Comparison of *Cis* and *Trans* Fatty Acid Containing Phosphatidylcholines on Membrane Properties†

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ABSTRACT: The ever-increasing amount of *trans* fatty acids in the human diet has been linked to a variety of afflictions, most notably coronary heart disease and arteriosclerosis. The mechanism of why the replacement of *cis* fatty acids with their *trans* counterparts can be detrimental to the health of an individual remains a mystery. Here, we compare the differences in membrane physical properties including molecular dynamics, lateral lipid packing, thermotropic phase behavior, "fluidity", lateral mobility, and permeability between model membranes (lipid monolayers and bilayers) composed of *cis*- and *trans*-containing phosphatidylcholines (PCs). The PCs tested have a total of zero, one, two, or four *cis* (oleic or linoleic) or *trans* (elaidic or linoelaidic) double bonds. These experiments all confirm the basic hypothesis that *trans* fatty acids produce membrane properties more similar to those of saturated chains than to those of acyl chains containing *cis* double bonds; i.e., *cis* double bonds induce much larger membrane perturbations than *trans* double bonds.

Since the first successful hydrogenation of oils was reported in 1897, there has been a steady increase in the amount of *trans* fatty acids appearing in the human diet (*1*). It has been estimated that *trans* fats contribute 4−12% of the total dietary fat intake (2−4% of the total energy) in the United States population (*2*). This corresponds to as much as 13.3 g of *trans* fats per person per day (*3*)! A good correlation between the consumption of *trans* fats and the occurrence of coronary heart disease and arteriosclerosis has been established (*4*, *5*). Although far less certain, it has been suggested that *trans* fats may also be carcinogenic (*6*). In addition, it is likely that, contrary to *cis* fats, *trans* fats may accumulate in the body over time (*7*). In one study, Laryea et al. (*7*) reported that the concentrations of most erythrocyte phospholipid fatty acids leveled off by year 2 but *trans* fats continued to increase until late childhood. The high dietary occurrence and undeniable link of *trans* fats with potential health complications underlies the importance of determining the modes of action of the compounds.

All fatty acids can be incorporated into phospholipids and thereby effect the hydrophobic interior of membranes. In diets devoid of hydrogenated oils, the major source of *trans* fats in the human diet, *trans* fatty acids comprise only a tiny fraction of the total fatty acyl chains present in membranes. *Trans* fatty acids do occur naturally at relatively low levels in meat and dairy products as a byproduct of fermentation in ruminant animals (*1*). Many studies have supported the idea that, once incorporated into membrane phospholipids, *trans* fats may alter basic membrane properties, thus effecting biochemical activities (*8*, *9*). Although the modern human diet may contain a bewildering array of *trans* fatty acid products resulting from partial hydrogenations of many oils, most studies to date have focused on supplementation with the natural *trans* fat elaidic acid ($18{:}1^{\Delta 9t}$).[1] Several general conclusions have emerged from these studies. *Trans* fat uptake into membranes was reported to directly reflect the dietary *trans* level (*8*−*10*), and the effect of adding elaidic acid to the diet was more similar to supplementation with saturated stearic acid (18:0) than to supplementation with the *cis* counterpart of elaidic acid, oleic acid ($18{:}1^{\Delta 9c}$) (*11*). Once incorporated into phospholipids, the gross membrane property of "fluidity" was shown to be altered (*12*), as well as the activity of several proteins (*13*−*15*). While these

