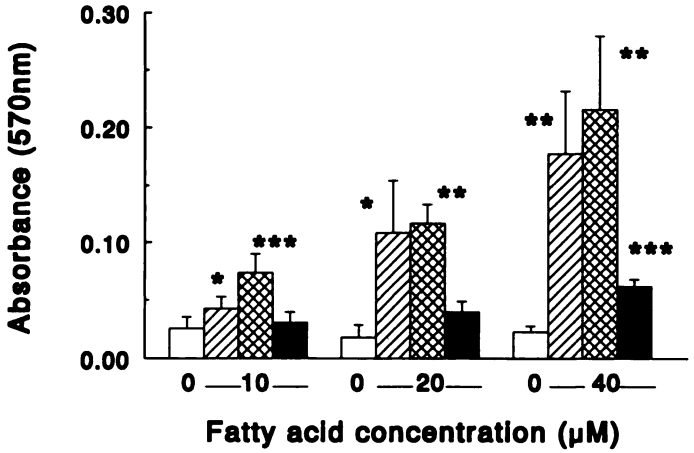

Fig. 1. Effect of 22:6, 20:4, and 20:5 on neutrophil adherence. Neutrophils (5×10^5/well) were coincubated for 30 min in microtiter plates with the indicated concentrations of 22:6 (hatched bars), 20:5 (crosshatched bars) or 20:5 (solid bars) as mixed DPC-fatty acid micelles and adherence was measured after staining with Rose Bengal. Control incubations (open bars) contained DPC alone. Incubations were performed in triplicate. Results are mean ± SD for four separate experiments. *,**,*** $P < .05, .02, .01$ compared to DPC control for each fatty acid concentration using a two-tailed *t*-test for paired data.

Alternative preparation of fatty acids

Where indicated, three alternative methods of preparation of 20:4 were used. First, 20:4 was dissolved in ethanol and diluted with HBSS to give a working solution of 100 μg 20:4/ml. The final concentration of ethanol in the adherence assay was 0.4% (v/v). Second, 20:4 was sonicated in a 1 mM solution of α-cyclodextrin (Sigma) in HBSS at a ratio of 1 to 10 (20:4 to cyclodextrin) by molarity. Third, 20:4 was resuspended in a 50 μM solution of essentially fatty acid-free bovine serum albumin (BSA) at a ratio of 2 to 1 (20:4 to BSA) by molarity. Controls contained ethanol, cyclodextrin, or BSA alone appropriately diluted.

Treatment of neutrophils

In some experiments neutrophils (50 μl, 5×10^5) were coincubated in microtiter plates for 30 min at 37°C with 25 μl of fatty acid dilutions (final concentration 10, 20, or 40 μM), DPC alone, TNF-α (50 μl, 50 U), or fMLP (5×10^{-7} M; Sigma) in a total volume of 100 μl. In other experiments neutrophils (1 ml, 1×10^7) were pretreated for 30 min at 37°C with 0.25 ml (25 μg) to 0.5 ml (50 μg) fatty acid, DPC alone, or TNF-α (1000 U) in a total volume of 2 ml of HBSS unless indicated otherwise. This gave final fatty acid concentrations of 40 or 80 μM, respectively. Cells were washed once with HBSS, counted, and resuspended at 1×10^7/ml before use in adherence assays. Recombinant human TNF-α (Genentech) was a gift from Dr. G. R. Adolf, Ernst Boehringer Institute, Vienna, Austria.

Measurement of adherence

Adherence was measured in 96-well microtiter plates previously coated with 250 μl of 10% autologous plasma for 30 min. Plates were washed twice and air dried before use. Neutrophils (50 μl, 5×10^5), either pretreated or in coincubation with fatty acids, were allowed to adhere for 30 min at 37°C (total volume in well, 100 μl HBSS). Nonadherent cells were removed by inversion of the plates and the wells were washed three times with 250 μl of HBSS and stained with Rose Bengal [10]. After release of the dye with 50% ethanol, the absorbance (570 nm) of each well was determined using an enzyme-linked immunosorbent assay reader. Parallel wells were run in the absence of neutrophils. Results are expressed after subtraction of absorbance values for wells without neutrophils.

