Trans Unsaturated Fatty Acids Are Less Oxidizable than *Cis* Unsaturated Fatty Acids and Protect Endogenous Lipids from Oxidation in Lipoproteins and Lipid Bilayers†

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ABSTRACT: Epidemiological data suggest that dietary *trans* unsaturated fatty acids increase the risk of heart disease; however, the underlying mechanisms are unclear. In this study, we investigated one possible mechanism, namely, their effect on LDL oxidation. Supplementation of LDL with 10% 16:1 *trans*-cholesteryl ester (CE) inhibited the oxidation compared to that with 16:1 *cis*-CE. Total replacement of core lipids with 18:2 *trans,trans*-CE decreased the rate of LDL oxidation by 19% compared to replacement with 18:2 *cis,cis*-CE. When the surface phosphoglycerides were replaced with either 16:0−18:2 *cis,cis*-phosphatidylcholine (PC) or 16:0−18:2 *trans,trans*-PC, the latter was found to inhibit the rate and increase the lag time of oxidation to a greater extent than the former. To confirm these findings, we studied the oxidation of PC liposomes by assessing the formation of conjugated dienes or the degradation of a fluorescently labeled PC. By both methods, the 16:0−18:2 *trans,trans*-PC exhibited greater resistance to oxidation than the 16:0−18:2 *cis,cis*-PC. Eliminating the fluidity differences did not completely eliminate the differences in oxidation rates, suggesting that the *trans* double bond is inherently resistant to oxidation. The composition of the conjugated hydroperoxy products formed after oxidation differed markedly for the two 18:2 isomers. Supplementation of 16:0−18:2 *cis,cis*-PC liposomes with 20 mol % di16:1 *trans*-PC retarded oxidation rates to a greater extent than supplementation with di16:1 *cis*-PC. These studies show that dietary *trans* unsaturated fatty acids decrease the rate of lipid peroxidation, an effect that may mitigate the atherogenic effect of these fatty acids.

Although the *trans* double bond configuration is thermodynamically more stable, most of the naturally occurring fatty acids have *cis* double bonds. One possible reason for this is the effect of *cis* double bonds on membrane properties. For instance, the introduction of a *cis* double bond at position 9 of stearic acid (18:0) to form oleic acid reduces the melting temperature from 69.6 °C to approximately 15 °C, whereas the introduction of a *trans* double bond at the same position reduces the melting temperature to only 46.5 °C (*1*). Some bacteria exploit these differences between *cis* and *trans* isomers to manage their membrane properties in response to environmental stresses through a *cis*/*trans* isomerase (*2*). The presence of additional double bonds increases membrane fluidity further; however, the effect of each additional bond is progressively smaller. Increasing the number of double bonds in fatty acids also increases their rate of peroxidation (*3*). This peroxidation of membrane and lipoprotein lipids is implicated in the pathogenesis of many diseases, including atherosclerosis, cancer, Alzheimer's, and HIV infection (*4*−*7*). The differential effects of *cis* and *trans* isomers on peroxidation reactions and disease progression are not well understood.

The connection between dietary *trans* unsaturated fatty acids (TUFA)[1] and coronary heart disease is largely based on studies that showed a correlation between dietary TUFA and serum cholesterol levels (*8*). Furthermore, the lipoprotein profile is altered with a greater intake of TUFA leading to an atherogenic increase in the LDL to HDL ratio (*9*). Little, however, is known about the physicochemical and biological properties of these lipoproteins. Since oxidation of LDL is believed to be a key initiating event in atherosclerosis (*4*), we investigated the effect of TUFA on lipid oxidation in LDL and liposomes.

In previous studies, we have shown that sphingomyelin (SM) inhibits the oxidation of lipoproteins and liposomes (*10*). Since SM does not have the properties of the prototypical antioxidant such as chelation of metal ions or scavenging

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[1] Abbreviations: A_2C, 2-(2-methoxyethoxy)ethyl-8-(*cis*-2-*n*-octylcyclopropyl)octanoate; AAPH, 2,2′-azobis(2-amidinopropane) dihydrochloride; AMVN, 2,2′-azobis(2,4-dimethylvaleronitrile); CE, cholesteryl esters; CLA, conjugated linoleic acids; DPH-PC, 2-[3-(diphenylhexatrienyl)propanoyl]-1-hexadecanoyl-*sn*-glycero-3-phosphocholine; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); FAME, fatty acid methyl esters; FC, free cholesterol; FFA, free fatty acids; HDL, high-density lipoproteins; HSA, human serum albumin; LDL, low-density lipoproteins; PC, phosphatidylcholine; PLA_2, phospholipase A_2; pTSA, *p*-toluenesulfinic acid; rLDL, reconstituted low-density lipoproteins; smLDL, surface-modified low-density lipoproteins; TG, triacylglycerols; TUFA, *trans* unsaturated fatty acids.

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of free radicals, we proposed that it inhibits oxidation through an alteration of the physical properties of the membrane (*11*). The proposed mechanism is based on two properties of SM. First, since it is less oxidizable than other membrane lipids, it can insulate oxidizable lipids from membrane free radicals. Second, by reducing the fluidity of the membrane, SM retards the lateral propagation of peroxy radicals, thereby inhibiting the overall oxidation of unsaturated lipids. This mechanism is similar to those proposed for tamoxifen and isoflavones (*12*−*14*). Other molecules that similarly affect the physical properties of biological membranes should also be expected to reduce the rate of oxidation.

TUFA could qualify as an antioxidant under both of the criteria proposed for SM. Compared to a similarly substituted *cis* double bond, a *trans* double bond exists at a lower-energy state as a consequence of the physical distribution of its substituents; therefore, they are generally less reactive. This assessment is supported by data showing TUFA to be less reactive than their *cis* isomers in substitution and Diels−Alder reactions (*15*). The implication is that TUFA would also be inherently more resistant to oxidation reactions. Since their physical properties are similar to those of saturated fatty acids, they can also be expected to reduce membrane fluidity when incorporated into phospholipid membranes, an effect believed to inhibit oxidation (*16*). Thus, membrane TUFA would be expected to retard oxidation compared to their *cis* isomers.

To explore the potential mechanisms of TUFA-related atherosclerosis, we have studied the effect of TUFA on the oxidation of lipoproteins and artificial membranes. The results presented here show a paradoxical protective effect of TUFA on lipoprotein oxidation, ruling out one possible mechanism for their apparent atherogenicity, namely, increasing the LDL oxidation.

EXPERIMENTAL PROCEDURES

Materials. 2,2′-Azobis(2,4-dimethylvaleronitrile) (AMVN) and 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) were obtained from Wako Pure Chemical Industries (Richmond, VA). Fatty acids, fatty acid methyl esters, and cholesteryl esters were purchased from Nu-Check Prep (Elysian, MN). 2-[3-(Diphenylhexatrienyl)propanoyl]-1-hexadecanoyl-*sn*-glycero-3-phosphocholine (DPH-PC) was purchased from Molecular Probes (Eugene, OR). 16:0−18:2 *cis*,*cis*-PC, di16:1 *cis*-PC, and di16:1 *trans*-PC were purchased from Avanti Polar Lipids (Alabaster, AL). OptiPrep was obtained from Greiner Bio-One (Longwood, FL). [1-^{14}C]-Linoleic acid was purchased from American Radiolabeled Chemicals (St. Louis, MO). All solvents (HPLC grade) and TLC plates were purchased from Fisher Scientific (Pittsburgh, PA). Boron trifluoride (14% in methanol) was obtained from Alltech, while 4-(dimethylamino)pyridine, *N*,*N*′-dicyclohexylcarbodiimide, *p*-toluenesulfinic acid (pTSA, sodium salt), 2-(2-methoxyethoxy)ethyl-8-(*cis*-2-*n*-octylcyclopropyl)octanoate (A_2C), and snake venom phospholipase A_2 (*Naja mocambique mocambique*) were purchased from Sigma-Aldrich (St. Louis, MO).

Methods. 16:0−18:2 *trans*,*trans*-PC was synthesized from 16:0 LPC and 18:2 *trans*,*trans*-FFA by the method of Paltauf and Hermetter (*17*). The PC was purified on silica gel TLC plates using the chloroform/methanol/water/ammonium hydroxide (65/25/4/2, v/v) solvent system, and was eluted with chloroform and methanol (*18*).

