Unsaturated fatty acids selectively induce an inflammatory environment in human endothelial cells

Michal Toborek, Yong Woo Lee, Rosario Garrido, Simone Kaiser, and Bernhard Hennig

ABSTRACT

Background: Activation of the vascular endothelium by dietary fatty acids may be among the most critical early events in the development of atherosclerosis. However, the specific effects of fatty acids on inflammatory responses in endothelial cells are not fully understood.

Objective: The present study focused on the induction of inflammatory genes in human endothelial cells exposed to individual dietary fatty acids. Because of the significance of nuclear factor κB (NF-κB) and activator protein 1 (AP-1) in the regulation of inflammatory gene expression, we also determined the effects of fatty acids on NF-κB and AP-1 transcriptional activation.

Design: Human umbilical vein endothelial cells were exposed to dietary mono- and polyunsaturated 18-carbon fatty acids. Transcriptional activation of NF-κB and AP-1 was determined in human umbilical vein endothelial cells transfected with reporter constructs regulated by these transcription factors. Induction of the inflammatory genes was studied by use of reverse transcription–polymerase chain reaction.

Results: Of the fatty acids studied, linoleic acid stimulated NF-κB and AP-1 transcriptional activation the most. In addition, treatment with this fatty acid markedly enhanced messenger RNA levels of tumor necrosis factor α, monocyte chemoattractant protein 1, vascular cell adhesion molecule 1, and intercellular adhesion molecule 1. Treatment with linolenic acid stimulated only a moderate induction of the genes encoding for these inflammatory mediators, and exposure to oleic acid either had no effect or resulted in decreased inflammatory gene messenger RNA. In addition, exposure to both linoleic and linolenic acids strongly stimulated induction of the phospholipid hydroperoxide glutathione peroxidase gene.

Conclusion: Specific unsaturated dietary fatty acids, particularly linoleic acid, can selectively stimulate the development of a proinflammatory environment within the vascular endothelium.


KEY WORDS: Fatty acids, inflammatory genes, transcription factors, human endothelial cells, atherosclerosis, nuclear factor κB, activator protein 1

INTRODUCTION

Activation or dysfunction of the vascular endothelium is one of the first events in the development of atherosclerosis (1, 2), and selected dietary fatty acids may be among the most critical factors that induce these processes. For example, lipids, including selective fatty acids, may cause injury to the endothelium (reviewed in reference 3). It has been proposed that hydrolysis of triacylglycerol-rich lipoproteins mediated by lipoprotein lipase, a key enzyme in lipoprotein metabolism that is associated with the luminal site of endothelial cells, may be an important source of high concentrations of fatty acid anions near the endothelium (4, 5). In support of this notion, it was shown that lipoprotein lipase activity is increased in atherosclerotic lesions (5–7). Lipoprotein lipase–derived remnants of lipoproteins isolated from hypertriglyceridemic subjects as well as selective unsaturated fatty acids can disrupt endothelial integrity (8, 9). Because the lipid composition of plasma and tissues is closely related to dietary fat intake (10), exposure of endothelial cells to individual fatty acids can be directly influenced by the types of fatty acids consumed in the diet (10, 11).

Strong evidence indicates that exposure to selected dietary unsaturated 18-carbon fatty acids can directly affect endothelial cell metabolism. Significant amounts of data have been accumulated to show that linoleic acid (18:2n–6) can induce marked injury to endothelial cells. For example, it was reported that this fatty acid can disrupt endothelial cell integrity, alter functions of gap-junctional proteins (12), increase concentrations of intracellular calcium, and induce cellular oxidative stress (13). In addition, the treatment of endothelial cells with linoleic acid and tumor necrosis factor α (TNF-α) can activate caspase 3 activity and induce apoptotic cell death (14, 15). The role of other dietary unsaturated 18-carbon fatty acids in endothelial cell metabolism is less well understood. However, evidence indicates that dietary oleic acid can protect endothelial cells against hydrogen peroxide–induced oxidative stress (16) and reduce the susceptibility of LDLs to oxidative modifications (17).

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Atherosclerosis is an inflammatory disease of the vascular wall (18). Inflammatory reactions in endothelial cells are regulated primarily through the production of chemokines [eg, monocyte chemoattractant protein 1 (MCP-1)], inflammatory cytokines (eg, TNF-α), and adhesion molecules [eg, intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1)]. Expression of these inflammatory mediators and their effects are closely interrelated. In addition, overexpression of MCP-1 (19), TNF-α (20), and ICAM-1 and VCAM-1 (21) is a common feature of atherosclerotic processes.