Immunofluorescent flow cytometric analysis

Pretreated neutrophils (5×10^5) were incubated for 30 min at 4°C with saturating concentrations of monoclonal antibodies (mAbs), washed with ice-cold phosphate-buffered saline (PBS), and then stained at 4°C for 30 min with fluorescein-conjugated second antibody (goat anti-mouse immunoglobulin G, Organon Teknika) in the presence of 25% (v/v) autologous plasma. Cells were washed twice with PBS and fixed with paraformaldehyde (1%, w/v). Fluorescence distribution was measured on a Becton Dickinson fluorescence-activated cell sorter (FACS) analyzer. Fluorescence values were corrected by subtraction of values for isotype-matched negative controls.

RESULTS

Effect of 22:6, 20:4, and 20:5 on neutrophil adherence

When neutrophils were coincubated in microtiter plates for 30 min with 22:6 or 20:4 at 10 to 40 μM, a significant increase in adherence was observed compared to cells treated with DPC alone (**Fig. 1**). For 20:5, the higher concentration of 40 μM was necessary to demonstrate a significant increase in adherence. Preincubation as well as coincubation (fatty acids in contact with neutrophils throughout the adherence assay) experiments were performed to establish whether the effect of the fatty acids persisted through centrifugation, washing, and recounting of the cells or whether continued presence of the fatty acid was necessary. When neutrophils were preincubated for 30 min with 22:6, 20:4, or 20:5 at either 40 or 80 μM a significant increase in adherence was observed compared to cells treated with DPC alone (**Fig. 2**).

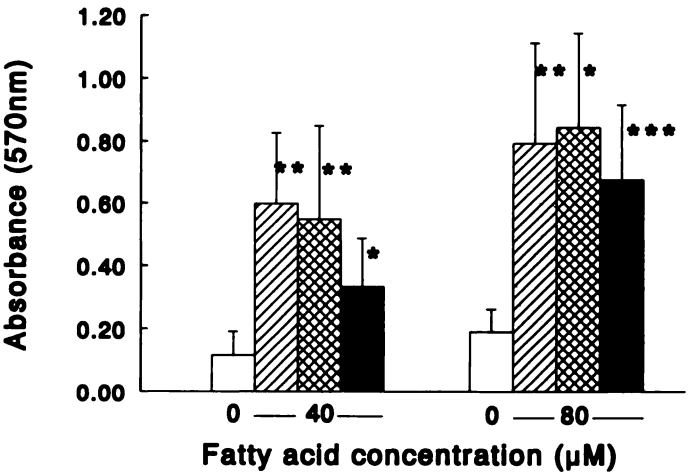

Fig. 2. Effect of preincubation with 22:6, 20:4, and 20:5 on neutrophil adherence. Neutrophils were preincubated at 5×10^6/ml for 30 min with the indicated concentrations of 22:6 (hatched bars), 20:4 (crosshatched bars), or 20:5 (solid bars) as mixed DPC-fatty acid micelles. Control preincubations (open bars) contained DPC alone. These pretreated neutrophils were washed and then incubated for 30 min in microtiter plates at 5×10^5/well and adherence was measured after staining with Rose Bengal. Incubations were performed at least in triplicate. Results are mean ± SD for 5 to 10 separate experiments. *,**,*** $P < .02, .01, .001$ compared to DPC control for each fatty acid concentration using a two-tailed *t*-test for paired data.