In vitro isomerization of 16:0−18:2 *cis*,*cis*-PC to 16:0−18:2 *trans*,*trans*-PC was performed essentially as described by Gibson and Strassburger (*19*). The free acid of pTSA was prepared from the commercially available sodium salt as previously described (*20*). For isomerization, 8 mg of 16:0−18:2 *cis*,*cis*-PC was added to a screw-cap tube as a chloroform solution, and the solvent was evaporated under nitrogen. To the tube was added 400 μL of a pTSA solution (5 mg/mL in dioxane). The tube was flushed with nitrogen, tightly closed with a Teflon-lined cap, and heated at 116 °C for 40 min. The dioxane was removed by nitrogen evaporation, and the material was redissolved in chloroform. The PC was isolated by silica gel TLC with the chloroform/methanol/acetic acid/water (50/30/8/4, v/v) solvent system, and eluted with the procedure of Bligh and Dyer (*18*). The concentration of isomerized PC was estimated by lipid phosphorus by the modified Bartlett method (*21*). The extent of isomerization was determined by gas chromatography of the fatty acid methyl esters (FAME), using heptadecanoic acid (17:0 FFA) as an internal standard (*22*). The FAME were separated using an isothermal program (160 °C for 2 h) on a Shimadzu GC-17A gas chromatograph equipped with an autoinjector using an Omegawax 250 column and a flame ionization detector. Retention times were compared with standards of 18:2 *cis*,*cis*-FAME and 18:2 *trans*,*trans*-FAME. FAME from pTSA-treated 16:0−18:2 *cis*,*cis*-PC showed that 82.5% of the *cis* double bonds were converted to the *trans* geometry. Of all the FAME formed during treatment, 78% were 18:2 *trans*,*trans* while 9% were either 18:2 *cis*-9,*trans*-12 or 18:2 *trans*-9,*cis*-12 (unresolved by our gas chromatography method). This degree of conversion is in agreement with published data (*19*).

Liposomes (large unilamellar vesicles) for oxidation studies were prepared by extrusion using a commercially available Mini-Extruder (Avanti) (*23*). Briefly, 2.5 μmol of PC (or 3.125 μmol of PC for preparations containing di16:1 PC) was added to a tube, and the solvent was removed by nitrogen evaporation. The lipid was redissolved in ethanol, and the solvent was again removed by nitrogen evaporation. To the dried lipid was added 1 mL of 10 mM Tris and 150 mM NaCl (pH 7.4) (Tris-NaCl buffer). The tube was flushed with nitrogen, covered with Parafilm, and vortexed vigorously for 1 min followed by heating at 45 °C for 20 min. The emulsion was then passed through a 0.2 μm polycarbonate membrane 11 times so that the final solution was in the syringe opposite from the one in which it started to prevent contamination. The liposomes were stored under nitrogen in Teflon-capped tubes. All liposomes were used within 1 week of preparation.

Liposomes for supplementation of LDL were prepared by a modification of the cholate dialysis method (*24*) in the absence of apoprotein AI. The preparations were dialyzed extensively against 10 mM Tris, 150 mM NaCl, 1 mM EDTA, and 0.01% NaN_3 (pH 7.4) (buffer A) at 4 °C.

LDL was prepared from fresh, never frozen human plasma obtained from a commercial source (Lifesource, Glenview, IL) by sequential density gradient ultracentrifugation ($1.019 < d < 1.063$). It was dialyzed against Tris-NaCl buffer, passed through a 0.45 μm filter, and stored at 4 °C.

Modification of the neutral lipid core of LDL was performed essentially by the method of Krieger *et al.* (*25*). Briefly, LDL was dialyzed extensively against 0.3 mM EDTA (pH 7.0) and lyophilized after binding to potato starch. The neutral lipids were extracted with heptane and replaced with either 100% 18:2 (*cis* or *trans*) CE or LDL/heptane extract supplemented with 16:1 CE. To each tube was added 6 mg of cholesteryl ester (CE) in 200 μL of heptane. The LDL/starch/CE mixture was incubated at −20 °C for 1.5 h after being vortexed. The heptane was then removed by evaporation under N_2 with the tubes on ice. One milliliter of 10 mM Tricine buffer (pH 8.4) was added, and the samples were vortexed. Tubes were flushed with nitrogen, covered with Parafilm, and stored overnight at room temperature. The rLDL was collected from the starch pellet by centrifugation. The supernatant was collected, passed through a 0.8 μm filter, and dialyzed extensively against Tris-NaCl buffer at 4 °C.

The LDL surface monolayer was modified by depletion of phospholipids by phospholipase A_2 treatment followed by replenishment with 16:0−18:2 PC geometric isomers. A reaction mixture containing 2.2 mg of LDL protein, 1 mM $CaCl_2$ (final concentration), 10 mg of human serum albumin (HSA, essentially fatty acid free), and 2 milliunits of snake venom phospholipase A_2 (*N. mocambique mocambique*) was prepared in Tris-NaCl buffer, to a final volume of 825 μL. The reaction mixture was incubated at 37 °C for 1 h, and the enzyme reaction was stopped by the addition of 12 mM EDTA followed by incubation of the mixture at 37 °C for 20 min. The phosphoglycerides were then replaced by incubating the LDL preparation with 828 μL of liposomes (5 μmol of 16:0−18:2 *cis*- or *trans*-PC/mL and 1.25 μmol of FC/mL) and 545 μL of the $d > 1.21$ plasma fraction as a source of phospholipid transfer protein in the presence of 0.7 mM DTNB at 37 °C for 44 h under N_2. The LDL was then isolated by density gradient ultracentrifugation at 400000*g* for 4 h at 4 °C in a Beckman TL-100 centrifuge, using 12% Optiprep overlaid with 200 μL of buffer A. The band corresponding to LDL was collected by syringe aspiration. The OptiPrep was removed from the LDL by dialyzing the mixture extensively against Tris-NaCl buffer at 4 °C.

Oxidation was assessed by measuring the increase in absorbance at 234 nm (formation of conjugated dienes) in a temperature-controlled spectrophotometer (Shimadzu UV-1601) (*26*). PC liposomes (0.5 μmol of 16:0−18:2 PC) were diluted to 3.0 mL with Tris-NaCl buffer, and oxidation was initiated by the addition of either AAPH (final concentration of 2 mM) or $CuSO_4$ (final concentration of 20 μM for liposomes or 5 μM for modified LDL). Oxidation rates were determined as the slope of a regression line drawn through the linear range of the absorbance versus time curve. Lag times were determined as the time corresponding to the intersection of the oxidation rate regression line with a regression line drawn through the initial phase of oxidation.

The isomers of the lipid hydroperoxides formed during the oxidation of 18:2 FAME were analyzed by normal phase HPLC (*27*). 18:2 FAME (1.25 mg of the *cis* or *trans* isomer) was dispersed in 0.6% Tween 20 and was oxidized for 2 h at 37 °C with 2.4 mM AAPH. The reaction was stopped by addition of 0.5 mL of ethanol, and the lipid was extracted twice with 3.0 mL of hexane. The pooled hexane layers were evaporated under nitrogen, and the lipid was redissolved in 40 μL of hexane. The lipid hydroperoxides were separated on a 4.6 mm × 150 mm Alltima 100 Å, 3 μm silica column (Alltech) with hexane and diethyl ether (250/12, v/v) as the mobile phase, at a flow rate of 0.6 mL/min. The peaks were identified by their known elution pattern (*27*). To determine decay rates of specific lipid hydroperoxide isomers, [1-^{14}C]-18:2 *cis*,*cis*-FAME were oxidized with 12 mM AAPH at 37 °C for 4 h as detergent-stabilized micelles in 0.6% Tween 20. The hydroperoxide products were isolated from each other by HPLC as described above while monitoring the absorbance at 234 nm. [1-^{14}C]-18:0 FAME was added to the isolated lipid hydroperoxides as an internal standard; the FAME mixture was solubilized in 0.6% Tween 20 as before, and the mixture was oxidized with 2 mM AAPH. Aliquots at various time points were extracted with ethanol and hexane, and the rates of decay were determined relative to the internal standard (18:0 FAME) by HPLC (flow rate of 1.0 mL/min) using a Packard Radiomatic Flo-One/Beta instrument with a scintillation fluid flow rate of 2.0 mL/min.