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[1] Abbreviations: 18:0−18:0 PC, distearoylphosphatidylcholine (DSPC); $18{:}0-18{:}1^{\Delta 9c}$ PC, 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; $18{:}0-18{:}1^{\Delta 9t}$ PC, 1-stearoyl-2-elaidoyl-*sn*-glycero-3-phosphocholine; $18{:}0-18{:}2^{\Delta 9c,12c}$ PC, 1-stearoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine; $18{:}0-18{:}2^{\Delta 9t,12t}$ PC, 1-stearoyl-2-linoelaidoyl-*sn*-glycero-3-phosphocholine; $18{:}1^{\Delta 9c}-18{:}1^{\Delta 9c}$ PC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; $18{:}1^{\Delta 9t}-18{:}1^{\Delta 9t}$ PC; 1,2-dielaidoyl-*sn*-glycero-3-phosphocholine; $18{:}2^{\Delta 9c,12c}-18{:}2^{\Delta 9c,12c}$ PC, 1,2-dilinoleoyl-*sn*-glycero-3-phosphocholine; $18{:}2^{\Delta 9t,12t}-18{:}2^{\Delta 9t,12t}$ PC, 1,2-linoelaidoyl-*sn*-glycero-3-phosphocholine; DPH, 1,6-diphenyl-1,3,5-hexatriene; DSC, differential scanning calorimetry; L_α, lamellar liquid crystalline phase; L_β, lamellar gel phase; LUV, large unilamellar vesicle; MD, molecular dynamics; MLV, multilamellar vesicle; PC, phosphatidylcholine; $\pi-A$, pressure−area; T_m, gel−liquid crystalline transition temperature.

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studies do implicate a possible membrane site of action for *trans* fatty acids, they do not indicate how *trans* fats affect membranes differently than *cis* fats.

To date, most studies have been performed with only elaidic acid versus oleic acid and have been reported in papers done at different times, under different conditions, and employing different techniques. Here, we attempt to eliminate some of the ambiguities by running parallel experiments that examine differences in molecular dynamics (MD), lateral lipid packing, thermotropic phase behavior, "fluidity", lateral mobility, and permeability between lipid monolayers and bilayers composed of 18:0−18:1$^{\Delta 9c}$ phosphatidylcholine (PC), 18:0−18:1$^{\Delta 9t}$ PC, 18:1$^{\Delta 9c}$−18:1$^{\Delta 9c}$ PC, 18:1$^{\Delta 9t}$−18:1$^{\Delta 9t}$ PC, 18:0−18:2$^{\Delta 9c,12c}$ PC, 18:0−18:2$^{\Delta 9t,12t}$ PC, 18:2$^{\Delta 9c,12c}$−18:2$^{\Delta 9c,12c}$ PC, and 18:2$^{\Delta 9t,12t}$−18:2$^{\Delta 9t,12t}$ PC.

EXPERIMENTAL PROCEDURES

Materials. 1-Stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (18:0−18:1$^{\Delta 9c}$ PC), 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (18:1$^{\Delta 9c}$−18:1$^{\Delta 9c}$ PC), 1-stearoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine (18:0−18:2$^{\Delta 9c,12c}$ PC), 1,2-dilinoleoyl-*sn*-glycero-3-phosphocholine (18:2$^{\Delta 9c,12c}$−18:2$^{\Delta 9c,12c}$ PC), and 1,2-dielaidoyl-*sn*-glycero-3-phosphocholine (18:1$^{\Delta 9t}$−18:1$^{\Delta 9t}$ PC) were purchased from Avanti Polar Lipids (Alabaster, AL). The other PCs, 1-stearoyl-2-elaidoyl-*sn*-glycero-3-phosphocholine (18:0−18:1$^{\Delta 9t}$ PC), 1-stearoyl-2-linoelaidoyl-*sn*-glycero-3-phosphocholine (18:0−18:2$^{\Delta 9t,12t}$ PC), and dilinoelaidoyl-*sn*-glycero-3-phosphocholine (18:2$^{\Delta 9t,12t}$−18:2$^{\Delta 9t,12t}$ PC) were synthesized as described below. All fluorescent probes were purchased from Molecular Probes (Eugene, OR). Lipid purity was assessed with thin-layer chromatography (TLC). Lipid concentrations were quantified using phosphate analysis (*16*). Carboxymethylcellulose, *N*,*N*-dicyclocarbodiimide, 4-(dimethylamino)pyridine, and butylated hydroxytoluene (BHT) were purchased from Sigma−Aldrich (Milwaukee, WI). All solvents were HPLC-grade and were purchased from Sigma−Aldrich. Water utilized for buffer solutions was deionized, glass-distilled, and run through a Milli-Q Plus Water Purification System (Millipore, Milford, MA).