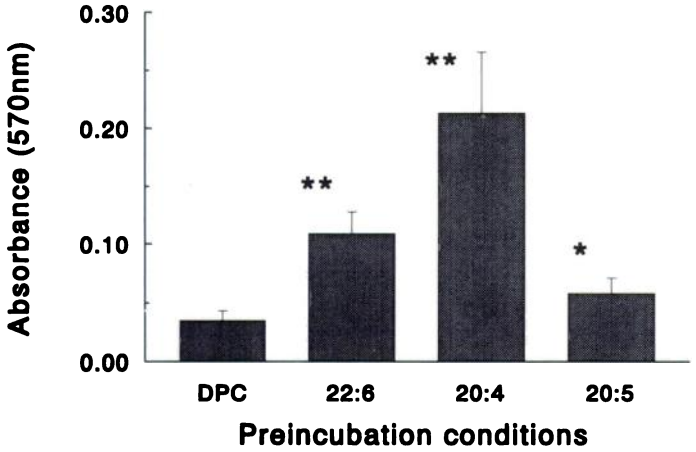

Fig. 3. Effect of fatty acid at 1 × 10^6 neutrophils/ml preincubation. Neutrophils were preincubated at 1 × 10^6/ml for 30 min with 10 μM fatty acids as mixed DPC-fatty acid micelles or DPC alone. These pretreated neutrophils were washed and then incubated for 30 min in microtiter plates at 5 × 10^5/well and adherence was measured after staining with Rose Bengal. Results are mean ± SD of six replicate wells from a representative experiment. *,**P < .01, .001 compared to DPC control using a two-tailed t-test for unpaired data.

In these experiments 22:6 was as active as 20:4, with 20:5 being somewhat less active. Stimulation by fatty acids was of the same order as that by TNF-α (100 U/10^6 cells), which is known to stimulate adherence [11-13], being 0.18 ± 0.10 and 0.72 ± 0.27 (mean ± SD, n = 7) for neutrophils preincubated for 30 min in its absence and presence. Glutaraldehyde fixation (to stabilize loosely adherent cells) did not alter the extent of neutrophil adherence in the presence of fatty acids (data not shown). In addition, visual assessment of wells demonstrated that stimulated adherence could not be attributed to neutrophil-neutrophil aggregates (data not shown).

Effect of lowering the concentration of neutrophils during fatty acid pretreatments

During the coincubation and preincubation experiments reported in Figures 1 and 2, the neutrophils were at a concentration of 5 × 10^6/ml while in contact with the fatty acids. Figure 1 demonstrates that at this cell concentration there was a small but significant effect of 10 μM 22:6 or 20:4 on neutrophil adherence and no effect of 20:5. However, when the number of neutrophils per ml in preincubation was reduced to 1 × 10^6/ml, the effect of 10 μM 22:6 or 20:4 was increased and now a small but significant increase in adherence was seen with 10 μM 20:5 (**Fig. 3**).

The lowest concentration of the fatty acids tested that could stimulate adherence was 5 μM at a neutrophil concentration of 5 × 10^5/ml preincubation. Under these conditions, absorbance (570 nm) values of 0.11 ± 0.05, 0.17 ± 0.04, 0.28 ± 0.05, and 0.22 ± 0.03 were obtained for DPC, 22:6, 20:4, and 20:5 respectively (P < .05 for 22:6 and P < .001 for 20:4 and 20:5 relative to DPC; n = 6).

Effect of incubation time and cell concentration on neutrophil adherence

When the time of incubation was varied in the adherence assay, fatty acid-stimulated adherence was maximal at 30 min (**Fig. 4A**). This followed closely the time course for TNF-α-stimulated cells but was somewhat delayed relative to fMLP-stimulated cells (Fig. 4A). The stimulation by fMLP (5 × 10^{-7} M) was midway between that of 22:6 or 20:5 and 20:4 (Fig. 3A). The ability of fMLP to stimulate neutrophil adherence is well known [12, 14]. The number of cells used in the adherence assay was kept at 5 × 10^5/well, there being a tendency for adherence to plateau above this number (Fig. 4B and C). The Rose Bengal assay does not allow routine assessment of the numbers of adherent cells [10]. However, a standard curve can be constructed after measurement of the absorbance of a known number of cells (cen-

