ω-3 Fatty acids suppress monocyte adhesion to human endothelial cells: role of endothelial PAF generation

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Mayer, Konstantin, Martina Merfels, Marion Muhly-Reinholz, Stephanie Gokorsch, Simone Rosseau, Jürgen Lohmeyer, Nicole Schwarzer, Matthias Krüll, Norbert Suttorp, Friedrich Grimminger, and Werner Seeger. ω-3 Fatty acids suppress monocyte adhesion to human endothelial cells: role of endothelial PAF generation. Am J Physiol Heart Circ Physiol 283: H811–H818, 2002; 10.1152/ajpheart.00235.2002.—Monocyte-endothelium interaction is a fundamental process in many acute and chronic inflammatory diseases. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are fish oil-derived alternative (ω-3) precursor fatty acids implicated in the suppression of inflammatory events. We investigated their influence on rolling and adhesion of monocytes to human umbilical vein endothelial cells (HUVEC) under laminar flow conditions in vitro. Exposure of HUVEC to tumor necrosis factor (TNF-α) strongly increased ω-3 fatty acid-induced lesion in vivo (1, 13, 45). In addition to the classical adhesion molecules, platelet-activating factor (PAF) expression in the endothelial membrane, interacting with monocyte PAF receptors, was suggested to contribute to the adhesive interaction of the mononuclear cells with the endothelium (47, 48).

Enhanced transmigration of circulating blood monocytes across the vascular endothelium is considered as an important contributor to the pathogenesis of acute and chronic systemic inflammatory diseases with sepsis and multiorgan failure (29) as well as atherosclerosis (24, 34) representing prototypic entities. In both diseases, lipid mediators have additionally been implicated in the pathogenesis of vascular abnormalities, and, of interest, supplementation with ω-3 fatty acid-rich diets is considered to be a therapeutic approach (10, 35). The family of ω-6 fatty acids, including arachidonic acid (AA), represents the predominant polyunsaturated fatty acids in common diets including β2-integrins (CD11/CD18 complex), the β1-integrin VLA-4 (very late antigen-4), selectins, intercellular adhesion molecule (ICAM-1), platelet endothelial cell adhesion molecule-1 (PECAM-1), and vascular cell adhesion molecule (VCAM-1) (1, 23, 27, 28, 33, 36, 38, 43). For firm monocyte-endothelium adherence, CD11/CD18-ICAM-1 and VLA-4-VCAM-1 interactions were noted to be particularly relevant, with directional motility through the interendothelial gaps into the subendothelial tissue apparently demanding reversible integrin-endothelial and subsequent monocyte-matrix interactions as, e.g., communicated via VLA-5 (43). Under conditions of inflammation, mimicked by endothelial cytokine pre-treatment in in vitro studies, endothelial adhesion molecules such as E-selectin, ICAM-1, and VCAM-1 are upregulated, and endothelial monocyte adhesion is markedly increased, with the role of VLA-4-VCAM-1 interaction being particularly prominent under these conditions (6–8, 33, 36, 43). This is consistent with in vivo studies addressing the role of both β1- and β2-integrins in monocyte migration into inflammatory sites or cytokine-induced lesions in vivo (1, 13, 45). In addition to the classical adhesion molecules, platelet-activating factor (PAF) expression in the endothelial membrane, interacting with monocyte PAF receptors, was suggested to contribute to the adhesive interaction of the mononuclear cells with the endothelium (47, 48).

