Fatty Acid–Mediated Activation of Vascular Endothelial Cells

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Vascular endothelial cell activation and dysfunction are critical early events in atherosclerosis. Selected dietary lipids (eg, fatty acids) may be atherogenic by activating endothelial cells and by potentiating an inflammatory response. Due to their prooxidant property, unsaturated fatty acids may play a critical role in endothelial cell activation and injury. To test this hypothesis, porcine endothelial cells were exposed to 18-carbon fatty acids differing in the degree of unsaturation, ie, 90 μmol/L stearic (18:0), oleic (18:1n-9), linoleic (18:2n-6), or linolenic acid (18:3n-3) for 6 to 24 hours and/or tumor necrosis factor alpha ([TNF-α] 500 U/L) for up to 3 hours. Compared with control cultures, treatment with 18:0 and 18:2 decreased glutathione levels, suggesting an increase in cellular oxidative stress. Both 18:2 and 18:0 activated the transcription factor nuclear factor κB (NF-κB) the most and 18:1 the least. This NF-κB–dependent transcription was confirmed in endothelial cells by luciferase reporter gene assay. The fatty acid–mediated activation of NF-κB was blocked by preenrichment of the cultures with 25 μmol/L vitamin E. All fatty acids except 18:1 and 18:3 increased transendothelial albumin transfer, and 18:2 caused the most marked disruption of endothelial integrity. Preenrichment of endothelial cells with 18:2 followed by exposure to TNF-α resulted in a 100% increase in interleukin-6 (IL-6) production compared with TNF-α exposure alone. In contrast, cellular preenrichment with 18:0, 18:1, or 18:3 had no effect on TNF-α–mediated production of IL-6. Cellular release of radiolabeled arachidonic acid (20:4) was markedly increased only by cell exposure to 18:2 and 18:3, and the release of 20:4 appeared to be mainly from the phosphatidylethanolamine fraction. These data suggest that oleic acid does not activate endothelial cells. Furthermore, linoleic acid and other omega-6 fatty acids appear to be the most proinflammatory and possibly atherogenic fatty acids.

EVIDENCE SUGGESTS that the mechanisms of vascular disease such as atherosclerosis involve damage to the endothelium, which then reduces its effectiveness as a selectively permeable barrier to plasma components.[1,2] The endothelium interacts with the blood and underlying tissues, serves as both a prothrombotic and antithrombotic surface, and releases regulatory factors important in modulating vascular tone. Factors implicated in the pathogenesis of atherosclerosis include chronic and cumulative metabolic alterations of the endothelium induced by numerous activating molecules, such as certain lipids, prooxidants, and inflammatory cytokines. These risk factors may contribute to an overall cellular imbalance of the oxidative stress/antioxidant balance, thus leading to chronic activation or stimulation of the endothelium, as well as endothelial barrier dysfunction, which can result in accelerated uptake of cholesterol-rich lipoproteins into the vessel wall.

There is ample evidence suggesting that serum cholesterol is a predictor of atherosclerosis and that serum cholesterol concentrations can be modified by varying the composition of dietary fat. However, less is known about the role of specific fatty acids in atherosclerosis. The role of saturated fatty acids in atherosclerosis has been questioned recently.[3-5] In fact, data obtained in subjects with varying degrees of coronary atherosclerosis support the hypothesis that high serum polyunsaturated fatty acid levels (eg, linoleic acid), when insufficiently protected by antioxidants (eg, vitamin E), may indicate a higher risk of atherosclerosis.[6] Recent research with a population from a country with one of the highest dietary polyunsaturated to saturated fat ratios in the world has concluded that diets rich in n-6 (or omega-6) fatty acids may contribute to an increased incidence of atherosclerosis, hyperinsulinemia, and tumorigenesis.[7]

A transcription factor implicated in many endothelial cell activation responses to injury and stress is nuclear factor κB (NF-κB).[8,9] NF-κB plays a central role in regulating the cytokine network, and hence its activation may be a major factor in the pathogenesis of atherosclerosis. NF-κB can be activated by a variety of pathogenic or pathogen-elicited stimuli including cytokines, lipids, mitogens, bacteria, and related products, with the common denominator apparently being reactive oxygen species. Many target genes in endothelial cells contain NF-κB or NF-κB–like binding sites in the promoter genes coding for inflammatory cytokines (eg, tumor necrosis factor [TNF] and interleukin-6 [IL-6] and adhesion molecules).[10]

In light of the evidence that oxidative stress plays a critical role in atherosclerosis[11,12] and that antioxidant nutrients such as vitamin E may provide protection against this disease,[13,14] one may speculate that the atherosclerotic risk of dietary lipids may be directly related to their degree of unsaturation. Thus, a focus of the present study was to examine the mechanisms of the effects of 18-carbon fatty acids, differing in degree of unsaturation, on endothelial cell activation.