Synthesis of trans-PCs. 1-Stearoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (18:0−OH PC) was purchased from Avanti Polar Lipids (Albaster, AL). The *trans* fatty acids, elaidic acid (18:1$^{\Delta 9t}$) and linoelaidic acid (18:2$^{\Delta 9t,12t}$) and methyl esters reference standards were purchased from Nu-Check Prep, Inc. (Elysian, MN). The PC synthesis is the same as described previously with some modifications (*17*).

Synthesis of 1-stearoyl-2-elaidoyl-*sn*-glycero-3-phosphocholine (18:0−18:1$^{\Delta 9t}$ PC) and 1-stearoyl-2-linoelaidoyl-*sn*-glycero-3-phosphocholine (18:0−18:2$^{\Delta 9t,12t}$ PC): Briefly, 2 eq (400 μmol) of either elaidic or linoelaidic acid were dissolved in 6 mL of dry, doubly distillated *N*,*N*-dimethyformamide (DMF), and the solution was stirred under nitrogen at 30 °C for 1 h. *N*,*N*-dicyclocarbodiimide (200 μmol) was then added, followed by 18:0−OH PC (200 μmol) and 4-(dimethylamino)pyridine (200 μmol). The resulting mixture was stirred under nitrogen at 25 °C for 18 h and then filtered. The solvent was evaporated under vacuum and redissolved in chloroform.

Synthesis of dilinoelaidoyl-*sn*-glycero-3-phosphocholine (18:2$^{\Delta 9t,12t}$−18:2$^{\Delta 9t,12t}$ PC): Briefly, 2 eq (400 μmol) of linoelaidic acid were dissolved in 4 mL of dry DMF, to which 200 μmol of *N*,*N*-dicyclocarbodiimide and 20 μmol of butylated hydroxytoluene (BHT) were added. The solution was stirred at 30 °C for 3 h under nitrogen, and then L-α-glycerophosphorylcholine (100 μmol) diluted in 2 mL of dry DMF and 4-(dimethyamino)pyridine (200 μmol) were added. The mixture was stirred at room temperature under nitrogen for a period of 16 h after which the resulting mixture was filtered. The solvent was evaporated under vacuum and redissolved in chloroform.

Purification of the PC products: The resultant PC products in chloroform were purified by CM-cellulose column chromatography using stepwise elution with solvents containing increasing percentages of methanol in chloroform. To assay purity, the synthesized PCs were tested by silica gel TLC and subjected to fatty acid analysis on a Shimadzu GC-17A gas chromatograph equipped with a 0.25 mm × 30 m Stabilwax capillary column (Restek, Bellefonte, PA), as previously described (*18*). The total phosphate content was determined spectrophotometrically after complete mineralization according to Chen et al. (*16*). The yield of the synthesis was 50−60% for 18:0−18:1$^{\Delta 9t}$ PC or 18:0−18:2$^{\Delta 9t,12t}$ PC relative to 18:0−OH PC and 80−90% for 18:2$^{\Delta 9t,12t}$−18:2$^{\Delta 9t,12t}$ PC relative to L-α-glycerophosphorylcholine.