Enhanced transmigration of circulating blood monocytes across the vascular endothelium is considered as an important contributor to the pathogenesis of acute and chronic systemic inflammatory diseases with sepsis and multiorgan failure (29) as well as atherosclerosis (24, 34) representing prototypic entities. In both diseases, lipid mediators have additionally been implicated in the pathogenesis of vascular abnormalities, and, of interest, supplementation with ω-3 instead of ω-6 fatty acid-rich diets is considered to be a therapeutic approach (10, 35). The family of ω-6 fatty acids, including arachidonic acid (AA), represents the predominant polyunsaturated fatty acids in common diets.
of the Western world. In contrast, ω-3 fatty acids make up an appreciable part of the fat in cold-water fish and
seal meat. In this family of fatty acids, the last double
bond is located between the third and fourth carbon
atom from the methyl end, with eicosapentaenoic acid
(EPA) and docosahexaenoic acid (DHA) being im-
portant representatives. AA is metabolized via multiple
metabolic pathways, including cyclooxygenases and
various lipoxygenases to prostanoids, leukotrienes,
and other lipoygenase products with well-described
vascular effects (12). The anti-inflammatory potency of
the ω-3 fatty acids is largely ascribed to the fact that
they serve as alternative lipid precursors for all meta-
bolic pathways hitherto recognized for AA. Arising
metabolites are trienoic prostanoids, thromboxane A3,
5-series cysteinyl-leukotrienes, and leukotriene B4,
which possess markedly reduced inflammatory and
vasomotor potencies compared with the AA-derived
lipid mediators and exert even antagonistic functions.
In addition to being precursors for different eicosanoid
formation, ω-3 versus ω-6 fatty acid incorporation into
membrane (phospho) lipid pools was suggested to have
impact on lipid-related intracellular signaling events
(9, 39, 44). Phosphatidylinositol and sphingomyelin
pools, but also subclasses of phosphatidylcholine such
as the PAF precursor pool, may be particularly rele-
vant in this respect.

In the present study performed on human endo-
thelial cells undergoing cytokine stimulation in vitro, the
impact of AA versus EPA and DHA on monocyte-
endothelial interaction was investigated under lami-
nar flow conditions. Incubation of the endothelial cells
with ω-3 fatty acids turned out to suppress monocyte
rolling and adhesion significantly, with DHA being
even more potent than EPA. Evidence is forwarded
that this ω-3 fatty acid effect is related to suppression of
endothelial PAF generation. Such an impact on
monocyte-endothelium interaction may contribute to
the dampening of inflammatory events observed under
ω-3-rich enteral or parenteral diets in acute and
chronic inflammatory diseases.

MATERIALS AND METHODS

Materials. AA, EPA, and DHA were obtained from Sigma
Chemical (Deisenhofen, Germany). Chromatographic sup-
plies included HPLC-grade solvents, glass-distilled (Fluka;
Heidelberg, Germany) octadecylsilyl (5

silica gel 5-μm column packing (Machery-Nagel; Duren, Ger-
mort), and C-18 Sep-Pac cartridges (Waters; Milford, MA).
RPMM-1640 medium and fetal calf serum were from Boehr-
gering Mannheim (Mannheim, Germany). Collagenase (type
CLS type II) was purchased from Worthington Biochemical
(Freehold, NJ). Medium 199, fetal calf serum, HEPES,
trypsin-EDTA solution, and antibiotics were obtained from
GIBCO (Karlsruhe, Germany). [3H]PAF, lypo-[3H]PAF,
[3H]acetate, and [3H]serotonin were obtained from Amer-
sch (Dreieich, Germany). The PAF receptor antagonist
WEB-2086 was generously supplied by Boehringer Ingelheim
(Ingelheim, Germany). Tissue culture plastic was purchased
from Becton-Dickinson (Heidelberg, Germany). 1-Octa-
decyl-2-acet-y1-sn-glycero-3-phosphocholine (PAP) and thom-
lin were obtained from Sigma Chemical. The PAF receptor
antagonist BN-50730 (46) was generously supplied by Dr. P.
Braquet (Institute Henri Beaufour, Le Plessis-Robinson,
France), and the PAF receptor antagonists CV-3988 and
CV-6209 were bought from Biomol (Hamburg, Germany). All
other biochemicals were obtained from Merck (Munich,
Germany).

Preparation of endothelial cells. Endothelial cells were
obtained from human umbilical veins (HUVEC) according to
the method described by Jaffe et al. (15).

Monocyte isolation. Human monocytes were isolated from
platelet pheresis residues by centrifugation on Ficoll-
Hypaque density gradient centrifugation, followed by
counterflow centrifugation elutriation using a Beckman JE-5.0
rotor. Monocyte purity (88–90%) was confirmed by light
scatter [fluorescence-activated cell sorter (FACS) scan; Bec-
ton Dickinson]. Cell viability ranged above 96% throughout
the study.