Inflammatory genes, such as those encoding for MCP-1, TNF-α, ICAM-1, and VCAM-1, are regulated by a variety of transcription factors (2, 22). It appears that nuclear factor κB (NF-κB) and activator protein 1 (AP-1) play critical roles in these regulatory processes. The binding sites for these transcription factors were identified in the promoter regions of various inflammatory genes (22–25), and increased amounts of NF-κB were found in atherosclerotic vessels (26, 27). In addition, selected fatty acids, such as linoleic acid, can activate NF-κB in endothelial cells (13). Moreover, effects mediated by NF-κB and AP-1 appear to be interrelated. For example, it was shown that TNF-α-mediated induction of VCAM-1 expression requires both activated NF-κB and AP-1 (23).

Phospholipid hydroperoxide glutathione peroxidase (PHGPx) is an antioxidant enzyme involved in detoxification of lipid hydroperoxides in cellular membranes and lipoproteins (28). Thus, this enzyme may play a critical role in antioxidant protection against oxidative stress induced by unsaturated fatty acids. Although it is known that selected fatty acids can induce oxidative stress and activate transcription factors responsive to oxidative stress (13), the specific effects of unsaturated fatty acids on inflammatory responses in endothelial cells are not fully understood. Therefore, the focus of the present study was to examine the induction of the inflammatory genes in human endothelial cells exposed to specific 18-carbon, mono- and polyunsaturated fatty acids. In addition, because of the significance of NF-κB and AP-1 in the regulation of the inflammatory genes, the effects of unsaturated fatty acids on the activity of these transcription factors were also determined.

MATERIALS AND METHODS

Human umbilical vein endothelial cell cultures and fatty acid treatments

Human umbilical vein endothelial cells (HUVECs) were isolated as described previously (29) and cultured in enriched M199 medium, which included 25 mmol HEPES/L, 54.3 × 10⁻⁵ U heparin/L, 2 mmol L-glutamine/L, 1 μmol sodium pyruvate/L, 200 × 10⁻⁵ U penicillin/L, 200 mg streptomycin/L, 0.25 mg amphotericin B/L (GibcoBRL, Grand Island, NY), 0.04 g endothelial cell growth supplement/L (Becton Dickinson, Bedford, MA), and 20% fetal bovine serum (HyClone, Logan, UT). Cells were determined to be endothelial in origin by their cobblestone morphology and uptake of fluorescently labeled acetylated LDL (1,1‘-dioctadecyl-3,3,3’,3‘-tetrachlorofluorescein diacetate) and DiI-acetylated LDL (Molecular Probes, Eugene, OR). All experiments were performed on cells from passage 2. Confluent cell cultures were treated with ≤180 μmol/L of oleic acid (18:1n−9), linoleic acid, or linolenic acid (18:3n−3) (Nu-Chek Prep, Elysian, MN). Fatty acid–enriched experimental media were prepared as described earlier (9).

To study the dose-dependent effects of specific unsaturated fatty acids on messenger RNA (mRNA) levels of genes critical in the endothelial cell inflammatory response, HUVECs were exposed to 60, 90, and 180 μmol fatty acids/L. Preliminary experiments showed that fatty acids consistently exerted a maximum effect on inflammatory gene induction at the concentration of 90 μmol/L. Therefore, experiments with 180 μmol fatty acids/L were discontinued and data are presented only from studies in which HUVECs were exposed to 60 and 90 μmol unsaturated fatty acids/L.

Transfections and reporter gene assay

Transfections were performed as described earlier (30). Briefly, HUVECs were seeded in 12-well plates and grown to 50–60% confluency in normal growth medium. Then, aliquots of normal M199 medium were mixed with 36 mg/L of a liposome pFx-7 (Invitrogen, Carlsbad, CA) and with 10 mg/L of NF-κB- or AP-1-responsive plasmids (pNFκB-Luc or pAP1-Luc, respectively) containing a luciferase reporter gene (Stratagene, La Jolla, CA). The transfection mixtures were incubated at 37°C for 30 min to allow DNA-lipid complexes to form. Endothelial cell cultures were washed with M199 medium to remove serum, and 1 mL transfection solution was added for 1.5 h to each well of the 12-well plate. After incubation, transfection solutions were aspirated and replaced with growth medium for 24 h. Then, transected cultures were treated with specific unsaturated fatty acids for 24 h. Control groups consisted of transfected HUVEC cultures that were not exposed to fatty acids.