MATERIALS AND METHODS

Cell Culture and Experimental Media

Porcine pulmonary artery–derived endothelial cells were isolated from porcine pulmonary arteries and cultured as described previously.[15]

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Cells were subcultured in medium 199 (M-199) containing 10% bovine calf serum (HyClone Laboratories, Logan, UT) using standard techniques. The purity of the cultures was determined by morphological criteria and by quantitatively measuring angiotensin-converting enzyme activity, or by the uptake of fluorescent-labeled acetylated low-density lipoprotein (1,1′-dioctadecyl-3,3,3′,3′-tetramethyl-indocarbocyanine perchlorate; Molecular Probes, Eugene, OR).

The experimental media were composed of M-199 enriched with 5% FBS and either fatty acids (90 μmol/L) or TNF-α (500 U/mL or 100 ng/mL; Knoll Laboratories, Whippany, NJ). Fatty acids (>99% pure) were obtained from Nu-Chek Prep (Elysian, MN). Preparations of experimental media with fatty acids and/or TNF were made as described previously.[15,16] Thus, fatty acids were introduced into the media bound to serum albumin. Assuming albumin concentrations of 30 μmol/L (in 5% serum) to 60 μmol/L (in 10% serum) in our culture media, the fatty acid concentrations are within physiological and metabolic relevance. Even though only about 5% of total free fatty acids in the experimental media are derived from the serum, fatty acid–mediated activation of endothelial cells may vary depending on the type of serum in which cells are cultured.[17] For most experimental settings, cells were treated with fatty acids for 6 to 24 hours and/or TNF for 3 hours before termination. Some cultures were preenriched with 25 μmol/L vitamin E (α-tocopherol). All experimental outcomes were confirmed more than twice.

Glutathione Assay

Glutathione assays were performed according to a modified method of Baker et al.[18] To determine total glutathione, cellular protein was precipitated by adding 100 μL ice-cold 0.09% sulfosalicylic acid (SSA) to cells collected from P-100 tissue culture plates. The culture plates were then incubated at 40°C for 15 minutes, after which the cell lysates were collected and centrifuged at 9,000× *g* for 5 minutes. Glutathione levels were determined spectrophotometrically using the glutathione-linked 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) recycling assay. The mixture for the assay contained 50 μL supernatant and 100 μL 125-mmol/L phosphate buffer containing 0.225 mmol/L DTNB, 0.302 mmol/L NADPH, and glutathione reductase at a concentration of 1.25 U/μL. The blank contained 50 μL 0.09% 5-SSA instead of the supernatant, and the control reaction contained the glutathione standard in place of the supernatant. The mixtures were equilibrated at room temperature for 3 minutes, and the reaction was started by the addition of 100 μL reaction buffer. Absorbance was measured at 405 nm in a 96-well plate reader.

Transcription Factor (NF-κB) Activation Studies: Electrophoretic Mobility Shift Assay

These transcription factors, which bind to enhancer elements on DNA, were determined in endothelial cells by an electrophoretic mobility shift assay as described by Sen and Baltimore.[19] Nuclear extracts containing the NF-κB active protein were prepared from cells according to the method of Dignam et al.[20] Nuclear extracts were incubated for 20 to 30 minutes with ^{32}P-end-labeled oligonucleotide probe (GIBCO/BRL, Gaithersburg, MD) containing the κB enhancer DNA element with a tandem duplicate of a NF-κB binding site (-GGGGACTTTCC-). Incubation at room temperature was performed in the presence of nonspecific competitor DNA. Following binding, the complexed and uncomplexed DNA in the mixture were resolved by electrophoresis in a 5% low–ionic-strength nondenaturing polyacrylamide gel and visualized by autoradiography. Control reactions using a 200-fold molar excess of unlabeled oligonucleotide probes or a supershift assay were performed to demonstrate the specificity of the shifted DNA-protein complexes for NF-κB.

Transfection and Luciferase Assay

The luciferase reporter gene assay reflects NF-κB–dependent transcription. Briefly, endothelial cells were transfected with 2 μg pNF-κB-Luc plasmid (Stratagene, La Jolla, CA) by the lipofection method (Invitrogen, Carlsbad, CA). Four hours after transfection, cells were washed with phosphate-buffered saline (PBS) and incubated with M-199 (with 10% serum) for 24 hours. Then, the endothelial cells were stimulated with 90 μmol/L fatty acid (18:0 or 18:2) for 24 hours. Luciferase activity was determined following the instructions described in the luciferase assay kit (Promega, Madison, WI) using a luminometer.