MD Simulations. Three MD simulations were carried out for lipid bilayers composed of 1-oleoyl-2-stearoylphosphatidylcholine, 1-elaidoyl-2-stearoylphosphatidylcholine, and distearoylphosphatidylcholine (DSPC). Each simulation cell contained 72 lipids (36 per monolayer) and 1930 water molecules, corresponding to full hydration. The simulations were at 45 °C. CHARMM (chemistry at Harvard molecular mechanics) (*19*) was used with the PARAM22b4b all-atom parameter set (*20*) and its extension to *cis* unsaturated lipids (*21*). New parameters for the elaidic acid chain were developed using the methods described by Feller et al. (*21*). Electrostatic interactions were included via the particle mesh Ewald summation (*22*). All bonds involving hydrogen were fixed at their equilibrium distances using the SHAKE algorithm (*23*). A time step of 2 fs was used with a leapfrog Verlet integration scheme. A flexible simulation cell was used with the *z* dimension (the bilayer normal) adjusted to maintain $P_{zz} = 1$ atm, and the *x* and *y* dimensions were adjusted to maintain a surface area of 65.9 Å^2/molecule. Coordinates were saved every picosecond for subsequent analysis. Simulations were carried out using eight processors on a Beowulf-type parallel computer. The total simulation length was 5 ns, with the first nanosecond discarded as equilibration.

Differential Scanning Calorimetry (DSC). DSC studies were conducted as described before (*24*). Briefly, phospholipids dissolved in chloroform were dried under nitrogen followed by vacuum pumping overnight to remove traces of organic solvent. Multilamellar vesicles (MLVs) were made by hydrating the appropriate phospholipids (10 mg/mL) in 10 mM sodium phosphate (pH 7.4) with subsequent removal of oxygen with vacuum pumping for 30 min. MLVs were frozen in dry ice and thawed three times in a water bath above the T_m of the lipids. The MLV solutions (500 μL) were added to each of the three chambers of a Hart Scientific differential scanning calorimeter (Provo, Utah), while the 4th chamber contained 500 μL of the buffer. Heating and cooling scans were made at 5−10 °C/hour.

Area/Molecule Determinations. Pressure−area ($\pi-A$) isotherms of lipid monolayers were obtained using a Mini Langmuir−Blodgett Trough (KSV Instruments, Helsinki, Finland) and a Wilhelmy plate. All equipment was rinsed with ethanol and distilled water prior to use. Lipid monolayers were spread on a 10 mM sodium phosphate buffer (pH 7.4) using hexane/2-propanol (3:2, v/v). The carrying solvent was allowed to evaporate for ~5 min, and compression rates were set at ≤5 mN/m per minute. $\pi-A$ isotherms were acquired at 23 ± 0.5 °C using a temperature-regulated circulating water bath. The lipid area/molecule was calculated by linear extrapolation from the target pressure of 30 mN/m.

Large Unilamellar Vesicles (LUVs). LUVs were made by extruding MLVs 10 times through 0.2-um Nucleopore Filters using a temperature-controlled Extruder (Lipex, Vancouver, BC, Canada).

Steady-State Fluorescence Measurements. Fluorescence polarization studies were conducted as described before (*25*). Prior to MLV formation, the fluorescent probes were included at a 200:1 lipid/probe ratio and LUVs were prepared subsequently as described above. The fluorescent probes used were either a series of stearic acids with a 9-anthroyloxy group attached at the 2 (2AS), 6 (6AS), or 9 (9AS) positions or 1,6-diphenyl-1,3,5-hexatriene (DPH). Fluorescence anisotropy (A) was calculated by

$$A = (I_{VV} - GI_{VH})/(I_{VV} + 2GI_{VH}) \quad (1)$$

where $G = I_{HV}/I_{VH}$. I_{VH} is the emission intensity detected when the sample is excited by vertically plane-polarized light and emission-measured through horizontal polarized light. I_{VV} and I_{HV} are defined analogously. Fluorescent intensities and A were measured on a Perkin-Elmer LS 50B Luminescence spectrometer. Excitation was at 351 nm and emission at 430 nm for DPH; for the AS probes, the excitation and emission wavelengths were 341 and 446 nm, respectively. Temperature was controlled to ±0.1 °C, and the A values presented are the average of six determinations.