Luciferase activity was measured by use of the Luciferase Assay System (Promega, Madison, WI). Briefly, culture media were removed and HUVECs were washed with phosphate-buffered saline and incubated with cell culture lysis reagent. Cell lysates were centrifuged (12000 × g, 2 min, 4°C) to remove membrane debris, and 10 μL of the cell extracts was mixed with 100 μL luciferase assay reagent containing luciferin and ATP in a luminometer with automatic injection. Values are expressed in relative light units (RLU)/μg protein.

Transfection efficiency was monitored as described earlier (30) by transfection of endothelial cells with the VR-3301 vector, which contains human placental alkaline phosphatase as the reporter gene. Under the described conditions, transfection efficiency was determined to be 32% (30). All transfection studies were repeated 3 times by using 6 wells in 12-well plates per experimental group.

Reverse transcriptase–polymerase chain reaction analyses

Reverse transcriptase–polymerase chain reaction (RT-PCR) was performed as described earlier (29, 31). Briefly, treated HUVECs were lysed and the total RNA was extracted with use of RNA STAT-60 (Tel-TEST, Inc, Friendswood, TX) according to the procedure supplied by the manufacturer. Isolated RNA was quantitated by measuring absorbance at 260 nm. A standard reverse transcription reaction was performed at 42°C for 60 min in 20 μL of 5 mmol MgCl₂/L, 10 mmol dCTP/L, pH 9.0, 50 mmol KCl/L, 0.1% Triton X-100; 1 mmol dNTP/L; 1 × 10⁻⁶ U recombinant RNasin ribonuclease inhibitor/L; 15 × 10⁻⁶ U AMV reverse transcriptase/L; and 0.5 μg oligo(dT)₁₅ primer (Promega). The sequences of the primer pairs used for PCR amplification of the studied genes are shown in Table 1. For quantitation, levels of mRNA of the studied inflammatory genes

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and the gene encoding for PHGPx were related to β-actin mRNA. The PCR mixture consisted of 2 μL of a product of the reverse transcription reaction, a Taq PCR Master Mix Kit (Qiagen, Valencia, CA), and 20 pmol of primer pairs in a total volume of 50 μL. For each individual gene, a linear range of PCR amplification was established and the induction of the target gene was studied within the range.

The following thermocycling conditions were used to determine the induction of the genes encoding for the studied inflammatory mediators:

- **MCP-1**: 94°C for 4 min; followed by 94°C for 45 s, 55°C for 45 s, 72°C for 45 s (repeated 25 times); followed by an extension at 72°C for 10 min;
- **TNF-α**: 94°C for 4 min; followed by 94°C for 45 s, 55°C for 45 s, 72°C for 45 s (repeated 28 times); followed by an extension at 72°C for 10 min;
- **ICAM-1**: 94°C for 4 min; followed by 94°C for 45 s, 60°C for 45 s, 72°C for 60 s (repeated 28 times); followed by an extension at 72°C for 7 min;
- **VCAM-1**: 94°C for 60 s, 55°C for 60 s, 72°C for 60 s (repeated 25 times); and
- **PHGPx**: 94°C for 4 min; followed by 94°C for 40 s, 66°C for 40 s, 72°C for 2 min (repeated 20 times); followed by an extension at 72°C for 7 min.

Induction of the β-actin gene was determined by using the same number of cycles and thermocycling conditions as for the target genes. Under these RT-PCR conditions, the β-actin transcript increased linearly in the range of 15–40 PCR cycles.

PCR products were separated by 2% agarose gel electrophoresis, stained with SYBR Green I (Molecular Probes), and visualized by using phosphorimaging technology (FLA-2000; Fuji, Stamford, CT). The relative intensity of fluorescence (ratio of the intensity of the band corresponding to the target gene to that corresponding to the β-actin gene) was quantified with IMAGE GAUGE 3.0 software (Fuji) and expressed as average pixel intensity. Experiments were repeated 4 times on different days, and the values of relative fluorescence from the 4 experiments were statistically analyzed.

### Statistical analysis

Statistical analysis was performed by using SYSTAT 8.0 (SPPS Inc, Chicago). One-way analysis of variance was used to compare mean values among the treatments. When the overall F values were significant, analysis of variance was followed by post hoc Bonferroni tests to compare means from different treatments. A P value <0.05 was considered significant.