IL-6 Production

After exposure to fatty acids and TNF, the media were removed from the wells and frozen immediately at −80°C until IL-6 analysis. The remaining cells were trypsinized and washed with PBS twice and resuspended in 0.2% sodium dodecyl sulfate with 0.2 mol/L NaOH for protein analysis.[21] IL-6 production and release into the medium was determined using the murine hybridoma cell line B9 (kindly supplied by Dr L.A. Aarden, Emeryville, CA) as described by Helle et al.[22] The B9 cell line viability is IL-6–dependent, and thus, the incorporation of ^{3}H-thymidine by viable cells is a reflection of the quantity of IL-6 produced by endothelial cells.

Endothelial Barrier Function (albumin transfer studies)

Endothelial barrier function was measured as transendothelial albumin transfer using polystyrene chambers with a 0.8-μm pore size polycarbonate membrane (Millipore, Bedford, MA) as described previously.[15] After achieving approximate confluence, endothelial monolayers were exposed to control or experimental media for 24 hours. Following treatments, chambers with endothelial cells attached to the membranes were washed with M-199 and exposed to 200 μmol/L bovine serum albumin (fatty acid–free; Sigma Chemical, St Louis, MO) in M-199 for 1 hour. After incubation, the albumin transferred across endothelial monolayers was determined using bromcresol green (Sigma) and recorded spectrophotometrically at 630 nm.

Lipid Analysis

Measurement of arachidonic acid release. Endothelial cells were cultured in M-199 enriched with 10% FBS and incubated with ^{3}H-arachidonic acid (0.2 mCi/mL medium) for 24 hours. Following incubation with radiolabeled 20:4, the cells were washed with serum-free M-199 medium and medium supplemented with 0.2% fatty acid–free BSA and then exposed to different 18-carbon fatty acids (90 μmol/L) for 6 hours. Subsequently, the media were collected and centrifuged at 3,000 rpm for 10 minutes to remove floating cells, and radioactivity was measured in the supernatant. The cells were immediately scraped in PBS, and lipids were extracted with chloroform: methanol (2:1) using a modified method of Takenaka et al.[23]

Separation of arachidonic acid and phospholipid fractions. Lipid extracts from each treatment were applied to a silica gel thin-layer chromatography (TLC) plate, and the separation of arachidonic acid and different phospholipids was performed using chloroform:methanol: ammonia (65:25:4) as a mobile phase. After identification of lipids in iodine vapor, arachidonic acid and phospholipid spots were scraped from the plate into scintillation vials with 10 mL scintillation cocktail (3a70B). The radioactivity of the samples was measured in a Tri-Carb2100TR liquid scintillation analyzer (Packard Instrument, Meriden, CT).

Statistical Analysis

Data were analyzed statistically using a 1-way ANOVA. For each endpoint, the treatment means were compared in pairs using the

Bonferroni procedure.[24] A *P* value of .05 or less was considered significant.

RESULTS

The effects of the 18-carbon fatty acids on cellular redox status were determined by measurement of cellular glutathione levels. Figure 1 demonstrates that both 18:0 and 18:2 significantly decreased glutathione levels. Compared with control cultures, treatment with 18:1 increased total glutathione, whereas 18:3 had no effect on intracellular glutathione levels.

The evidence suggests that oxidative stress can affect cellular metabolism by an increased expression of genes regulated by NF-κB. Interestingly, 18:0, the only saturated fatty acid, and 18:2 activated the transcription factor NF-κB most markedly (Fig 2), whereas 18:1 exposure to endothelial cells had little effect on the activation of this transcription factor. To test whether vitamin E can protect against fatty acid–induced activation of NF-κB, endothelial cells were pretreated with vitamin E for 24 hours before coexposure to fatty acids for an additional 6 hours (Fig 3). Vitamin E markedly decreased NF-κB binding induced by 18:0 or 18:2.

To determine whether 18:0- or 18:2-activated NF-κB can induce gene expression, endothelial cells were transfected with a plasmid (pNF-κB-Luc) encoding the bacterial protein luciferase. The expression of this construct is controlled by a promoter responsive to NF-κB. Results of the luciferase reporter gene assay are shown in Fig 4. Both 18:0- and 18:2-mediated activation of NF-κB were sufficient to induce NF-κB–dependent transcription in cultured endothelial cells. Compared with control cultures, luciferase activity was significantly higher in both 18:0- and 18:2-treated cells.