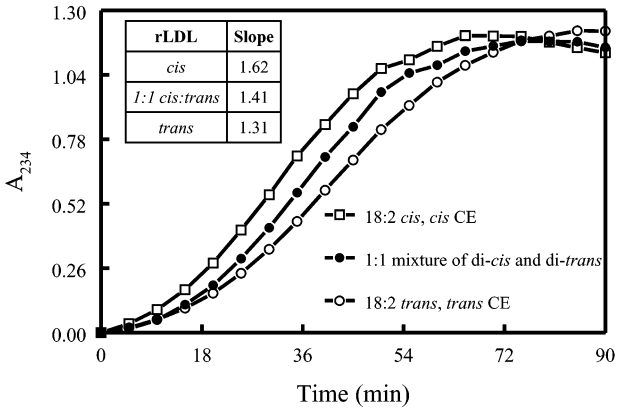

FIGURE 1: Effect of core lipid replacement with 18:2 CE isomers on LDL oxidation. LDL (600 μg of protein) was diluted to 3.0 mL in Tris-NaCl buffer and oxidized at 37 °C with 5 μM $CuSO_4$. The formation of conjugated dienes was monitored at 234 nm. The inset shows the oxidation rates as slopes (ΔA_{234}/h).

Protein concentrations were estimated by the Markwell modification of the Lowry method (*28*). The levels of total and free cholesterol were measured by commercially available enzymatic kits from Sigma and Wako, respectively. Phospholipid levels were estimated by the modified Bartlett procedure (*21*). Statistical significance was determined by the two-tailed, paired Student's *t* test.

RESULTS

Oxidation of LDL: Effect of Total Core Replacement with 18:2 CE Isomers. To determine the effect of TUFA on the oxidation of LDL, we first replaced the neutral lipid core of LDL with 18:2 CE geometric isomers. It should be noted that the neutral lipid extraction used in this technique removes the FC and the triacylglycerol (TG) as well as the CE; therefore, the reconstituted LDL (rLDL) does not contain either FC or TG. The rate of oxidation was determined from the formation of conjugated dienes by measuring the absorbance at 234 nm at 37 °C upon addition of $CuSO_4$ (final concentration of 5 μM). Figure 1 shows a typical oxidation curve. Reconstituted LDL containing 18:2 *trans*,*trans*-CE was oxidized 19% slower than rLDL containing 18:2 *cis*,*cis*-

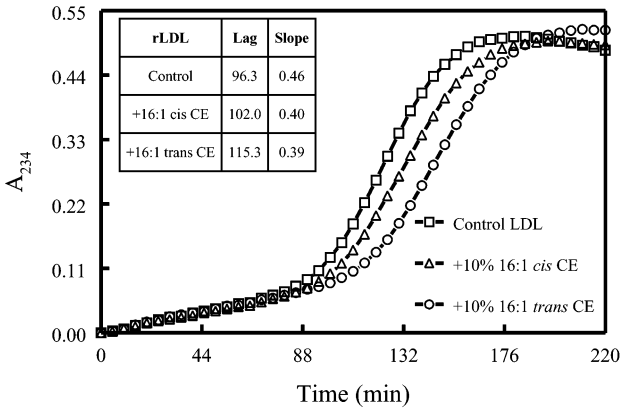

FIGURE 2: Effect of supplementation with 16:1 CE isomers on LDL oxidation. LDL (600 μg of protein) was diluted to 3.0 mL in Tris-NaCl buffer and oxidized at 37 °C with 5 μM $CuSO_4$. The inset shows the lag times (in minutes) and the oxidation rates as slopes (ΔA_{234}/h).

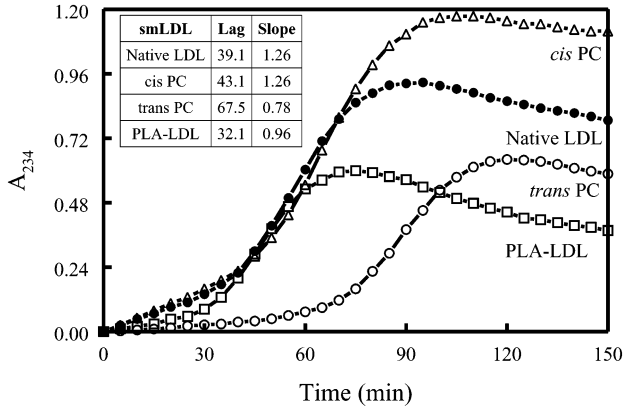

FIGURE 3: Effect of modification of the surface phosphoglycerides on LDL oxidation. LDL (200 μg of protein) was diluted to 3.0 mL in Tris-NaCl buffer and oxidized at 37 °C with 5 μM $CuSO_4$. The phospholipids of native LDL were first depleted by treatment with snake venom PLA_2 and then replaced with either 16:0−18:2 *cis,cis*-PC or 16:0−18:2 *trans,trans*-PC. The inset shows the lag times (in minutes) and the oxidation rates as slopes (ΔA_{234}/h) for each of the preparations.

CE; the 1/1 mixture of the two isomers exhibited an intermediate rate of oxidation (13% slower than the rate of 18:2 *cis,cis*-CE). Unlike the oxidation of native LDL, that of rLDL exhibited a negligible lag phase, possibly the consequence of removal of the endogenous antioxidant during the lipid extraction process.

Core Supplementation with 16:1 CE Isomers. To test whether *trans* unsaturated CE could inhibit the oxidation of endogenous lipids in LDL, we supplemented LDL core lipids with 10% 16:1 CE isomers. For this purpose, we extracted the neutral lipids as described above, and then added them back after supplementation of the extract with 16:1 CE at a concentration of 10% of the total CE. Because the endogenous lipids were added back to the depleted LDL, these preparations contain FC, TG, and endogenous antioxidants unlike the case in which the whole LDL core is replaced. The presence of a lag period reflects the reintroduction of endogenous antioxidants. As shown in Figure 2, supplementation with 16:1 *trans*-CE increased the lag time from 96 to 115 min, whereas supplementation with 16:1 *cis*-CE caused an only modest change from the control (102 min). The rate of oxidation, however, was decreased to a similar extent by both the isomers (88% of the control for the *cis* isomer and 86% of the control for the *trans* isomer). Therefore, it appears that incorporation of *trans* unsaturated CE into LDL inhibits the overall oxidation rate both by being more resistant to oxidation and by protecting endogenous lipids (and antioxidants) from oxidation.

Effect of Alteration of the LDL Surface Monolayer. To determine the effect of TUFA on the surface lipids of LDL, the surface monolayer of native LDL was depleted of phosphoglycerides by treatment with snake venom phospholipase A_2 (PLA_2) followed by repletion with the 16:0−18:2 PC *cis* or *trans* isomer. Figure 3 illustrates the effect of the two isomers on surface-modified LDL (smLDL) oxidation. The calculated lag time and rates of oxidation are shown in the inset. Although the PLA_2 treatment was performed in the presence of excess HSA, all LDL preparations retained significant amounts of lysoPC, in agreement with previous studies (*29*). The amount of lysoPC, however, was the same in all preparations. The amount of 16:0−18:2 *trans,trans*-PC incorporated was only 33% of the PC concentration in native LDL, whereas the amount of the *cis* isomer was 36% greater than the native LDL PC concentration. Compared to those of native LDL, the lag time for PLA_2-treated LDL was reduced by 18% while the oxidation rate was reduced by 16%. Incorporation of 16:0−18:2 *cis,cis*-PC increased the lag time by 4 min, compared to that of native LDL, whereas the *trans* isomer increased it by 28 min. The rate of oxidation was increased after replacement with the *cis*-PC isomer, possibly reflecting the increased amount of oxidizable lipid. LDL with 16:0−18:2 *trans,trans*-PC, however, was oxidized at a slower rate than the native (62%) as well as PLA_2-treated (81%) LDL. The increase in the lag time may reflect the protection of the endogenous antioxidants by the TUFA-containing PC. Since the measured oxidation rates reflect the oxidation of both the core and surface lipids (with the former contributing the bulk of oxidation), the inhibition of the rate of oxidation by TUFA-PC indicates the protection of core lipids by the surface phospholipids.