Lateral Mobility Measurements. Pyrene decanoate (1-pyrene hexadecanoate) is employed to follow lateral diffusion, another way of estimating relative membrane "fluidity". The ability of pyrene decanoate to form highly fluorescent excimers is known to be, at least over short distances, directly proportional to its lateral diffusion rate (*26*, *27*). Pyrene decanoate in tetrahydrofuran/DMSO (1:1, v/v) was incubated with LUVs for 30 min at 37 °C. The probe/PC ratio was 1:100. The LUVs were excited at 340 nm, and the emission spectra followed from 360 to 560 nm on a Perkin-Elmer LS50B Fluorescence spectrophotometer. The excimer (475 nm)/monomer (375 nm) ratio was calculated for each PC.

Carboxyfluorescein (CF) Permeability. Carboxyfluorescein (CF) leakage measurements were conducted as described previously (*17*). Permeability of PC LUVs was monitored by recording the increase in fluorescence intensity resulting from CF leakage. Nonsequestered CF was removed using a Sephadex G-50 column with 90 mM KCl and 10 mM Na_2HPO_4 at pH 7.0. CF permeability measurements were started by rapidly mixing small aliquot of LUVs with 2.5 mL of 90 mM KCl/10 mM Na_2HPO_4 at pH 7.0 and 25 °C. Fluorescence was measured using a Perkin-Elmer LS 50B Luminescence spectrometer at 25 °C. Excitation was set to 490 nm with a resulting emission at 520 nm. Total sequestered CF was obtained following LUV breakage with the addition of 50 μL of 5% Triton X-100. Percent CF leakage was determined by

$$\% \text{ CF leakage} = [(F - F_i)/F_{max}] \times 100 \quad (2)$$

where F and F_i are the fluorescence intensity at time t and $t = 0$, respectively, and F_{max} is the maximum fluorescence after Triton X-100 release.

Statistical Analysis. Fluorescence results (Figures 2−4) are the average of at least six separate determinations, and statistical analysis is as described in Zerouga et al. (*17*). For comparison of the values, the Student's t test was employed. Statistical significance between all groups was determined by analysis of variance (ANOVA) and the Scheffe F test. Differences were considered significant if $P < 0.05$.

RESULTS AND DISCUSSION

While there are now a variety of reports indicating that *trans* unsaturated fatty acids may effect membranes more like saturated fats than *cis* unsaturated fats, many contradictory studies have also appeared. For example, Engelhard et al. (*12*) found that the addition of *trans* fatty acids to a culture medium actually increased the "fluidity" of choline-supplemented membranes. Csordas and Schauenstein (*28*) demonstrated that oleic and elaidic acids similarly protect against osmotic rupture of chicken and sheep erythrocytes. Linoleic and linolenic acid, however, were much less protective indicating a specificity for one double bond regardless of whether it is *cis* or *trans*. Finally, Kummerow et al. (*29*) reported that elaidic and linoelaidic acids increased incorporation of calcium into cells, whereas stearic and oleic acids did not.

To more systematically assess the effect of acyl chain double bonds on the membrane physical properties, we have employed a diverse set of techniques, namely, MD simulations, thermotropic phase behavior, area/molecule determinations, "fluidity", lateral mobility, and membrane permeability. Our experiments are designed to test the general assumption that chains with *trans* double bonds are straighter than "kinked" *cis* chains, thus effecting lipid packing (*30*).