Figure 5 shows the effect of cellular incubation with control medium and media enriched with 18-carbon fatty acids on endothelial barrier function. Compared with control cultures, all fatty acids except 18:1 and 18:3 increased albumin transfer across endothelial monolayers. However, treatment with 18:2 disrupted endothelial barrier function most markedly.

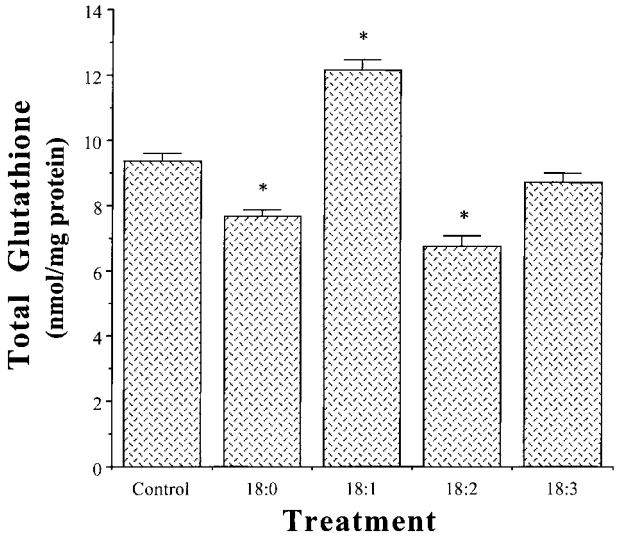

Fig 1. Effect of treatment with different 18-carbon fatty acids (90 μmol/L) on total glutathione levels in cultured endothelial cells. Cells were exposed to experimental media for 6 hours. Values are the mean ± SEM (n = 3). *Significantly different *v* control cultures.

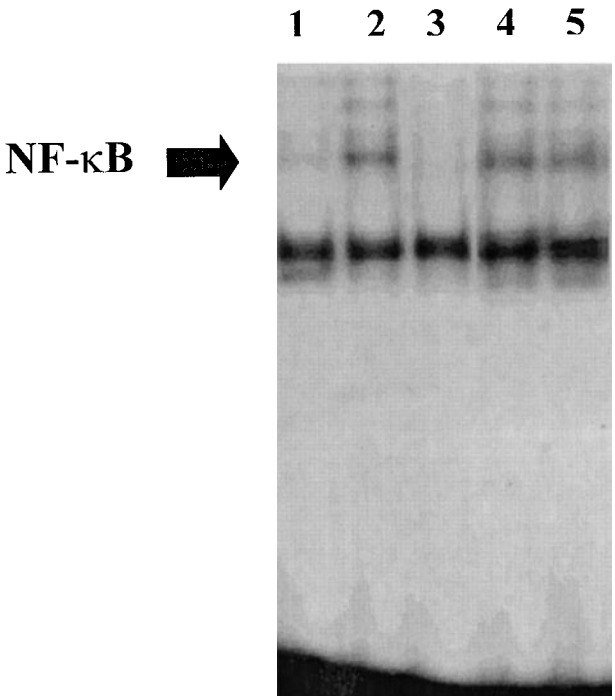

Fig 2. Effect of different 18-carbon fatty acids on activation of NF-κB. Cells were treated with the different fatty acids (90 μmol/L) for 6 hours. Lane 1, control; lane 2, stearic acid (18:0); lane 3, oleic acid (18:1); lane 4, linoleic acid (18:2); lane 5, linolenic acid (18:3). The specific binding of NF-κB was confirmed by both competitive (excess unlabeled oligonucleotide) and supershift assays.

Figure 6 shows IL-6 production in endothelial cells during fatty acid treatment for 9 hours followed by TNF exposure for an additional 3 hours. These data show that the cellular lipid environment can modify TNF-mediated inflammatory properties by selectively promoting endothelial cell–mediated production of IL-6. Compared with TNF treatment alone, preenrichment of endothelial cells with 18:2 followed by exposure to TNF resulted in a 100% increase in IL-6 production. In contrast, cellular preenrichment with 18:0, 18:1, and 18:3 had no further effect on the TNF-α–mediated production of IL-6.