### RESULTS

**Unsaturated fatty acids selectively induce NF-κB and AP-1 transcriptional activation**

The effects of specific unsaturated fatty acids on NF-κB transcriptional activation are shown in Figure 1A. Treatment of
endothelial cells with oleic acid did not significantly affect luciferase activity in cells transfected with pNFκB-Luc. Compared with control cultures, linolenic acid exerted only a moderate effect on NF-κB transcriptional activation; however, treatment of transfected endothelial cells with linoleic acid resulted in a pronounced increase in luciferase activity, indicating a marked increase in transcriptional activation of NF-κB.

Similar results were observed in endothelial cells transfected with pAP1-Luc (Figure 1B). Among the fatty acids tested, linoleic acid stimulated AP-1 transcriptional activation most markedly compared with control cultures. In contrast, linolenic acid exerted more moderate effects, and oleic acid did not significantly affect luciferase expression.

Unsaturated fatty acids selectively induce the genes encoding for MCP-1 and TNF-α

The effects of treatment with selected unsaturated fatty acids on MCP-1 mRNA levels are shown in Figure 2. Among the tested fatty acids, linoleic acid at the concentration of 90 μmol/L stimulated the most pronounced induction of the MCP-1 gene (51 ± 1.97% above the control values as measured by the density of the fluorescent bands). Indeed, MCP-1 mRNA levels in endothelial cells treated with 90 μmol linoleic acid/L for 3 h were in the range observed in cells exposed to 20 μg TNF-α/L, which was used as a positive control. MCP-1 mRNA levels also increased in endothelial cells treated with 60 and 90 μmol linolenic acid/L (by 24 ± 2.46% and 30 ± 5.25%, respectively). In contrast, induction of the MCP-1 gene in endothelial cells exposed to oleic acid was approximately at the range observed in unstimulated endothelial cells.

The effects of treatment with selected unsaturated fatty acids on TNF-α mRNA levels are also shown in Figure 2. Similarly to the results for MCP-1 gene induction, treatment of HUVECs with linoleic acid markedly induced TNF-α mRNA levels (21 ± 3.22% above control values). In addition, linolenic acid at the dose of 90 μmol/L stimulated similar induction of the TNF-α gene. Independent of the dose used, treatment with oleic acid did not significantly affect TNF-α mRNA levels in cultured HUVECs.

Unsaturated fatty acids selectively induce the genes encoding for adhesion molecules

The effects of treatment with selected unsaturated fatty acids on ICAM-1 mRNA levels is shown in Figure 2. Exposure to both linoleic acid and linolenic acid induced similar dose-dependent increases in ICAM-1 mRNA levels. Specifically, linoleic and linolenic acids at the concentration of 90 μmol/L stimulated
inhibition of the ICAM-1 gene by 32 ± 2.54% and 30 ± 3.34%, respectively. In contrast, exposure of HUVECs to oleic acid decreased ICAM-1 mRNA levels to ~50% of control values.

The effects of specific unsaturated fatty acids on VCAM-1 mRNA levels in HUVEC are also shown in Figure 2. The most significant induction of the VCAM-1 gene (by 38 ± 2.20%) was observed in cells treated with 90 μmol linoleic acid/L. Exposure to 90 μmol linolenic acid/L resulted in a slight increase in VCAM-1 mRNA levels (14 ± 1.88%). Treatment with oleic acid had no significant effect on VCAM-1 gene induction compared with control cultures.

**Unsaturation fatty acids induce the gene encoding for PHGPx**

The effects of selected fatty acids on PHGPx mRNA levels in HUVECs are shown in Figure 3. Compared with the control, treatment with oleic acid increased PHGPx mRNA levels by ~30%. However, both linoleic and linolenic acids strongly, and dose dependently, stimulated induction of PHGPx gene in HUVECs. In fact, treatment with 60 and 90 μmol linoleic acid/L increased PHGPx mRNA expression by 60 ± 7.58% and 104 ± 5.04%, respectively. Furthermore, exposure to 60 and 90 μmol linolenic acid/L increased the induction of the PHGPx gene by 108 ± 6.48% and 121 ± 4.36%, respectively.