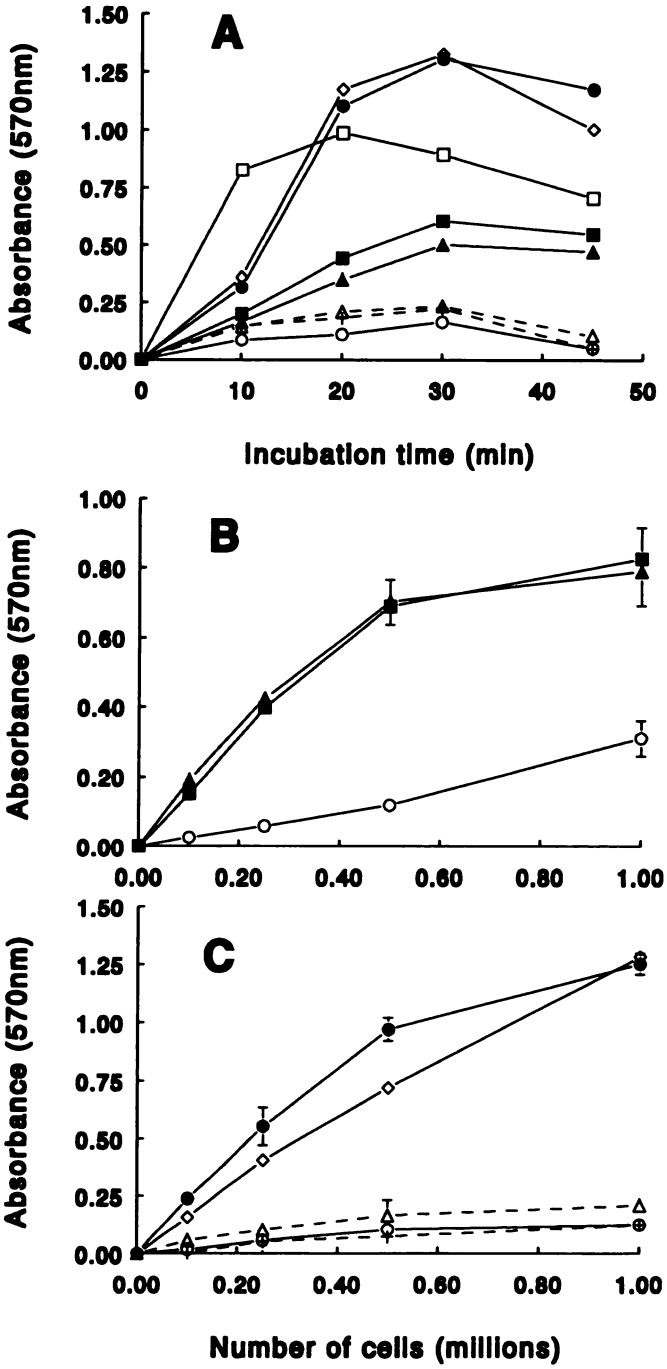

Fig. 4. Effect of incubation time and cell concentration on adherence. (A) Neutrophils (5 × 10^5/well) were coincubated for the indicated times in microtiter plates in the presence of HBSS (+), DPC (○), 22:6 (80 μM, ▲) 20:4 (80 μM, ●), 20:5 (80 μM, ■), 22:0 (80 μM, △, behenic acid), TNF-α (50 U, ◇), fMLP (5 × 10^{-7} M, □), and adherence was measured after staining with Rose Bengal. (B and C) Neutrophils were preincubated at 5 × 10^6/ml for 30 min with 22:6 (▲), 20:4 (●), 20:5 (■), 22:0 (△) (each at 80 μM), DPC (○), HBSS (+), or TNF-α (100 U/10^6 cells; ◇) and washed, and adherence to microtiter plates was measured after staining with Rose Bengal. Results are mean ± SD of triplicate wells from representative experiments.

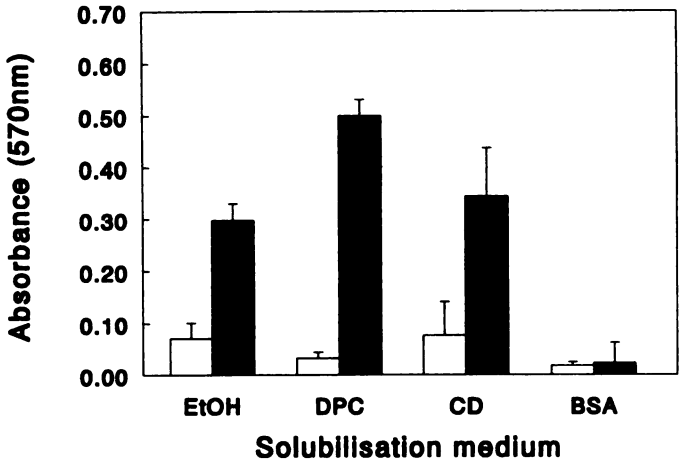

Fig. 5. Effect of solubilization method on the adherence due to 20:4. Neutrophils (5×10^5/well) were coincubated for 30 min in microtiter plates with 80 μM 20:4 (solid bars) prepared either in ethanol (EtOH), as micelles with DPC, with cyclodextrin (CD), or BSA. Controls (open bars) contained EtOH, DPC, CD, or BSA alone. Results are from a representative experiment and are mean ± SD of triplicate wells.