The fatty acid–mediated changes in oxidative stress and other observed mediators of endothelial cell activation may be due to an increase in phospholipase A_2 activity and thus an increase in available arachidonic acid (20:4n-6) for metabolic activity. To test this hypothesis, cells were preenriched with radiolabeled 20:4 for 24 hours, carefully washed, and then treated with the 18-carbon fatty acids for an additional 6 hours. The surrounding media then were tested for cellular release of radiolabeled 20:4 (Fig 7). Cells were also harvested and analyzed for radioactivity in various lipid fractions, including phospholipids (Fig 8). Compared with control cultures (cells not enriched with 18-carbon fatty acids), cellular release of radiolabeled 20:4 was markedly increased only by cell exposure to 18:2 or 18:3 (Fig 7). Neither 18:0 nor 18:1 affected 20:4 release. Preenriching cultures with vitamin E decreased the fatty acid–mediated release of 20:4 into the media in all cultures independently of

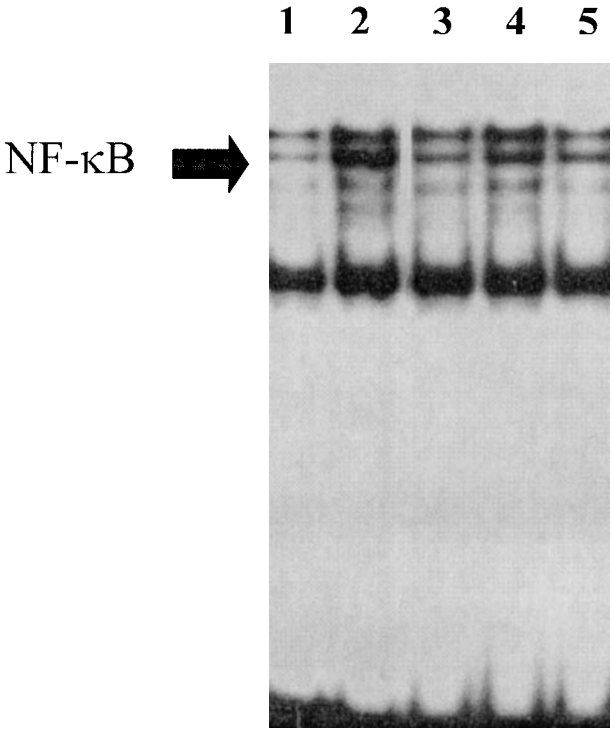

Fig 3. Effect of preenrichment with vitamin E on fatty acid–mediated activation of NF-κB. All cells were exposed to the different fatty acids (90 μmol/L) for 6 hours, and some cultures were first preenriched with vitamin E for 24 hours. Lane 1, control + vitamin E; lane 2, stearic acid (18:0); lane 3, 18:0 + vitamin E; lane 4, linoleic acid (18:2); lane 5, 18:2 + vitamin E.

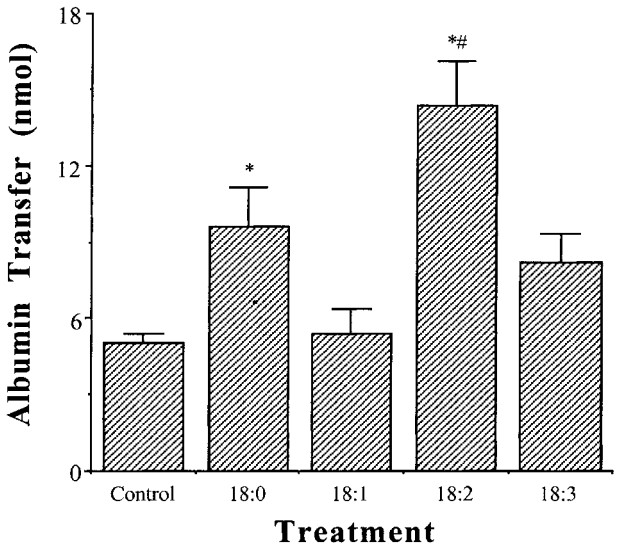

Fig 5. Effect of fatty acid exposure on albumin transfer across endothelial monolayers. Cultures were exposed to different 18-carbon fatty acids (90 μmol/L) for 24 hours. Subsequently, albumin transfer was measured over a 1-hour period. Values are the mean ÷ SEM (n = 6). *Significantly higher *v* control cultures. #Significantly higher *v* cultures treated with 18:0.

the type of 18-carbon fatty acid to which the endothelial cells were exposed (data not shown). When analyzing for radiolabeled 20:4 in several types of cellular phospholipids, only its level in the phosphatidylethanolamine fraction was affected to a significant extent by 18-carbon fatty acid treatment. The most marked decrease in 20:4 incorporation into this phospholipid fraction was in cultures treated with 18:2, followed by cultures treated with 18:3. Neither 18:0 nor 18:1 treatment affected the 20:4 content in the phosphatidylethanolamine fraction. Thus, it appears that treatment with 18:2 or 18:3 can stimulate the release of 20:4 specifically from phosphatidylethanolamine.