Oxidation of 16:0−18:2 PC Isomers Incorporated into Liposomes. To more closely examine the mechanism by which TUFA reduce LDL oxidation rates, we investigated the oxidation of TUFA incorporated into defined liposomes (large unilamellar vesicles). To determine the relative oxidizability of TUFA, we first assessed the oxidation of 16:0−18:2 PC containing either *cis* or *trans* 18:2 fatty acid. The PC was incorporated into extruded liposomes and oxidized with 20 μM $CuSO_4$. Figure 4 shows a typical oxidation curve at 37 °C. The 16:0−18:2 *cis,cis*-PC was oxidized, on average, more than 4 times faster than the 16:0−18:2 *trans,trans*-PC at 37 °C. A 1/1 mixture of the two isomers showed an intermediate rate of oxidation, approximately 2.3 times greater than the rate of 16:0−18:2 *trans,trans*-PC.

Effect of Isomerization of 16:0−18:2 cis,cis-PC on Liposome Oxidation. To further determine whether the differences in oxidation rate could be attributed to the geometry of the double bond, we isomerized 16:0−18:2 *cis,cis*-PC to 16:0−18:2 *trans,trans*-PC by treatment with pTSA, a reagent known to isomerize *cis* double bonds to their *trans* isomers without changing the position of the double bond (*19*). The pTSA-treated 16:0−18:2 PC contained approximately 78%

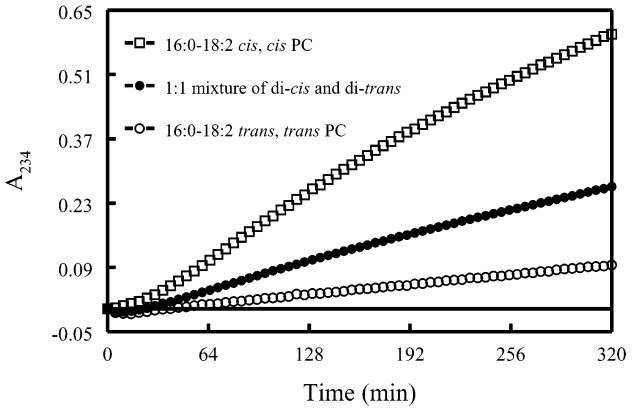

FIGURE 4: Oxidation of 16:0−18:2 PC geometric isomers. Liposomes (0.5 μmol of PC/3.0 mL) were oxidized at 37 °C with 20 μM $CuSO_4$.

trans,*trans* isomer and 9% of either the *cis*-9,*trans*-12 or *trans*-9,*cis*-12 isomer. Oxidation of liposomes prepared with pTSA-treated and untreated 16:0−18:2 *cis*,*cis*-PC showed that the isomerization inhibited the rate of oxidation in the presence of 20 μM $CuSO_4$ by 32% (results not shown).

Temperature Dependence of 16:0−18:2 PC Isomer Oxidation. The decreased rate of oxidation of the *trans*-PC isomer may be due to either its supramolecular effects on the liposomes (e.g., fluidity and lateral diffusion rates) or the inherent resistance of the *trans* double bond to oxidative modification. To determine whether the membrane rigidifying effect of the *trans* fatty acid is responsible, we measured the oxidation rates at increasing temperatures (16, 25, 37, 45, and 55 °C) for the three liposome preparations (16:0−18:2 *cis*,*cis*-PC, 16:0−18:2 *trans*,*trans*-PC, or a 1/1 mixture of the two isomers). Since both the *cis* and *trans* isomers would be in the liquid-disordered state at higher temperatures (above their melting temperatures), any difference in oxidation rates could be attributed to the nature of the double bond *per se*. Figure 5 shows the oxidation rates of the three preparations. Panel A shows the mean absolute oxidation rates of the three preparations as a function of temperature. As expected, the oxidation rates of all the preparations increased with increasing temperature; however, the increase in rate was not uniform for all three preparations. From 16 to 45 °C, the rate of oxidation of the 16:0−18:2 *cis*,*cis*-PC increased by a factor of 3, whereas the rate of oxidation of 16:0−18:2 *trans*,*trans*-PC increased by a factor of nearly 16. In panel B, the oxidation rates are normalized to the rate of the 1/1 mixture of the two PC isomers (means ± standard error of the mean). From the normalized data, it can be seen that the differences in the oxidation rates between the two preparations are temperature-dependent; the relative rates were statistically significant at all temperatures ($p < 0.05$). The difference is greatest at the lowest temperature and lowest at the highest temperature. The ratio of normalized oxidation rates of the 16:0−18:2 *cis*,*cis*-PC to the 16:0−18:2 *trans*,*trans*-PC decreased from 7.1 at 16 °C to 3.0 at 45 °C. This suggests that the physical state of the membrane is an important factor in the differential rates of oxidation since the increasing temperature should act to minimize differences in supramolecular structure; however, this cannot account for all the observed difference, indicating that *trans* double bonds are inherently more resistant to oxidation than the corresponding *cis* isomers.

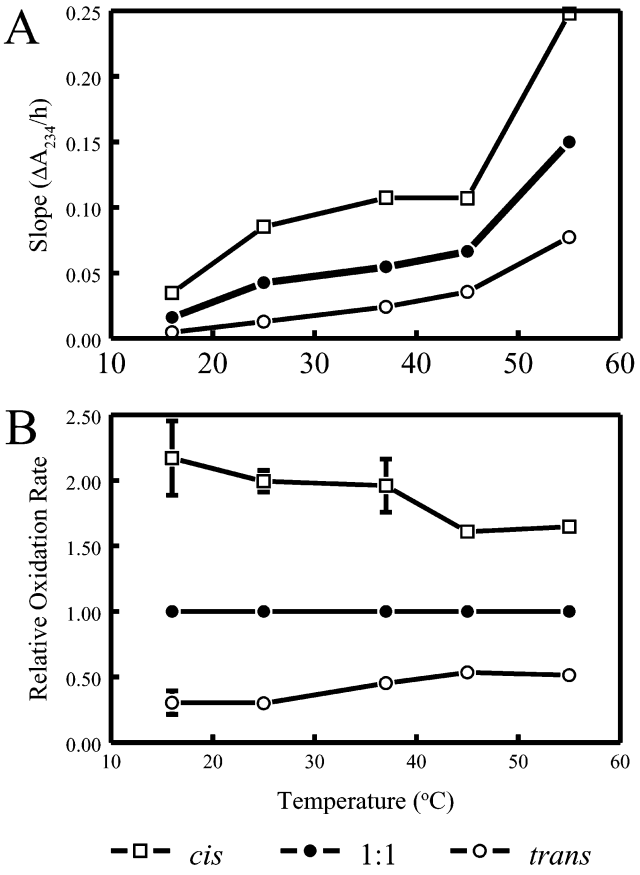

FIGURE 5: Temperature dependence of 16:0−18:2 PC isomer oxidation. 16:0−18:2 PC isomers (0.5 μmol) were oxidized as extruded liposomes with 20 μM $CuSO_4$ at 16, 25, 37, 45, and 55 °C in a final volume of 3.0 mL. The oxidation rates were calculated as the slopes of linear regression lines drawn through the linear region of the absorbance vs time curves. Panel A shows the mean ($n = 3$) absolute oxidation rates as a function of temperature. Panel B shows the oxidation rates normalized to the rate of the 1/1 mixture of 16:0−18:2 *cis*,*cis*-PC and 16:0−18:2 *trans*,*trans*-PC (mean ± standard error of the mean, $n = 3$). At all temperatures, the differences in relative rates between the pure 16:0−18:2 PC isomers are significant ($p < 0.05$).