Analysis of the MD simulation trajectory provides an atomic-level picture of changes in chain packing because of unsaturation. The effect of the *cis* double bond at the center of the oleic acid chain is often described as placing a "kink" in the fatty acid chain, thus inhibiting close packing of the chains. This destabilization of the ordered gel state thus leads to lower temperatures for the gel−liquid crystalline phase transition. In discussing fluid phase lipids, it is unclear as to the pre*cis*e meaning of a "kink", given that the chains are most accurately represented by an immense ensemble of conformations and cannot be described by a single conformer or even a small group of conformers. We have chosen to characterize the fatty acid chains by a pair of vectors stretching from C2 to C9 and from C10 to C17. For each lipid, the dot product of these vectors was calculated and the results were averaged over all lipids and all time steps of the simulation to provide an ensemble averaged measure of the relative orientations of the upper- and lower-chain segments. Figure 1 shows the probability distribution for this quantity observed in the simulation for stearic, oleic, and

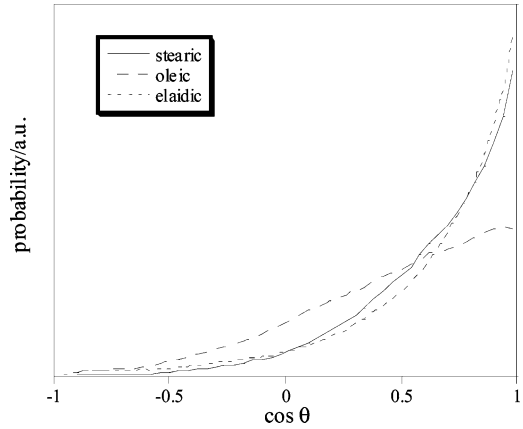

FIGURE 1: Probability distribution function for the angle made between vectors representing the upper and lower halves of the fatty acids in the *sn*-1 position of the phosphatidylcholine bilayer.

elaidic acid chains. Not surprisingly, the distribution for the saturated stearic chain shows a maximum probability at 1, indicating a parallel orientation as the most likely state. The distribution function for the *cis* unsaturated oleic acid, however, is distinctly different. While the probability still takes on a maximum value at the parallel orientation, the distribution is considerably broader, indicating a higher proportion of the bent conformations where the upper and lower halves of the chain are not aligned with each other. The distribution function for the *trans* unsaturated elaidic acid chain is nearly identical to the saturated stearic acid result. Thus, the effect of *trans* unsaturation on the bend of the chains is minimal. These observations can be further quantified by calculating the average angle made by the chain segments. While the results of the stearic and elaidic acids differ by less than one degree, the oleic acid segments are found to be 13° further from parallel than that of the case for stearic acid.

The MD simulations are in general agreement with two companion molecular simulation studies by Pasenkiewicz-Gierula and co-workers (*31*, *32*). These investigators could detect no significant difference between 16:0−18:1$^{\Delta 9c}$ PC and 16:0−18:1$^{\Delta 9t}$ PC at the aqueous interface (*31*) but did observe a difference between the two PCs within the bilayer hydrophobic interior (*32*), reporting that the *trans*-PC was more similar to the disaturated DMPC standard than the *cis*-PC.

In general, the more tightly packed the phospholipid bilayers are the higher their gel (L_β)−liquid crystalline (L_α) phase transition temperatures T_m are. In an early report, Small (*33*) showed that adding a *cis* double bond to stearic acid to form oleic acid reduces the melting temperature from 69.6 to 15 °C, whereas adding a *trans* double bond to form elaidic acid only reduces the melting temperature to 46.5 °C. In Table 1, we report the L_β−L_α transition (T_m) for the various *cis*- and *trans*-PCs. As predicted by the MD simulations, the *cis*-PCs have a considerably lower T_m than the *trans*-PCs. The largest decrease in T_m is noted upon the addition of a first double bond to the 18-carbon saturated stearic acid (18:0−18:0 PC, T_m = 58 °C). The measured decrease in T_m of 18:0−18:1$^{\Delta 9c}$ PC (from 58 to 5.5 °C = −52.5 °C) is 25.6 °C larger than the decrease measured for 18:0−18:1$^{\Delta 9t}$ PC (from 58 to 31.1 °C = −26.9 °C). Similar but smaller trends were measured upon inclusion of additional double bonds.