Leukocyte adhesion assay. Leukocyte adhesion was deter-
mmed as described previously (16) using a parallel plate flow
chamber according to Lawrence and Sussman (19). Confluent
endothelial monolayers were preincubated with fatty acids
and tumor necrosis factor-α (TNF-α) according to the experi-
mental protocol. A suspension of 4 × 10⁶ monocytes per
milliliter was perfused through the chamber at a constant
wall shear stress of 1.0 dyn/cm² (syringe pump sp100I, WPI;
Sarasota, FL). Interactions were visualized using a phase-
contrast video microscope (IMT-2, Olympus Optical, Ham-
burg, Germany, with a KP-C551 CCD camera, Hitachi,
Rodgau, Germany) and videotaped (JVC HR-S7000, JVC;
Friedberg, Germany) over the entire time period of leukocyte
perfusion. Rolling in the parallel plate flow chamber was
measured in a high-power field for each experiment. “Rolling”
was expressed as the number of rolling cells per high power
field (×20 objective) during a 10-min observation period.
Leukocytes were considered to be adherent after 30 s of
stable contact with the monolayer. Adhesion was determined
after 10 min of perfusion by analysis of five random high
magnification fields (×20) from videotape (16, 19). Results
are expressed as adherent cells per high magnification
field.

Measurement of PAF by bioincorporation of radiolabel.
Endothelial cell PAF production was quantified by post-
HPLC liquid scintillation counting using the radiochromatog-
amic imaging system (5LS Raytest). Endothelial cells were
stimulated in the presence of 50 μCi [3H]acetate (7.75 Ci/
mol) with 0.1 U/ml thrombin according to Tessner et al. (42)
as adapted by Suttorp et al. (41). Reactions were stopped by
addition of three volumes of chloroform:methanol (2:1 vol/
vol), and extraction was performed according to Bligh and
Dyer (31).

Post-HPLC PAF Bioassay. In addition, PAF production in
HUVEC was quantified by induction of [3H]serotonin release
from prelabeled rabbit platelets. After HUVEC incubation,
the total cellular and extracellular PAF content was lipid-
extracted and subjected to straight phase HPLC separation
as described above. Eluate fractions were collected at the
appropriate PAF retention time, again lipid extracted for
removal of phosphoric acid present in the mobile phase,
evaporated to dryness, and redissolved in 50 μl of assay
buffer for induction of platelet serotonin release. Preparation
of platelets and the protocol of the bioassay were essentially
as published by Pinkard et al. (30) and Suttorp et al. (41).
Aliquots of each sample were used to ascertain the specificity
of platelet secretion by the inhibitory effect of the PAF-
receptor antagonist BN-50730.

Immunofluorescence staining of endothelial cells. Immuno-
fluorescence labeling of HUVEC was performed as previously
described (21). Antibodies directed against ICAM-1 [clone R1/1 (CD54); Bender MedSystems; Vienna, Austria], VCAM-1 [clone 1G11 (CD106), Coulter-Immunotech; Marseille, France], E-selectin [clone BBIG-E1 (CD62E), R&D Systems], major histocompatibility complex-I (MHC-I, positive control, W6/32.HL, generously provided by A. Ziegler, Berlin, Germany), and isotype controls (negative control; Dianova) were used.

Cell surface ELISA for P-selectin. Expression of P-selectin was determined by cell surface ELISA as previously described (17). The primary monoclonal antibody against P-selectin [clone 9E1 (CD62P), R&D] was used.

Experimental protocol. HUVEC were grown to confluence, the culture medium was exchanged, and free fatty acids (AA, EPA, and DHA) dissolved in ethanol (final volume <1%, vol/vol) were admixed to the culture medium at a final concentration of 10 μmol/l and incubated for 6 h. Controls were sham incubated with solvent only. Without exchange of incubation medium, admixture of TNF-α (0, 1, or 10 ng/ml, as detailed) was then performed, and HUVEC were incubated for another 20 h. Expression of the endothelial adhesion molecules was then carried out after treatment with trypsin and transfer of the HUVEC to a FACS. For the monocyte adhesion experiments, HUVEC were grown on slides under the detailed experimental conditions, the incubation medium was discarded, and cells were gently washed directly before use in the flow chamber.

Statistics. For statistical comparison, one-way analysis of variance was performed. A level of P < 0.05 was considered to be significant. Analysis was carried out with SPSS for Windows (Release 8.0.0, SPSS; Chicago, IL).