Effect of the Membrane Mobility Agent A_2C on the Oxidation of 16:0−18:2 PC Isomers. To further determine whether membrane supramolecular structure is an important factor in lipid oxidation rates, we tested the effect of 2-(2-methoxyethoxy)ethyl-8-(*cis*-2-*n*-octylcyclopropyl)octanoate (A_2C), a membrane-fluidizing agent that decreases the lipid packing density through its cyclopropyl ring (*30*). Because the cyclopropyl group is not a target for oxidation, A_2C can be used to increase membrane fluidity without altering the amount of oxidizable lipid present in the liposome or increasing the temperature. With both 16:0−18:2 PC isomers, the A_2C (at 10 and 25 mol % PC) increased the oxidation rate in a dose-dependent manner; however, the effect on the oxidation of 16:0−18:2 *trans*,*trans*-PC was greater than on its *cis* isomer (results not shown). At 37 °C, the incorporation of 25 mol % A_2C increased the rate of oxidation of 16:0−18:2 *cis*,*cis*-PC by 35%, whereas the same amount of A_2C increased the rate of oxidation of the *trans* isomer by 55%. These differences are magnified at lower temperatures and diminished at higher temperatures. In the most fluid state that was tested (55 °C and 25 mol % A_2C), the oxidation of the 16:0−18:2 *trans*,*trans*-PC was carried

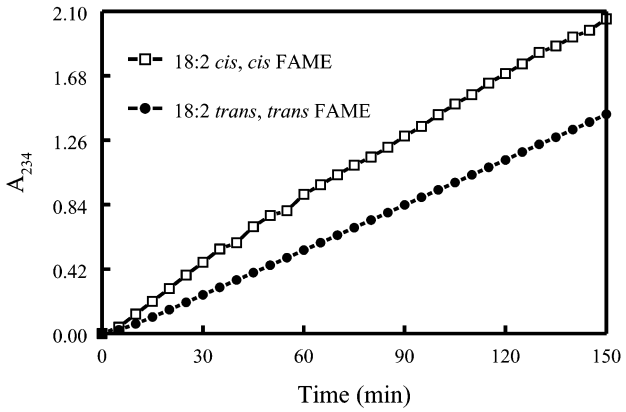

FIGURE 6: Oxidation of monomeric 18:2 FAME isomers. 18:2 FAME isomers were dissolved in 2-propanol at a concentration of 0.3 mM and oxidized with 17 mM AMVN at 45 °C.

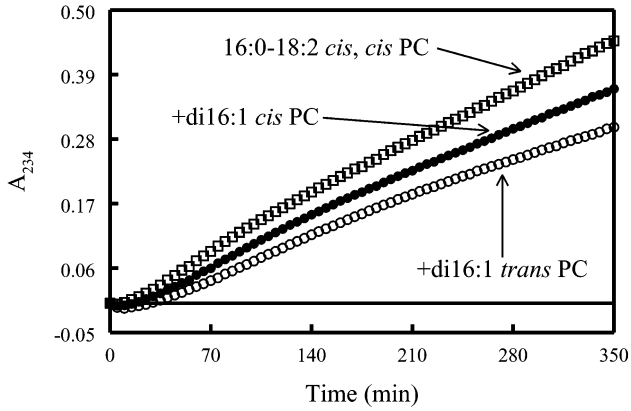

FIGURE 7: Effect of di16:1 PC isomers on the oxidation of 16:0−18:2 *cis,cis*-PC. Liposomes were prepared with 16:0−18:2 *cis,cis*-PC (2.5 μmol/ml) alone or with either the *cis* or *trans* di16:1 PC isomer (0.625 μmol/mL). The liposomes (0.5 μmol of 16:0−18:2 *cis,cis*-PC) were oxidized at 37 °C with 20 μM $CuSO_4$ in a final volume of 3.0 mL.

out at only 73% of the rate of the *cis* isomer without A_2C, indicating that removing most of the supramolecular effects cannot completely eliminate the difference in oxidation rates between the two 16:0−18:2 PC isomers.

Oxidation of 18:2 Isomers in the Monomeric State. To assess the oxidizability of *cis* and *trans* double bonds in the absence of supramolecular effects, we oxidized 18:2 FAME geometric isomers in 2-propanol at 45 °C using the free radical generator AMVN. Under the experimental conditions that were used, the concentration of FAME is expected to be below the critical micellar concentration. Thus, the oxidizable substrate should exist as monomers in solution, free from the supramolecular effects of the bilayer or other ordered structures. Furthermore, since the oxidizing agent is in molar excess, the oxidation rate is dependent on the interaction of the oxidizing agent with the FAME and largely independent of propagation of the peroxy radicals. Figure 6 shows a representative oxidation study. Here, the rate of formation of conjugated dienes for the 18:2 *trans,trans*-FAME was only 67% of the rate of the 18:2 *cis,cis*-FAME. A similar pattern was observed when the oxidation was conducted in methanol or ethanol (results not shown). Thus, the *trans* double bond appears to be inherently more resistant to oxidation than the *cis* isomer.

Effect of Supplementation with Monounsaturated Isomers. Since modification of the physical state of the membrane appears to alter oxidation rates, the incorporation of relatively nonoxidizable PC geometric isomers into 16:0−18:2 *cis,cis*-PC liposomes should also alter the oxidation rates by rigidifying the membrane. To address this hypothesis, liposomes were prepared with 16:0−18:2 *cis,cis*-PC alone or supplemented with 20 mol % di16:1 *cis*-PC or di16:1 *trans*-PC. Oxidation was assessed by monitoring the formation of conjugated dienes after addition of $CuSO_4$ (final concentration of 20 μM). In this experimental system, only the oxidation of 16:0−18:2 *cis,cis*-PC is being measured for three reasons. First, only the diunsaturated PC can form the conjugated dienes measured in our system. Second, it is unlikely that much of the di16:1 PC is being oxidized in the background because diunsaturated fatty acids are approximately 40-fold more oxidizable than monounsaturated fatty acids (*3*), and the amount of diunsaturated fatty acids in this system is 2-fold greater than the amount of monounsaturated fatty acids. Third, on the basis of the oxidation curves, it is unlikely that more than 50% of the diunsaturated fatty acid is oxidized over the course of the experiment because the plateau phase, when the oxidizable substrate becomes limiting, was not reached. Thus, the results mainly reflect the supramolecular effects of TUFA on membrane oxidation.

Figure 7 shows a typical oxidation pattern for the three preparations at 37 °C. The incorporation of either monounsaturated PC inhibited the oxidation of the 16:0−18:2 *cis,cis*-PC; however, the di16:1 *trans*-PC inhibited oxidation to a greater extent than the di16:1 *cis*-PC (on average, 74% of control compared to 87% at 37 °C), and di16:0 PC exhibited an inhibition of oxidation similar to that of the di16:1 *trans*-PC (results not shown). Since only the oxidation of the 16:0−18:2 *cis,cis*-PC is being measured in this experiment, these results show that the presence of less oxidizable lipids in the membrane inhibits the overall rate of oxidation, with stronger inhibition in the presence of *trans* isomers.

To explore the relationship between the physical state of the membrane and the oxidation rates of the liposome preparations, oxidation rates were measured over a wide temperature range. Figure 8 shows the temperature dependence of the oxidation rates. Panel A shows the increase in absolute rate with increasing temperature for all three preparations. Panel B shows the data normalized to the rate of the unsupplemented 16:0−18:2 *cis,cis*-PC liposomes. As was the case with the 16:0−18:2 PC geometric isomers, the difference in oxidation rates among the preparations decreases with increasing temperature. Figure 8B shows that the oxidation rates converge at higher temperatures, implying that the differences in the oxidation rates are minimized as the differences in the physical state of the membranes are minimized. The relative rate of oxidation of the liposomes supplemented with di16:1 *trans*-PC increases from 62% of the control at 16 °C to 85% at 55 °C, while the liposomes supplemented with di16:1 *cis*-PC exhibit a range of 73−90% of the control. At all the temperatures that were assayed, the liposomes supplemented with di16:1 *trans*-PC were oxidized less rapidly than the liposomes containing di16:1 *cis*-PC or the controls. The fact that there remains a difference between the oxidation of the unsupplemented control and the di16:1 PC-containing liposomes even at 55 °C may reflect the insulating properties of the less oxidizable lipids, or the temperature may be insufficient to completely remove the differences in membrane fluidity.