**DISCUSSION**

Mono- and polyunsaturated 18-carbon fatty acids provide a unique model for studying the cellular effects of fatty acids that differ in unsaturation independent of carbon length (9). In addition, the unsaturated fatty acids used in the present study are major dietary fatty acids. Endothelial cells were exposed to fatty acids at concentrations of 60 or 90 μmol/L, with an albumin concentration in the experimental media of 60 μmol/L. Normal plasma fatty acid concentrations can range from ~90/1200 μmol/L; however, most fatty acids are bound to plasma components, mostly albumin (32, 33). In fact, the main factor in the availability of fatty acids for cellular uptake is determined by the ratio of fatty acids to albumin. Normally, this ratio can range from 0.15 to 4 under various conditions, with an average of ~1 (32, 33). Thus, the experimental conditions used in the present study, which resulted in a ratio of fatty acids to albumin of 1 or 1.5, were within the physiologic range.

One of the most important functions of the vascular endothelium is to regulate inflammatory reactions (1). The development of inflammatory reactions is a normal defense mechanism in response to injury or activation of the vessel wall. The physiologic significance of such reactions is to maintain and repair the normal structure and function of the vessel wall. However, excessive inflammatory reactions with the development of a positive feedback inflammatory cycle can lead to severe tissue damage and are associated with vascular pathology, including the development of atherosclerotic plaques (34).

Induction of genes encoding for mediators of the inflammatory response, ie, inflammatory cytokines, chemokines, and adhesion molecules, can initiate leukocyte infiltration of the vessel wall. These mediators of the inflammatory response interact closely with each other in vivo. For example, ICAM-1 and VCAM-1 facilitate leukocyte adhesion to the vascular endothelium and both MCP-1 (35) and, to a lesser extent, TNF-α (36, 37) are potent chemoattractant factors that play a significant role in recruiting lymphocytes and monocytes into the vessel wall. In addition, TNF-α is a strong inducer of inflammatory reactions and can stimulate overexpression of MCP-1, inflammatory cytokines, and the adhesion molecules ICAM-1 and VCAM-1 (38). In fact, these strong proinflammatory properties of TNF-α were the reason that this cytokine was used as the positive control in our present study. In addition, the inflammatory genes examined in the present study, ie, those encoding for VCAM-1, ICAM-1, TNF-α, and MCP-1, are regulated by similar transcription factors, with dominant roles of NF-κB and AP-1 (22–25).

The importance of NF-κB and AP-1 in the induction of inflammatory reactions prompted us to study the effects of specific fatty acids on the transcriptional activity of these transcription factors in human endothelial cells. Among the unsaturated fatty acids studied, linoleic acid induced both NF-κB and AP-1 transcriptional activation most markedly. These data agree with our previous results in which the use of an electrophoretic mobility shift assay showed a marked activation of NF-κB (13) and AP-1 (39) in endothelial cells exposed to linoleic acid. It is possible that fatty acid–induced endothelial cell oxidative stress and disturbances in the glutathione redox status are responsible for the activation of these oxidative stress–responsive transcription factors. Intracellular glutathione is the major nonprotein thiol compound that regulates the cellular redox status. Depletion of glutathione concentrations and alterations in the equilibrium between the reduced and oxidized derivatives of glutathione can stimulate activation of NF-κB (40). To support this notion, we showed that exposure of endothelial cells to unsaturated fatty acids can result in a marked decrease in cellular glutathione concentrations and activation of NF-κB (9, 13). In addition, the glutathione precursor N-acetylcysteine prevented fatty acid–induced activation of NF-κB (41).

Glutathione peroxidases are a family of antioxidant enzymes that utilize glutathione in the reduction of hydrogen peroxide and alkyl hydroperoxides. Among the various glutathione peroxidases, PHGPx plays a unique role. In addition to reducing hydrogen peroxide and soluble hydroperoxides, PHGPx is the only antioxidant enzyme that can reduce hydroperoxy fatty acids that are integrated in cellular membranes (42) or lipoproteins (43).
PHGPx was also shown to be involved in silencing activities of cyclooxygenase or 5- and 15-lipoxygenases (44, 45), enzymes involved in the metabolism of unsaturated fatty acids. Results of the present study showed that exposure of endothelial cells to specific unsaturated fatty acids can markedly stimulate induction of PHGPx mRNA. In addition, the fatty acid–stimulated increases in PHGPx mRNA levels appeared to be correlated with the amount of unsaturated bonds in fatty acid molecules. For example, linolenic acid, followed by linoleic acid, enhanced induction of the PHGPx gene most markedly.