RESULTS

Influence of TNF-α and fatty acids on monocyte-endothelium rolling and adhesion. Superfusing monocytes over HUVEC monolayer incubated under control conditions resulted in a low number of rolling and adherent monocytes (~5–9 and 10–12 monocytes/high magnification field, respectively). Monocyte rolling and adhesion were enhanced by nearly one order of magnitude by a preceding 20-h exposure of the HUVEC to TNF-α (10 ng/ml; Fig. 1). Preincubation of the HUVEC for 6 h with free EPA or DHA (10 μmol/l each), followed by the stimulation with TNF-α (20 h), significantly reduced this increase in monocyte rolling compared with TNF-α alone to 52% and 38%, respectively. In contrast, free AA (10 μmol/l) increased monocyte rolling to 149% (Fig. 1). Adhesion of monocytes to endothelial cells was significantly reduced by both ω-3 fatty acids. Compared with TNF-α alone set at 100%, EPA reduced this adhesion to ~61%, and DHA to ~55%, respectively. AA led to some reduction of monocyte adhesion but did not reach level of significance. The higher potency of the ω-3 fatty acids compared with AA was statistically highly significant (P < 0.005, Fig. 1).

Influence of TNF-α and fatty acids on endothelial adhesion molecule expression. To address the hypothesis that fatty acids might suppress monocyte adhesion by reducing the expression of endothelial adhesion molecules, FACS analysis of EC was performed. E-selectin, VCAM-1, and ICAM-1 were upregulated by TNF-α (Fig. 2); however, none of the fatty acids exerted an significant effect on this increase. In addition, preincubation of HUVEC with AA, EPA, or DHA in the absence of TNF-α also did not affect the expression of E-selectin, VCAM-1, or ICAM-1. Analysis of endothelial P-selectin showed no signal on these cultured cells even after TNF-α challenge, which did not change in response to fatty acid preincubation.

Influence of PAF-receptor antagonists on monocyte adhesion to TNF-α-activated endothelial cells. To assess a putative role of PAF in the TNF-α- and fatty acid-induced alterations of monocyte-endothelium interaction, the effect of the selective PAF-receptor an-
agonists BN-50730, CV-3988, and CV-6209 were investigated. BN-50730 dose dependently inhibited the adhesion of monocytes to TNF-α-activated HUVEC (Fig. 3). Used in concentrations of 0.01, 0.1, and 1 μmol/l, BN-50730 reduced monocyte adhesion to 86%, 70%, and 39%, respectively. The solvent control was without influence (data not shown). CV-3988 (10 nmol/l-10 μmol/l) and CV-6209 (10 nmol/l-10 μmol/l) dose dependently reduced TNF-α-enhanced adhesion of monocytes, with a maximal reduction to 49% and 52%, respectively. In the presence of 1 μmol/l BN-50730, neither EPA nor DHA further suppressed the amount of adherent monocytes to TNF-α-activated endothelial cells (Fig. 4). Under these conditions, AA even slightly increased the monocyte-endothelium adhesion (TNF+AA+BN compared with the TNF+BN in Fig. 4).

Influence of ICAM-1 or VCAM-1 blockade on monocyte adhesion to TNF-α-activated endothelial cells. In separate experiments, saturating amounts of adhesion-blocking antibodies against ICAM-1 [clone R1/1 (CD54)] and VCAM-1 [clone 1G11 (CD106)] were admixed to the endothelial incubation medium of TNF-α-pretreated HUVEC 30 min before the adhesion assay. Monocyte adhesion was reduced to 57.0 ± 4.2% (anti-ICAM-1) and 46.3 ± 3.8% (anti-VCAM-1) compared with the corresponding control group in any of the fatty acid-incubated cells.
with TNF-α controls in the absence of antibodies monoclonal antibodies.

**Fatty acids and endothelial cell PAF generation.** To address the impact of the fatty acids on endothelial PAF generation in a more direct fashion, short-term provocation of PAF synthesis by challenge of HUVEC with thrombin was performed, because examination of HUVEC after superfusion of monocytes yielded PAF levels below the detection limit of our assays. Pretreatment of the endothelial cells with TNF-α and fatty acids was undertaken as described above. Thrombin provoked a dose-dependent generation of PAF in control HUVEC, as demonstrated by both PAF bioassay (Fig. 5) and release of bioincorporated [3H]acetate (data not shown). Thrombin-induced PAF-synthesis was increased by approximately equal to one order of magnitude when endothelial cells were pretreated with TNF-α. A further increase in PAF quantities in lipid-extracted HUVEC was found after preincubation with AA (Fig. 5). In contrast, incubation with EPA and even more with DHA resulted in a suppression of thrombin-induced PAF synthesis.