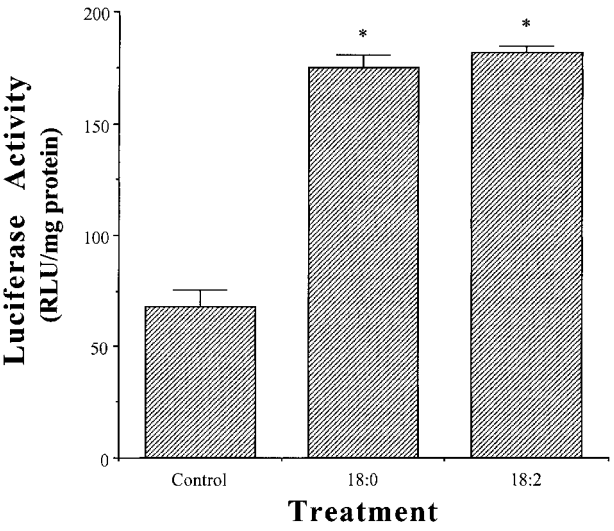

Fig 4. Effect of a 24-hour exposure to 18:0 or 18:2 on NF-κB–dependent transcription as measured by luciferase reporter gene assay. Data are expressed as relative light units (RLU) per mg protein. Values are the mean ± SEM (n = 3). *Significantly different *v* control cultures.

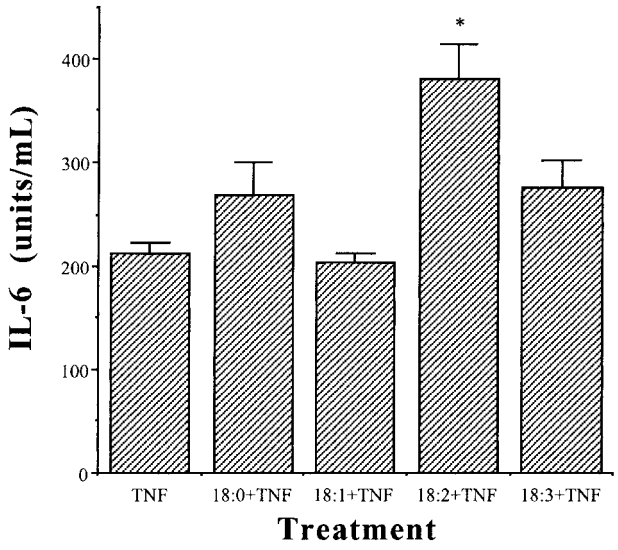

Fig 6. IL-6 production in endothelial cells after exposure to different 18-carbon fatty acids. Endothelial cells were treated with the different fatty acids (90 μmol/L) for 9 hours and with added TNF-α (500 U/mL) for an additional 3 hours. Values are the mean ± SEM (n = 3). *Significantly higher *v* control cultures.

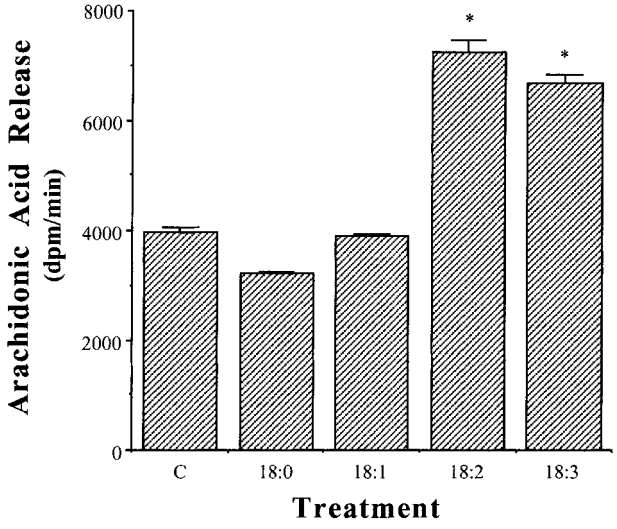

Fig 7. Release of radiolabeled 20:4 from endothelial cells following exposure to 18-carbon fatty acids (90 μmol/L) for 6 hours (cells were labeled with ^{3}H-arachidonic acid for 24 hours prior to fatty acid exposure). The media were collected and radioactivity was counted and expressed as dpm/min. Values are the mean ± SEM (n = 3). *Significantly higher *v* control cultures.