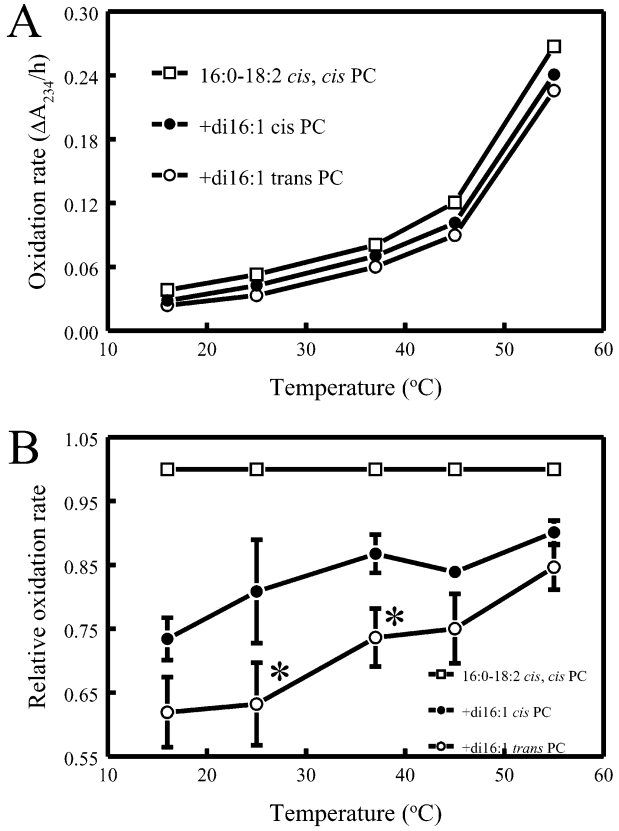

FIGURE 8: Temperature dependence of the di16:1 PC inhibition of 16:0−18:2 *cis,cis*-PC oxidation. 16:0−18:2 *cis,cis*-PC liposomes (0.5 μmol) with and without 20 mol % di16:1 PC were diluted to 3.0 mL and oxidized with 20 μM $CuSO_4$ at 16, 25, 37, 45, and 55 °C. The oxidation rates were calculated as described in the legend of Figure 5. Panel A shows the mean ($n = 3$) absolute oxidation rates as a function of temperature. Panel B shows the oxidation rates normalized to the rate of oxidation of the unsupplemented 16:0−18:2 *cis,cis*-PC liposomes (mean ± standard error of the mean, $n = 3$). An asterisk denotes a significant difference between relative oxidation rates of the two supplements ($p < 0.05$). The difference at 16 °C approached significance ($p = 0.07$).

Identification of the Hydroperoxide Isomers Formed during Oxidation. The major products in the triplet oxidation of 18:2 fatty acids are the hydroperoxy derivatives with a conjugated diene structure (*31*). This structure is introduced into a nonconjugated fatty acid when one of the double bonds shifts position (from position 9 to 10 or from position 12 to 11 in the case of linoleic acid) upon addition of molecular oxygen to a lipid radical. The translocating bond adopts the *trans* configuration, while the second bond can either retain the original geometry or adopt the *trans* configuration as well. To assess whether there were qualitative, as well as quantitative, differences in the hydroperoxide products of the two geometric isomers, 18:2 FAME isomers were oxidized as detergent-stabilized micelles, and the structures of the hydroperoxide products were determined by HPLC. Four products were identified on the basis of the known retention times (Figure 9) (*27*). In the order of elution, these peaks are methyl 13-hydroperoxy-9-*cis*-11-*trans*-octadecenoate, methyl 13-hydroperoxy-9-*trans*-11-*trans*-octadecenoate, methyl 9-hydroperoxy-10-*trans*-12-*cis*-octadecenoate, and methyl 9-hydroperoxy-10-*trans*-12-*trans*-octadecenoate. As shown in Figure 9A, AAPH oxidation of 18:2 *cis,cis*-FAME produced all four products in nearly equal amounts while the *trans,trans* isomer yielded two predominant products (13-hydroperoxy-9-*trans*-11-*trans* and 9-hydroperoxy-10-*trans*-12-*trans*) (Figure 9B). Enzymatic oxidation with soybean lipoxidase produced a single product for the 18:2 *cis,cis*-FAME (methyl 13-hydroperoxy-9-*cis*-11-*trans*-octadecenoate), whereas the 18:2 *trans,trans*-FAME was not a substrate for the enzyme under identical conditions (results not shown).

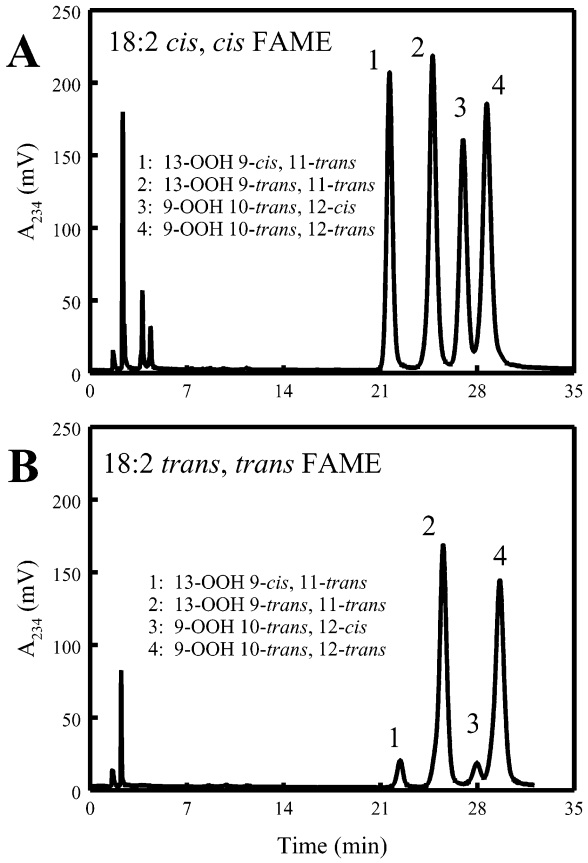

FIGURE 9: Hydroperoxide distribution of oxidized 18:2 FAME isomers. 18:2 FAME hydroperoxides were produced by oxidation of detergent-stabilized micelles with 5 mM AAPH at 37 °C for 2 h in Tris-NaCl buffer. Lipids were extracted with hexanes and ethanol and separated on normal phase HPLC as described in the text. Peaks were monitored at 234 nm.

Degradation of Hydroperoxide Isomers. Because our method of assessing oxidation rates is based on a steady state measurement of conjugated products, it is affected by rates of degradation as well as rates of formation; therefore, it is conceivable that the apparent resistance of the TUFA to oxidation could be the consequence of an increased rate of degradation of the hydroperoxide products. To determine whether this was the case, we isolated all four hydroperoxide products from oxidized [1-^{14}C]-18:2 *cis,cis*-FAME by HPLC, incorporated them into detergent-stabilized micelles, and oxidized them further with 2 mM AAPH. Lipid extracts of the oxidized micelles were taken at various time points to assess the degradation rates of the four hydroperoxide isomers relative to a [1-^{14}C]-18:0 FAME internal standard. Figure 10 illustrates the absolute rate of disappearance of radioactivity over time for the four isolated hydroperoxides. There was no significant difference in the rate of disappearance of the four isomers under the experimental conditions; however, the *trans,trans* isomers exhibited a tendency for slower degradation. Figure 11 shows the relative concentration of the hydroperoxide isomers during oxidation of a single hydroperoxide starting material. The data show a redistribu-