Table 1: Gel−Liquid Crystalline Phase Transition Temperature (T_m) for *cis*- and *trans*-PCs as Determined by DSC

cis-PC	T_m	*trans*-PC	T_m
18:0−18:1$^{\Delta 9c}$	5.5	18:0−18:1$^{\Delta 9t}$	31.1
18:1$^{\Delta 9c}$ −18:1$^{\Delta 9c}$	−18.1	18:1$^{\Delta 9t}$ −18:1$^{\Delta 9t}$	11.1
18:0−18:2$^{\Delta 9c,12c}$	−15.2	18:0−18:2$^{\Delta 9t,12t}$	9.7
18:2$^{\Delta 9c,12c}$−18:2$^{\Delta 9c,12c}$	−57.0	18:2$^{\Delta 9t,12t}$−18:2$^{\Delta 9t,12t}$	nd[a]
18:0−18:0	58.0[b]		

[a] nd = not determined. [b] Value obtained from ref *42*.

Table 2: Area/Molecule Derived from π−*A* Isotherms of Select *cis*- and *trans*-PCs at 30 mN/m of Surface Pressure[a]

cis-PC	area/molecule (Å^2)	*trans*-PC	area/molecule (Å^2)
18:0−18:1$^{\Delta 9c}$	63.5	18:0−18:1$^{\Delta 9t}$	55.9
18:1$^{\Delta 9c}$ −18:1$^{\Delta 9c}$	65.2	18:1$^{\Delta 9t}$−18:1$^{\Delta 9t}$	58.1
18:0−18:2$^{\Delta 9c,12c}$	66.3	18:0−18:2$^{\Delta 9t,12t}$	59.5
18:2$^{\Delta 9c,12c}$−18:2$^{\Delta 9c,12c}$	70.7	18:2$^{\Delta 9t,12t}$−18:2$^{\Delta 9t,12t}$	62.3
18:0−18:0	48.6[b]		

[a] Standard error = ±0.1−1.5 Å^2. [b] Value obtained from ref *43*.

In all cases, the incorporation of *cis* double bonds has a larger influence on T_m than that of homologous *trans* double bonds.

Tighter lipid packing with *trans* versus *cis* double bonds was suggested by Funari et al. (*34*). In an X-ray study, these authors compared the effect of oleic, elaidic, and stearic acid as free fatty acids on phosphatidylethanolamine polymorphism. Oleic acid modulated the membrane structure (H_{II} phase facilitation), whereas the closely related elaidic and stearic acid did not. In Table 2, we directly compare the lipid packing for our series of *cis*- and *trans*-PCs by pressure−area isotherms on lipid monolayers. The results are expressed as areas/molecule for the PCs (Table 2). The areas/molecule increase with an increasing number of double bonds. As with the T_m's, a larger increase in area/molecule is found with the *cis*-PCs compared to the homologous *trans*-PCs. The largest increase in area/molecule is noted upon the addition of a first double bond to the saturated stearic acid (18:0−18:0 PC, area/molecule = 48.6 A^2). The measured increase in area/molecule of 18:0−18:1$^{\Delta 9c}$ PC (from 48.6 to 63.5 A^2 = 14.9 A^2) is twice the increase measured for 18:0−18:1$^{\Delta 9t}$ PC (from 48.6 to 55.9 A^2 = 7.3 A^2). Again, similar but smaller increases were measured for additional *trans* double bonds than for homologous *cis* double bonds. The conclusion from the MD simulations is in agreement with the area/molecule experiments. The *trans*-PCs are more closely packed than the homologous *cis*-PCs.