The present study provides compelling evidence that linoleic acid can induce profound inflammatory responses in cultured human endothelial cells. In fact, among all the unsaturated fatty acids studied, linoleic acid stimulated induction of inflammatory gene mRNA most markedly. Because expression of the inflammatory genes is regulated primarily by NF-κB and AP-1, a strong induction of NF-κB and AP-1 transcriptional activation by linoleic acid may explain the marked induction of the studied genes. In addition, not only linoleic acid but also specific oxidative products of this fatty acid can exert proinflammatory effects (46, 47). However, we observed that the lipoxygenase metabolites of linoleic acid, such as 13-hydroperoxyoctadecadienoic acid (13-HPODE) or 13-hydroxyoctadecadienoic acid (13-HODE), induce a different pattern of inflammatory responses in endothelial cells than does free linoleic acid. Specifically, exposure of HUVECs to 13-HPODE or 13-HODE does not induce the expression of VCAM-1 or E-selectin (48). In addition, polyunsaturated fatty acids, such as linoleic acid, can be nonenzymatically converted to 4-hydroxynonenal. However, exposure of HUVECs to 4-hydroxynonenal markedly stimulates apoptosis of vascular endothelial cells but does not result in activation of NF-κB or induction of adhesion molecules (49). Thus, even though linoleic acid can be converted to oxidized metabolites, it appears unlikely that 13-HPODE, 13-HODE, or 4-hydroxynonenal can contribute significantly to inflammatory reactions induced by this fatty acid. On the other hand, the effects of other metabolites of polyunsaturated fatty acids, eg, derivatives of the cytochrome P450 pathway, on inflammatory reactions in human endothelial cells remain to be determined. Our recent data suggest that epoxide metabolites of linoleic acid may have proinflammatory properties (50).

Although our data clearly indicate that specific unsaturated fatty acids can induce proinflammatory effects in endothelial cells, opposite results were reported when cells were exposed to selected n-3 or n-6 fatty acids for ≤72 h and coexposed to inflammatory cytokines, such as interleukin 1β (IL-1β) or TNF-α, for an additional 12 h. When such experimental approaches were used, preexposure to fatty acids inhibited cytokine-induced expression of inflammatory mediators, such as VCAM-1, on the surface of endothelial cells (51). Similar inhibition of ICAM-1 expression was also observed in cells pretreated with 13-HPODE before stimulation with IL-1β. However, simultaneous administration of 13-HPODE with IL-1β or TNF-α resulted in additive effects on ICAM-1 production (48). We showed that preexposure of endothelial cells to linoleic acid can cross-amplify TNF-α–mediated induction of cellular oxidative stress and endothelial cell dysfunction (13) but does not potentiate or even inhibit NF-κB–dependent transcription (13, 41). To explain this phenomenon, it was proposed that fatty acid–induced activation of NF-κB could lead to increased numbers of NF-κB inhibitory subunits, which, in turn, could prevent further activation of this transcription factor in cells exposed to cytokines at later time points (13).

In contrast with linoleic and linolenic acids, which exerted strong or moderate proinflammatory responses, respectively, oleic acid diminished inflammatory gene mRNA levels in endothelial cells. These data agree with previous reports on antioxidant effects mediated by oleic acid. For example, a diet enriched in oleic acid markedly decreases LDL susceptibility to oxidation and LDL-protein modification in mildly hypercholesterolemic patients (10). Similar results were obtained in experimental animals fed a diet enriched in oleic acid (52). Extensive evidence also indicates the protective and antioxidant effects of oleic acid on endothelial cell activation. Cellular treatment with this fatty acid protects endothelial cells against cytokine-induced VCAM-1, ICAM-1, or E-selectin overexpression (53). In addition, supplementation with oleic acid protects endothelial cells against hydrogen peroxide–induced cytotoxicity (16) and against dysfunction of the endothelial barrier as mediated by oxidized LDL (54).

In conclusion, the present study showed that specific unsaturated dietary fatty acids can induce highly individual effects on endothelial cell activation and contribute differently to induction of the inflammatory genes in vascular endothelial cells. Among the fatty acids studied, linoleic acid stimulated inflammatory gene mRNA most markedly. In contrast, oleic acid appeared to silence the induction of various proinflammatory genes in endothelial cells. These results showed that specific unsaturated dietary fatty acids, such as linoleic acid and to a lesser extent linolenic acid, can stimulate the development of proinflammatory environments within the vascular endothelium.

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