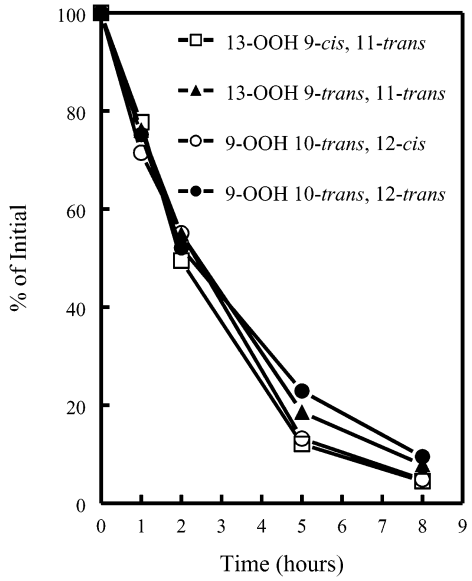

FIGURE 10: Degradation of positional and geometric FAME hydroperoxide isomers. [1-^{14}C]-18:2 *cis*,*cis*-FAME were oxidized as detergent-stabilized micelles with 12 mM AAPH for 4 h at 37 °C. The individual hydroperoxide isomers were isolated by normal phase HPLC and supplemented with [1-^{14}C]-18:0 FAME (internal standard). Each hydroperoxide was then further oxidized with 2 mM AAPH at 37 °C. Aliquots were extracted, and hydroperoxides were analyzed by HPLC using a radioactivity detector. Concentrations were estimated relative to the internal standard and are expressed relative to the initial concentration.

tion of the positional and geometric isomers over time, indicating that both bond isomerization and hydroperoxide migration occur under the conditions that were assayed. The initial *trans*,*trans*-hydroperoxides (Figure 11, right panels) remain a larger percentage of the total hydroperoxides over time than do the *cis*,*trans* isomers (Figure 11, left panels). When the starting material is a *cis*,*trans*-hydroperoxide, there is an increase in the amounts of both *trans*,*trans* isomers, whereas starting with a *trans*,*trans* isomer mainly produces its hydroperoxide positional isomer with only a minor formation of *cis*,*trans* isomers. These data imply a slight relative stability of the *trans*,*trans*-hydroperoxide isomers to further oxidative decay under these experimental conditions. This finding rules out the possibility that the 18:2 *trans*,*trans* isomer (in 18:2 FAME or 16:0−18:2 PC) is only apparently more stable to oxidation because of an increased rate of degradation of its conjugated products.

Oxidative Decay of Fluorescence. Because there is a slight difference in the absorbance spectra of the hydroperoxide products (*cis*,*trans* compared to *trans*,*trans*) and because the 18:2 geometric isomers differ in their product distribution and their degradation, we also explored the influence of 16:0−18:2 PC isomers on the oxidative degradation of the fluorescent probe DPH-PC (*32*); the response factor for DPH-PC is independent of these factors. Figure 12A illustrates the fluorescence decay of DPH-PC incorporated into liposomes of 16:0−18:2 PC isomers at a concentration of 0.1 mol % and oxidized with 2 mM AAPH at 37 °C. The oxidative destruction of DPH-PC was slower in the liposomes composed of 16:0−18:2 *trans*,*trans*-PC than in 16:0−18:2 *cis*,*cis*-PC liposomes. While the absolute oxidation rate for both preparations increased with increasing temperature, the relative oxidation rate of the 16:0−18:2 *trans*,*trans*-PC

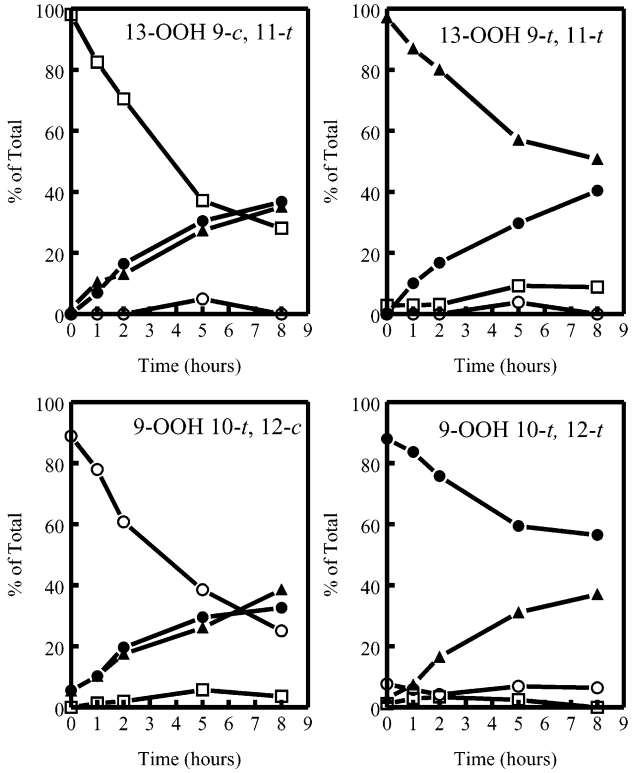

FIGURE 11: Distribution of geometric and positional isomers of 18:2 FAME hydroperoxides upon oxidation of specific isomers. The experiment was conducted as described in the legend of Figure 10. The hydroperoxide concentrations are expressed as the percent of the total at each time point. The starting hydroperoxide is given at the top of each panel: (□) 13-OOH 9-*cis*,11-*trans*, (▲) 13-OOH 9-*trans*,11-*trans*, (○) 9-OOH 10-*trans*,12-*cis*, and (●) 9-OOH 10-*trans*,12-*trans*.

increased from 67% of the *cis* isomer at 37 °C to 77% at 55 °C (results not shown).

Fluorescently labeled liposomes were also prepared with 16:0−18:2 *cis*,*cis*-PC supplemented with 25 mol % di16:1 PC isomers. As shown in Figure 12B, addition of either di16:1 PC isomer reduced the oxidation rate compared to the control; however, di16:1 *trans*-PC reduced the rate to a greater extent, 39% versus 18% for the di16:1 *cis*-PC compared to unsupplemented liposomes. These studies confirm that the introduction of less oxidizable lipids into a phospholipid membrane reduces the oxidizability of that membrane. Furthermore, TUFA-containing lipids reduce the oxidizability to a greater extent than do their *cis* isomers.

DISCUSSION

The average dietary intake of TUFA in the United States has been reported to be as high as 13.3 g per person per day (*33*), and much epidemiological data suggest that dietary TUFA intake is correlated with heart disease (*34*). This connection is often attributed to the cholesterol boosting effect of TUFA as well as the increase in the LDL-to-HDL ratio (*8*, *9*). Less is known about other possible mechanisms that could account for the correlation between TUFA and heart disease. Oxidative modification of lipoproteins is thought to play an important role in atherogenesis by exerting chemotactic and cytotoxic effects, by promoting the formation of foam cells by the unregulated uptake of oxidized LDL by macrophages, and by limiting the ability of HDL to

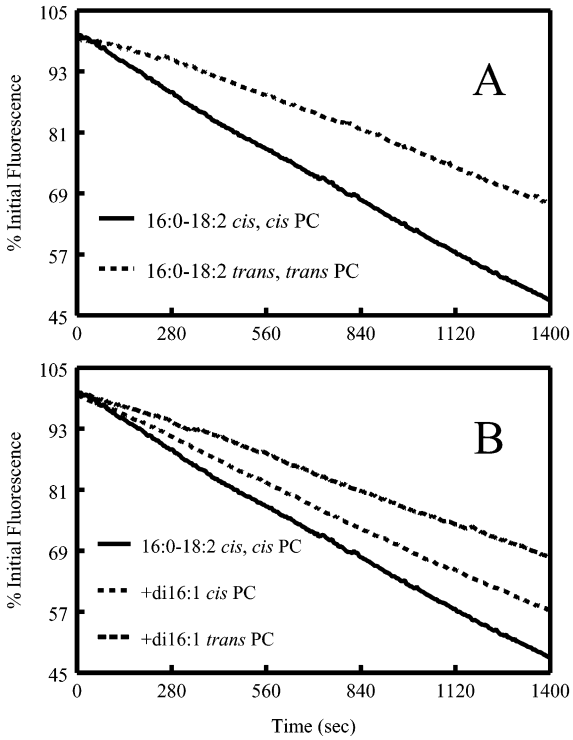

FIGURE 12: Effect of *cis*- and *trans*-PC isomers on the oxidative decay of DPH-PC. DPH-PC was incorporated into liposomes at 0.1 mol % of the total PC and oxidized with 2 mM AAPH at 37 °C. Liposome preparation was otherwise the same as that described in the legends of Figures 4 and 7. The oxidative decay of DPH-PC was monitored in a spectrofluorometer (excitation at 360 nm, emission at 430 nm). Panel A shows the effect of 16:0−18:2 PC isomers on DPH-PC oxidation. Panel B shows the effect of supplementation with di16:1 PC on DPH-PC oxidation in a matrix of 16:0−18:2 *cis*,*cis*-PC liposomes.

facilitate cholesterol efflux (*4*, *35*−*37*). In this study, we investigated the effect of TUFA-containing lipids on the oxidation of lipoproteins and liposomes in an effort to better understand the relationship between TUFA and heart disease.