trifugation of plates being used to retain both adherent and nonadherent cells). By this method, the data at 30 min in Figure 4A can be converted to percent adherences of 14 (DPC), 38 (22:6), 86 (20:4), 44 (20:5), 18 (22:0), 18 (HBSS), 88 (TNF-α), and 62 (fMLP).

Effect of alternative methods of solubilization

When 20:4 was dissolved in ethanol or incorporated with cyclodextrin, comparable increases in adherence were seen (**Fig. 5**). However, when BSA was used to solubilize 20:4 there was no increase in absorbance due to 20:4 (Fig. 5).

Effect of saturated fatty acids on adherence

The structure of the fatty acid is important, because removal of double bonds removes its stimulatory capacity (Fig. 4A–C). In a separate experiment, absorbance values for DPC and the saturated fatty acid 20:0 (arachidic acid) were 0.114 ± 0.021 and 0.109 ± 0.028, respectively (n = 4).

Expression of neutrophil integrin receptors

Table 1 shows the relative expression of neutrophil integrin receptors after pretreatment with 22:6, 20:4, 20:5, 20:0, or TNF-α. There was a two- to threefold increase in the expression of CD11b with polyunsaturated fatty acids and TNF-α. This was not seen with the saturated fatty acid 20:0. CD11a and CD11c were not increased by the fatty acids. TNF-α caused a significant increase in CD11c.

When the number of neutrophils/ml and the fatty acid concentration were decreased, increased expression of CD11b was apparent at 5 μM 22:6 or 20:4 and 5×10^5 cells/ml preincubation (mean fluorescence intensity 73 ± 4, 98 ± 12, and 129 ± 23 for DPC, 22:6, and 20:4, respectively; representative experiment, n = 2). At this concentration 20:5 did not alter the expression of CD11b, although at 10 μM 20:5 a 26% increase in expression was seen (data not shown).

DISCUSSION

This paper is the first to demonstrate that polyunsaturated fatty acids of both the n-3 series (22:6 and 20:5) and n-6 series (20:4) can stimulate neutrophil adherence. For this effect the structure of the fatty acid is important because removal of the double bonds or conversion of the carboxyl group to a methyl ester inhibits the response (data not shown). Previously, we demonstrated that 22:6, 20:4, and 20:5 stimulate the neutrophil oxygen-dependent respiratory burst [6, 7] but that saturated fatty acids of equivalent chain length as well as methyl esters of these polyunsaturated fatty acids were without effect. Steinbeck et al. [15] have also shown that in addition to the partitioning of the polyunsaturated fatty acid in the neutrophil plasma membrane, activation or assembly of the NADPH oxidase requires the ionized fatty acid carboxyl group.

There is little information in the literature about the concentration of dietary polyunsaturated fatty acids in the blood. Sperling et al. [16] reviewed the literature and stated that "it is possible that plasma concentrations of nonesterified 20:5 in subjects consuming a diet greatly enriched with certain fish oils could reach 5–10 μg/ml." This represents 16–33 μM. In addition, it has been reported that the concentration of linoleate (18:2) in plasma free fatty acids increases from 49 to 247 μM 6 h after feeding a single meal of safflower oil [17]. One must also consider the possibility of local high concentrations of polyunsaturated fatty acids due to lipoprotein lipase activity on endothelial cells. Here we found that significant stimulation of adherence occurs between 5 and 80 μM 22:6, 20:5, or 20:4. Above 40 μM there was no difference in the ability of the three fatty acids to stimulate adherence. However, at or below 40 μM 20:5 was less active than 22:6 or 20:4. At 10 μM or below 22:6 was less active than 20:4. It is possible that dietary fish oils (20:5 and 22:6) may, at low concentrations, stimulate neutrophil adherence less than 20:4. However, once a certain concentration is reached this anti-inflammatory effect may be lost, with 20:5, 22:6, and 20:4 stimulating adherence to a similar extent. These polyunsaturated fatty acids are nontoxic to neutrophils (measured by cytoplasmic lactate dehydrogenase release and trypan blue exclusion) even at 160 μM (data not shown).