**DISCUSSION**

Monocytes spontaneously adhere to endothelial cell monolayers under static conditions; however, substantial monocyte-endothelial adhesion under flow demands preceding cytokine stimulation of the endothelial cells: E-selectin-L-selectin, ICAM-1-β2 integrin, and in particular VCAM-1-VLA-4 interactions were shown to represent predominant adhesive forces under these conditions (6–8, 14, 33, 36, 43). It is well in line with these preceding observations that monocyte rolling and firm adhesion as currently addressed under laminar flow conditions was increased by approximately one order of magnitude after TNF-α pretreatment of the HUVEC, in companion with markedly enhanced endothelial ICAM-1 and VCAM-1 expression. Moreover, the monocyte adhesion to the cytokine-stimulated endothelial cells was reduced to 46% by blocking antibodies against VCAM-1 and to 57% by antibodies against ICAM-1.

In addition to these adhesion molecule interactions, the present data suggest endothelial PAF synthesis as an important contributor to monocyte-HUVEC adhesion. First, endothelial PAF synthesis as probed by thrombin challenge was increased by one order of magnitude upon prolonged TNF-α incubation. Second, monocyte adhesion to cytokine-stimulated endothelial cells was dose dependently inhibited by PAF-receptor antagonists. PAF synthesis in endothelial cells was first described in HUVEC (5, 31) but is in fact a function of endothelial cells from all vascular beds activated by receptor-operated stimuli or undergoing injurious attack (3, 18). Endothelial PAF remains associated with the cell surface, even in the presence of albumin for binding of this hydrophobic molecule, and studies from Zimmerman and co-workers (20, 48, 49) suggested that binding of endothelial PAF and the leukocyte PAF receptor contribute to adhesive interactions between endothelial cells and monocytes, followed by a juxtacrine activation of the adherent mononuclear cells. This concept is fully supported by the
presently noted efficacy of the PAF receptor antagonist. A role of P-selectin coexpression with PAF for the endothelial attraction of monocytes under condition of laminar flow (49) may be excluded for the present investigation. Cultured HUVEC demonstrate hardly any P-selectin expression after stimulation with TNF-α. This study did not address the question whether adhesive forces between PAF and its receptor may directly contribute to the firm monocyte attachment on the endothelial cells or whether the PAF system is largely operative via monocyte activation and enhanced mononuclear integrin expression and/or avidity as suggested as juxtacrine PAF-induced mechanisms (22, 49). Receptor-operated regulation of integrin affinity has, indeed, been disclosed as an important mechanism inducing rapid leukocyte adhesion to endothelial cell surfaces (26, 40, 43).

The most important finding of the present study is the fact that ω-3 fatty acid preincubation of the HUVEC markedly reduced monocyte rolling and adhesion to endothelial cells, with DHA being even more potent than EPA; monocyte adherence was reduced to 55% and 61%, respectively, compared with control cells incubated with TNF-α in the absence of these fatty acids. As assessed by random videotape analysis, firmly adhering monocytes all subsequently transmigrate the endothelial barrier, and this feature was not changed by endothelial fatty acid incubation (data not shown). Thus the ω-3 fatty acid-effect decrease in the amount of rolling and adhering monocytes directly translates into a reduction (in absolute numbers) of transmigrating monocytes.

The suppression of monocyte adherence to the EPA- or DHA-preexposed endothelial cells might be exerted by a reduction of adhesion molecule expression on the endothelial surface. Previous investigations indeed supplied evidence that ω-3 fatty acid incubation of cytokine-stimulated endothelial cells reduced VCAM-1 expression (9, 44). In the present study in HUVEC, however, quantification of endothelial surface expression of VCAM-1, ICAM-1, E-selectin, and P-selectin by FACS analysis did not detect any change in response to AA, EPA, or DHA pretreatment. This discrepancy may be explained by the fact that the fatty acid concentrations in these previous studies ranged between 100 and 300 μmol/l, whereas 10 μmol/l was employed in the present study to meet the physiological plasmatic fatty acid concentrations. The current finding of unchanged adhesion molecule expression on the EPA- or DHA-exposed HUVEC does, however, not exclude the possibility that the ω-3 fatty acids might have impact on the affinity of these adhesion molecules. Moreover, we did not address the endothelial surface expression of a L-selectin ligand suggested to contribute to monocyte-endothelial adherence under flow conditions (23).