We have found that TUFA incorporated into LDL and liposomes are less oxidizable than their *cis* counterparts and protect endogenous lipids from oxidation. Two important aspects of the experimental design should be noted. First, the molar extinction coefficients of the *cis*,*trans*-hydroperoxide products are lower than those of the *trans*,*trans* products (*38*, *39*). Thus, the oxidation of the 18:2 *cis*,*cis* isomer is actually underrepresented, and the actual differences in oxidation rates would be greater than those reported here. Second, the absorbance levels for controls containing no oxidizing agent were subtracted from the experimental samples prior to calculation of slopes. Because the controls are subject to atmospheric oxidation, there is a background oxidation that differs among the isomers. The *cis* unsaturated controls are atmospherically oxidized more rapidly than the TUFA controls. Since these controls are subtracted from the experimentals, the differences in the oxidation rates of those experimentals are actually diminished by our mathematical manipulation; therefore, the differences in oxidation rates are probably greater than those reported here.

In liposome studies, we showed that the differences in the relative rates of oxidation decrease when the differences in the physical state of the membrane are minimized through the addition of a fluidizing agent or by increasing the temperature above the lipid melting temperatures. This suggests that the effect on the physical state of the membrane is at least partly responsible for the antioxidant properties of TUFA. The similarity of TUFA to saturated fatty acids in their structure and melting temperatures implies that TUFA rigidify the membrane. Membrane rigidification has been proposed to be the mechanism responsible for the antioxidant properties of tamoxifen and other sterols as well as isoflavones (*12*−*14*). We have proposed a similar mechanism for sphingomyelin that would explain studies from our laboratory that have shown sphingomyelin to retard LDL and membrane oxidation (*10*, *11*). We hypothesize that decreasing membrane fluidity retards the lateral propagation of lipid radicals across the surface of the membrane, thereby limiting the rate and extent of oxidative damage. Our studies also indicate an inherent resistance of the *trans* double bond to oxidation. This inherent stability of the *trans* double bond to modification is consistent with other studies that have shown this geometry to be less reactive in substitution and Diels−Alder reactions (*15*). The relative resistance of TUFA to oxidation allows them to insulate neighboring *cis* unsaturated lipids from oxidative modification, contributing to the overall antioxidant effect.

In addition to showing a quantitative difference in the oxidation of *cis* and *trans* isomers, we have shown that the initial oxidation products also differ in their isomeric distribution as well as their stability to further oxidation. Since the products of TUFA oxidation are degraded more slowly than the products of *cis* unsaturated fatty acids, the conjugated diene measurements underestimate the differences in oxidation rates. This is based on the fact that our oxidation studies are based on steady state measurements of conjugated diene concentration that are affected by rates of degradation as well as rates of formation.

Another implication of the differential production of oxidation products is potential effects on signal transduction pathways. The hydroperoxide products of fatty acid oxidation may exhibit different biological effects through perturbations in cellular signaling. Studies with conjugated linoleic acid (CLA) have shown that positional isomers of CLA differ in their biological properties (*40*, *41*). CLA are structurally similar to the hydroperoxide products of 18:2 fatty acid oxidation in both the position and geometry of their double bonds; however, they lack the hydroperoxide groups. The differential effects of the hydroperoxide (and hydroxide) oxidation products will need to be further studied to determine whether this is a potential mechanism for the deleterious effects of TUFA. In addition to differences among the products themselves, the relative stability of the *trans*,*trans* product may allow it more time to exert potential effects on cellular metabolism, providing another mechanism by which TUFA may promote atherogenesis.

On the basis of our studies, dietary TUFA should not be expected to increase the susceptibility of LDL to oxidation, and therefore, this mechanism does not account for the increase in atherosclerosis risk associated with these fatty acids. In fact, the observed inhibition of LDL oxidation may mitigate the atherogenic propensity mediated through other mechanisms. These studies indicate that, while dietary TUFA increase LDL levels, these lipoproteins qualitatively differ from normal LDL and may be less atherogenic. Some early animal studies on dietary TUFA that specifically investigated

both lipid levels and atherosclerosis support such a hypothesis. For example, Weigensberg *et al*. (*42*) showed that rabbits fed elaidic acid (18:1 *trans*) exhibited a significantly increased serum cholesterol level when compared to those fed either oleic acid or corn oil. This increase in serum cholesterol levels, however, did not lead to a significantly increased level of atherosclerosis. Similarly, McMillan *et al*. (*43*) showed that feeding of elaidinized olive oil to rabbits significantly increased serum cholesterol levels compared to levels in controls receiving untreated olive oil; however, the level of atherosclerosis in the aortic arch was increased only slightly, while the level of atherosclerosis in the thoracic and abdominal aorta showed no difference from the control. The study of Kritchevsky (*44*) also showed that rabbits fed partially hydrogenated vegetable oils were hypercholesterolemic without a significant difference in atherosclerosis compared to controls. While these studies did not look at the distribution of cholesterol in the lipoproteins, they do point to the fact that increases in serum cholesterol levels caused by TUFA feeding may not correlate directly with an increased risk of atherosclerosis.

Previous studies that specifically investigated the oxidizability of LDL after TUFA feeding reported no change compared to the equivalent *cis* unsaturated fatty acid diet (*45*−*47*). The assumption inherent in these studies was that the oxidizability of the LDL in the TUFA-fed group would be greater than in the control group. When data from feeding studies are reassessed in light of the expected decrease in the level of oxidation for TUFA-fed subjects, there is evidence to support a tendency toward an antioxidant effect for TUFA. Nestel *et al*. reported that feeding subjects an elaidic acid-rich diet did not increase the susceptibility of LDL to oxidation. In fact, the data suggest a slight, although not statistically significant, increase in lag times in the elaidic acid-rich diet compared to the oleic-acid rich diet or habitual control (*46*). In a study by Cuchel *et al*. (*45*), a comparison was made between subjects fed corn oil or corn oil margarine. Once again, there was a tendency for the TUFA-fed group to exhibit a slight increase in lag times to oxidation. In this same study, animals fed corn oil margarine also exhibited a significantly higher concentration of α-tocopherol ($p < 0.005$). Nicolosi *et al*. (*47*) reported a similar increase, if not statistically significant, in α-tocopherol content for hamsters fed 18:1 *trans* and 18:1 *cis* fatty acids. This increase in α-tocopherol concentration in LDL could be explained by a TUFA-mediated protection of the endogenous antioxidants from oxidative degradation. In our studies on LDL with 16:0−18:2 PC isomers replacing the normal phosphoglycerides on the surface of the membrane, we found a significant increase in the lag time for the LDL replenished with the *trans* isomer, suggesting that the TUFA is protecting the endogenous antioxidants. Furthermore, we have demonstrated that the incorporation of TUFA into membrane lipids retards the oxidative decay of the fluorescent probe DPH-PC, which could be considered a surrogate for natural antioxidants; the protection from oxidation conferred by TUFA-containing lipids supports a similar protection of membrane antioxidants.

While our studies show that an increase in the oxidizability of lipids is not responsible for the purported atherogenic effect of dietary TUFA, other mechanisms may be indicated. Previous studies from our laboratory showed that TUFA-containing PC are poor substrates for lecithin:cholesterol acyltransferase and inhibit the enzyme activity against *cis*-PC (*48*). Others have proposed additional mechanisms, including increased cholesterol ester transfer protein activity (*49*), increased Lp[a] levels (*50*), altered enzyme activity (*51*), perturbed mitochondrial function (*52*), and disrupted cell surface receptors (*53*). It is also possible that differences in the oxidation products of *trans* and *cis* unsaturated fatty acids may lead to differential effects on cellular signaling and gene transcription. Further studies will be required to investigate these possibilities.

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