That the effect of the fatty acid persisted after its removal suggests that a neutrophil triggered at one site might still be

TABLE 1. Neutrophil Integrin Receptor Expression[a]

Treatment	Mean fluorescence intensity			
	CD11a	CD11b (1)	CD11b (2)	CD11c
HBSS	336 ± 103	972 ± 130	1118 ± 56	148 ± 38
DPC	298 ± 118	1009 ± 122	1182 ± 21	154 ± 58
22:6	288 ± 75	2498 ± 1922	3771 ± 1398*	273 ± 108
20:4	206 ± 116	2461 ± 835*	3040 ± 761**	176 ± 115
20:5	284 ± 48	2279 ± 476**	2951 ± 1294	216 ± 55
20:0	314 ± 119	1026 ± 167	1244 ± 101	160 ± 54
TNF-α	347 ± 89	1821 ± 180***	2210 ± 166***	267 ± 45*

[a]Neutrophils were preincubated at 5×10^6/ml for 30 min with HBSS, DPC, 40 μM fatty acids, or TNF-α (100 U/10^6 cells), washed, and labeled with mAbs for FACS analysis as described in Materials and Methods. Results are expressed as mean fluorescence intensity and are mean ± SD of three experiments. *,**,***P < .05, .02, .005 for incubations with fatty acids compared to DPC or for TNF-α compared to HBSS (two-tailed t-test for unpaired data). Anti-CD11a (HB202, IgG1) was TS1/22 [8], anti-CD11b (1) (WM20, IgG2b) was from Dr. K. Bradstock, Westmead Centre, Sydney, Australia [50], anti-CD11b (2) (IOM-1, IgG1) was from Immunotech, France, and anti-CD11c (KB90, IgG1) was from Dakopatts, Denmark. Negative controls were X63, an IgG1 [51], and Sal 4, an Ig2b [52]. Values for these controls were 54 to 85 and 39 to 92 for X-63 and Sal4, respectively, over the three experiments. There was no increase in fluorescence intensity with an unrelated mAb (FMC16, IgG2a) against HLA/β_2-microglobulin [53] after treatment with fatty acids or TNF-α.

active after migration to another site. The ability of these fatty acids to stimulate adherence varies with the concentration of neutrophils. Thus the extent of stimulation may be dependent on the stage of an inflammatory response during which neutrophil numbers may alter dramatically.

When 20:4 was solubilized with cyclodextrin, stimulation of adherence was also seen. This compound solubilizes fatty acids by incorporating them centrally into a doughnut-like structure of cyclodextrin [18], thus preventing the formation of fatty acid micelles. Fatty acids solubilized with cyclodextrin have proved suitable substrates for enzymatic studies [19]. Therefore the adherence of neutrophils stimulated by fatty acids was not a result of bridging of cells to the wells through micelles of DPC and fatty acid. In addition, fatty acid-stimulated adherence occurs only with 22:6, 20:4, or 20:5 but not with saturated fatty acids of equivalent chain length or the methyl ester of 22:6, which are also incorporated into DPC micelles. That 20:4 was unable to stimulate adherence when bound to BSA demonstrated that the stimulation normally seen was not due to a contaminant such as bacterial lipopolysaccharide in the preparations. It is well known that the effects of fatty acids on neutrophil morphology and superoxide release are reversed by treatment of the cells with delipidated albumin [20, 21].

CD11b/CD18, a major adhesion-related molecule of human neutrophils, was first reported as a receptor against complement component C3bi [22] but is also known to interact with fibrinogen [23], coagulation factor [24], bacterial lipopolysaccharide [25], and ICAM-1 on endothelial cells [26]. There is evidence that the interaction of stimulated neutrophils with unactivated endothelium involves CD11b [12, 27, 28]. Our data suggest that CD11b could be involved in fatty acid-stimulated neutrophil adherence. It is of interest that TNF-α stimulated expression of CD11c, not previously reported. TNF-α is known to increase CD11b [11, 13, 29].