DISCUSSION

Although the mortality from coronary heart disease has declined recently, atherosclerosis and related vascular disorders still are the leading cause of death in the United States and other Western countries. Injury to or abnormal mechanisms of the vascular endothelium may be initiating events in the etiology of atherosclerosis. Dietary fat affects plasma lipids and lipoproteins and thus is linked to atherosclerosis.[25] The question then arises as to whether dietary saturated fats should be replaced by unsaturated fats. Unsaturated fats, especially monounsaturated[26,27] and n-3 or omega-3[28,29] fatty acids, may be beneficial to human health. However, replacing saturated lipids with unsaturated and especially polyunsaturated lipids may not be desirable because of their ability to oxidize easily. The evidence supports the hypothesis that low-density lipoprotein undergoes oxidative modifications that increase its uptake by macrophages.[11] In fact, data from subjects with varying degrees of coronary atherosclerosis support the hypothesis that high serum polyunsaturated fatty acid levels, when insufficiently protected by antioxidants (eg, vitamin E), may indicate a higher risk of atherosclerosis.[30]

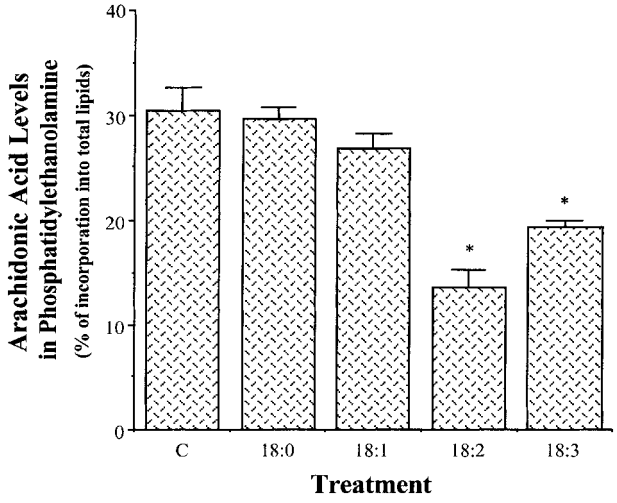

Fig 8. Incorporation of radiolabeled 20:4 into phosphatidylethanolamine. The experimental setup was the same as described in Fig 7. Lipids were obtained from total cell extracts and separated by TLC. Radioactivity was counted and expressed as dpm/min. Values are the mean ± SEM (n = 3). *Significantly lower *v* control cultures.

High levels of circulating triglyceride-rich lipoproteins (chylomicrons and very–low-density lipoprotein [VLDL]) have been implicated in the injury process of the endothelium.[31,32] Plasma chylomicron levels are elevated in humans after consuming a high-fat meal, and hepatic synthesis of VLDL is increased when the caloric intake is in excess of body needs. The hydrolysis of triglyceride-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 in the proximity to the endothelium.[33] It has been hypothesized that high levels of diet-derived fatty acids can cause endothelial injury or dysfunction and thus disrupt the ability of the endothelium to function as a selective barrier.[33,34] This would result in lipid deposition by allowing increased penetration of cholesterol-rich remnant lipoproteins into the arterial wall. In fact, the activity of lipoprotein lipase is increased in atherosclerotic lesions.[35,36] A recent report also provides evidence that lipoprotein lipase may be a chemoattractant for activated macrophages.[37] Lipoprotein lipase–derived remnants of lipoproteins isolated from hypertriglyceridemic subjects, as well as selective unsaturated fatty acids such as linoleic acid, were demonstrated to disrupt endothelial integrity.[38,39] In fact, a recent study has provided the first evidence that the lipolytic remnant products of triglyceride-rich lipoproteins produced after a meal rich in polyunsaturated fat are more injurious to arterial wall cells than those produced after a meal rich in saturated fat.[40] Furthermore, activated lipoprotein lipase induces TNF gene expression in macrophages and TNF production by this type of cell.[41] Thus, endothelial cells may be simultaneously exposed to free fatty acids and TNF.

As mentioned before, there is evidence that selected fatty acids, derived from the hydrolysis of triglyceride-rich lipoproteins, may be atherogenic by causing endothelial injury or dysfunction and subsequent endothelial barrier dysfunction.[42] In support of this hypothesis, we again confirm in the present study that, compared with all 18-carbon fatty acids, 18:2 disrupted endothelial barrier function most markedly. These findings agree with our earlier findings that when comparing fatty acid extracts derived from different animal fats and plant oils, the fat-induced disruption of endothelial barrier function was related to the amount of 18:2 present in the fat source.[43] These data suggest that among different fatty acids, linoleic acid may play a critical role in the pathogenesis of atherosclerosis.[44] This hypothesis is supported by the fact that adipose tissue levels of 18:2, which reflect the intake of this fatty acid over time, were positively associated with the degree of coronary artery disease.[45] In addition, concentrations of 18:2 were increased in the phospholipid fractions of human coronary

arteries in cases of sudden cardiac death due to ischemic heart disease.[46]