The present study did, however, forward evidence that the prominent impact of the ω-3 fatty acids on the monocyte-HUVEC interaction is related to endothelial PAF generation. First, coapplication of the PAF-receptor antagonist BN-50730 and EPA or DHA did not exert additive effects on the rate of monocyte adhesion, but the levels of suppression achieved by either BN-50730 or EPA/DHA or a combination of ω-3 fatty acid and BN-50730 were not statistically different from each other. Second, when probing with thrombin, an established stimulus for rapid PAF synthesis in endothelial cells (48), the appearance of this lipid mediator in the cytokine preexposed HUVEC was found to be markedly suppressed by EPA and DHA. These findings are well compatible with the concept that preincubation of the HUVEC with EPA or DHA inhibits endothelial PAF generation and subsequently reduces monocyte adhesion. Interestingly, recent studies of the lipid composition of the endothelial membrane pools under cytokine challenge demonstrated marked loss of long-chain polyunsaturated fatty acids within 22 h, mimicking an “essential fatty acid deficiency” syndrome, with exogenous ω-3 fatty acids being rapidly incorporated into the sn-2 position of the phosphatidylcholine, the phosphatidylethanolamine, and the phosphatidylinositol pool, including their PAF precursor subclasses under these conditions (K. Mayer and W. Seeger, personal communication). This is well in line with data in eosinophilic leukocytes, where preincubation with DHA resulted in incorporation into the phosphatidylcholine pool, a reduction of PAF generation, and free AA release (37). Moreover, the activity of the phospholipase A2, hydrolyzing the sn-2 acyl residue from the PAF precursor as an initial step in endothelial PAF synthesis, is known to be dependent on the type of fatty acid located in the sn-2 position (32, 37), and DHA and EPA might well exert their suppressive effect on PAF synthesis via this route. This suggestion for the mode of action of EPA and DHA does, of course, not exclude that these ω-3 fatty acids may have major impact on additional lipid-related signaling events finally contributing to the rate of monocyte adherence to endothelial cells.

In contrast to EPA and DHA, the endothelial PAF liberation as probed by thrombin challenge was increased upon preincubation of HUVEC with AA, which is well compatible with the finding that similar to the ω-3 fatty acids, AA is rapidly incorporated into PAF precursor pools of cytokine-stimulated HUVEC, and that PAF precursors with AA in the sn-2 position are preferred substrates for the phospholipase A2 attack, the rate-limiting step in PAF synthesis (32). In parallel, an increase of rolling monocytes on HUVEC was found. Accordingly, AA preincubation of the endothelial cells significantly antagonized the BN-50730-affected decrease in monocyte-HUVEC adherence. When given as sole agent, AA significantly differed from EPA and DHA with respect to the suppressive effect on endothelial monocyte adhesion; however, still some not significant reduction of adherence was noted. Thus further effects of AA, not related to its impact on PAF synthesis, must be assumed to underlie the influence of this fatty acid on the monocyte-HUVEC interaction.

In conclusion, firm adhesion of monocytes on cytokine-stimulated HUVEC was found to be largely depressed by preincubation of the endothelial cells with EPA and DHA. This effect of the ω-3 fatty acids oc-
ocurred independent of the endothelial expression of the adhesion molecules ICAM-1, VCAM-1, E-selectin, and P-selectin but is probably related to the suppression of endothelial PAF synthesis by EPA and DHA. The applied concentrations of ω-3 fatty acids (10 μmol/l) are in the range of free plasmatic EPA/DHA levels appearing under long-term oral supplementation with fish oil (4) and under short-term infusion of fish oil-based ω-3-based lipid emulsions; the latter approach even provoked plasmatic-free EPA/DHA concentrations of >100 μmol/l in septic patients (11, 25). The impressive effect of ω-3 fatty acids on monocyte-endothelium interaction may be of interest for dampening inflammatory processes in acute and chronic diseases, in which activation and transmigration of mononuclear cells largely contribute to the pathogenic sequels.

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