Patients genetically deficient in the CD18 β_2 chain, leading to lack of expression of all three CD11 molecules, suffer recurrent soft tissue infections with their neutrophils exhibiting defects in adhesion-related functions [30]. Normal neutrophils treated in vitro with anti-CD11b mAbs exhibit defects indistinguishable from those of neutrophils from such patients [31]. However, there is also evidence that the regulation of neutrophil adherence does not necessarily correlate with changes in expression of CD11a and CD11b [32, 33], and it has been suggested that conformational changes in CD11/CD18 molecules may be important. In addition, aggregation of CD11b receptors within the membrane may be a prerequisite for binding [34]. Integrin-modulating factor-1 has been described [35] and is suggested to be an unsaturated fatty acid or an isoprenoid acid. This molecule alters the avidity of CD11b and CD11a without a change in the total number of receptors. Our data would be in support of such qualitative changes in CD11/CD18 in addition to quantitative changes in expression, as we have found 2- to 3-fold increases in CD11b under conditions in which adherence is stimulated 6- to 10-fold (data not shown). Thus polyunsaturated fatty acids may be another class of lipids that alter integrin receptor affinity.

The mechanisms by which these fatty acids stimulate adherence and CD11b expression are not known. Intracellular storage pools for CD11b have been demonstrated in phagocytes (reviewed in ref. 33). Unsaturated fatty acids produce perturbations in the cytoskeleton of lymphocytes [36] and cytoskeletal components are known to participate in the adhesion process [37], so it is possible that 22:6, 20:4, and 20:5 exert their effects via the cytoskeleton and thus receptor translocation. It has been suggested that 20:5 and 22:6 may exert their anti-inflammatory and cardioprotective effects by interference with prostaglandin and leukotriene metabolism [4]. Leukotrience B_4 (LTB_4), produced from 20:4 after its release from membrane phospholipids or supplied exogenously, stimulates neutrophil adherence [38]. Dietary enrichment with both 20:5 and 22:6 results in decreased production of LTB_4 in neutrophils in vitro and appearance of LTB_5, which has decreased biological activity [4]. Dietary 22:6 alone has been found to produce small amounts of LTB_5 but not to alter LTB_4 production [39]. A similar profile of leukotriene synthesis is seen when 20:5 and 22:6 are incubated in vitro with neutrophils [4]. Our data suggest that the ratio of LTB_5 to LTB_4 produced is not of importance in fatty acid-stimulated adherence because 20:5 stimulated both adherence and CD11b expression to the same extent as 20:4 at concentrations that would be expected to inhibit completely LTB_4 formation [4]. Abramson et al. [40] reported that 20:4 has direct effects on a variety of cells that do not depend on its metabolism via cyclooxygenase or lipoxygenase pathways. Likewise, the inhibition of lymphocyte proliferation by 20:4, 20:5, and 22:6 is independent of eicosanoid production [41]. Direct effects of polyunsaturated fatty acids are certainly feasible in our experimental system, resulting in increased expression and possibly avidity of integrin receptors.

Despite much epidemiologic and clinical data [1-3] suggesting a protective and anti-inflammatory effect of fish oils, a number of generally ignored reports state that fish oils may raise low-density lipoprotein cholesterol, producing a potentially atherogenic lipid profile [42, 43], and not reduce mortality from coronary heart disease [44, 45]. In addition, the unexplained increased incidence of stroke in populations with a high intake of n-3 fatty acids [46] and apparent genetic abnormality in essential fatty acid desaturation in Eskimos [47] serve to confuse the picture. In vivo, fish oils could influence many parameters, such as plasma concentrations, lipoprotein/cholesterol levels, platelet aggregation, cytokine production, and blood viscosity/pressure. Our data suggests that under certain conditions fish oils like 20:4 may also have a proinflammatory effect on neutrophils. However, at the lower concentrations used 22:6 and 20:5, in particular, were less stimulatory than 20:4, which could be considered anti-inflammatory. Our findings that adherence and expression of neutrophil CD11b are up-regulated by these fatty acids may be of more importance in relation to neutrophil functions such as phagocytosis and microbial killing [48, 49].

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