Several mechanisms were proposed to explain the injurious effects of 18:2 to endothelial cells. Due to the very low basal activity of endothelial cell elongases and delta 5 and delta 9 desaturases, arachidonic acid is not produced from 18:2 significantly in this type of cell.[47,48] Consequently, 18:2 accumulates within endothelial cells.[47,49] Moreover, 18:2 decreases the level of intracellular ATP[50] and proteoglycans,[51] enhances elastase-like activity,[52] and can yield nitrated oxidation species by reacting with nitric oxide–derived products.[53] The 18:2-mediated disruption of endothelial barrier function also may be caused by its ability to inhibit gap-junctional intracellular communication[54,55] and to induce intracellular oxidative stress.[40] Furthermore, 18:2, but not 18:0, can activate phospholipase A_2, as measured by the cellular release of 20:4 in neutrophils.[56] In fact, polyunsaturated free fatty acids that are liberated by phospholipase A_2 increased the formation of bioactive phospholipids in LDL, which stimulated endothelial cell activation and monocyte–endothelial cell interactions.[57]

In recent years, the role of oxidative stress has gained much attention in studies of lipid- and/or inflammatory cytokine–mediated endothelial cell dysfunction or injury. It is now generally accepted that LDL oxidation plays one of the most critical roles in atherogenesis. LDL can be oxidized in the subendothelial space, which lacks many of the antioxidants present in the whole blood. Furthermore, dietary oxidized lipids can be absorbed by the small intestine, be incorporated into chylomicrons, appear in the bloodstream, and thus contribute to the total body pool of oxidized lipids.[58] Including oxidized corn oil (a rich source of 18:2) in a diet accelerated the development of fatty streaks in cholesterol-fed rabbits,[59] suggesting that the consumption of oxidized lipids (eg, high–corn oil diets) may be an important risk factor for atherosclerosis. Our data support the notion that omega-6 fatty acids, and especially fats rich in 18:2, are atherogenic by activating vascular endothelial cells and by promoting an inflammatory response. We clearly show that 18:2 most markedly amplifies TNF-mediated IL-6 production by endothelial cells. An increase in oxidative stress and subsequent activation of NF-κB may be one of the main mechanisms of the inflammatory properties of 18:2. However, there appears to be no relationship between the degree of unsaturation of fatty acids and endothelial cell activation. In fact, stearic acid (18:0) appears to activate endothelial cells more markedly than either 18:1 or 18:3. Furthermore, 18:1 had little or no effect on endothelial cell activation. Interestingly, when studying lipoproteins from subjects consuming different types of dietary fat, eg, oleic acid or linoleic acid, only the percentage of 18:2 in LDL correlated strongly with the extent of oxidizability and macrophage degradation of these lipoproteins.[60]

It is not clear why 18:0 decreased cellular glutathione and increased NF-κB activation so markedly. Although 18:0, as a saturated fatty acid, does not undergo peroxidative modifications, it may induce perturbations in cellular metabolism, which secondarily can result in oxidative stress and be responsible for the observed decreases in glutathione concentrations. On the other hand, 18:0 may influence gene expression or signal transduction pathways that are more substantial than its unknown or secondary effects on oxidative stress. The fact that preenrichment of cultures with vitamin E can block the activation of NF-κB suggests that this fatty acid can modify the cellular lipid milieu, leading to an imbalance in oxidative stress/antioxidant status and to endothelial cell activation. Because of its lack of double bonds, 18:0 may affect the membrane properties of endothelial cells differently compared with fatty acids with *cis* double bonds. 18:0 also may be taken up and metabolized differently than fatty acids that contain double bonds. In fact, once taken up by endothelial cells, 18:0 is randomly distributed among membrane phospholipids,[61] whereas unsaturated fatty acids are initially preferentially incorporated into phosphatidylcholine and then can undergo a time-dependent transfer to phosphatidylethanolamine.[61] Furthermore, using electron-spin resonance studies, we found that of all 18-carbon fatty acids, only 18:0 increased membrane fluidity.[62] In that same study, a relationship between membrane fluidity and fatty acid compositional alterations in cellular phospholipids was observed, ie, only the unsaturated fatty acids, not 18:0, decreased the cellular arachidonic acid content. These and our present data suggest that 18:0 may have unique membrane-modifying effects.

In summary, our data suggest that omega-6 fatty acids appear to be most effective in activating endothelial cells and in contributing to an inflammatory response. In contrast, 18:1 does not appear to activate endothelial cells, and in fact may protect endothelial cells against oxidative insult.[63] These data support the concept that the substitution of dietary monounsaturated fatty acids and not polyunsaturated fatty acids for saturated fatty acids might be preferable for the prevention of cardiovascular disease.

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