Steady-state fluorescence polarization measurements (expressed as *A*) of the probes DPH (diphenylhexatriene) and AS (anthracene stearic acid) are often employed to determine the general "fluidity" for the interior region of membranes. Figure 2 reports *A* (defined in the Experimental Procedures) for the *cis*- and *trans*-PCs as determined by DPH. For each phospholipid, the *cis*-PC displays a lower *A* (is more "fluid") than the homologous *trans*-PC. Also, for the *trans*-PCs with a total of two double bonds, the PC with one double bond in each chain is more "fluid" than the PCs with the two double bonds in the *sn*-2 chain. This trend is not observed with the *cis*-PCs. A series of AS probes with anthracene linked to positions 2 (2AS, A), 6 (6AS, B), and 9 (9AS, C) of stearic acid are used to compare "fluidity" (*A*) at various

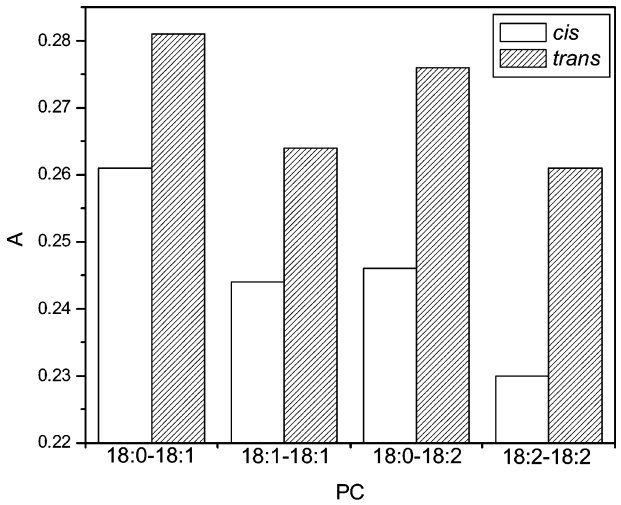

FIGURE 2: Comparative membrane "fluidity" of LUVs made from *cis*- and *trans*-PCs at 37 °C determined by steady-state fluorescence polarization of the probe DPH. Results are the average of six separate determinations, and statistical significance is as described in the Experimental Procedures.

depths in the membrane (Figure 3). In agreement with the DPH measurements, the *cis*-PC membranes are more "fluid" (have a lower *A*) than the *trans*-PC membranes at all depths and the "fluidity" increases from one to two to four double bonds. Again, for the *trans*-PCs with a total of two double bonds, there is a larger increase in "fluidity" when the double bonds are on both chains than when they are on the same chain. Once more, the trend is not observed with the *cis*-PCs.

A different way of estimating "fluidity" is through lateral mobility of pyrene-containing probes (*26*, *27*). The ability of pyrene decanoate to form highly fluorescent excimers is, over short distances, directly proportional to its lateral diffusion rate. The more "fluid" a membrane is the higher its excimer/monomer ratio. The results presented in Figure 4 are in agreement with those reported in Figures 2 and 3. For all membranes, the *cis*-containing PCs are more "fluid" than the *trans*-PC counterparts. Also, there is a general increase in "fluidity" with increasing number of double bonds. However, with pyrene decanoate no difference was observed between the 2-double bond PCs, whether they are *cis* or *trans*.

It may be assumed that more tightly packed membranes should be less permeable than membranes whose lipids are loosely packed. Bilayers composed of disaturated phospholipids are well-known to exhibit very low permeability in the gel state. In the carboxyfluorescein leakage experiments reported here (Figure 5), the DSPC bilayer permeability was too low to detect. For all membranes tested, the *cis*-PC bilayers were much more permeable (1.8−2.5× greater) than their *trans*-PC counterparts. Consistent with the "fluidity" measurements, permeability also increased with double-bond content.

There is no doubt that *trans* fatty acids can be incorporated into cell membranes (*9*), and the results presented here for model membranes clearly demonstrate a significant difference on membrane behavior between *cis* and *trans* fatty acids. However, what impact could large quantities of *trans* fatty acids have on a biological membrane? It has been suggested that perhaps *trans* fatty acyl chains are chemically

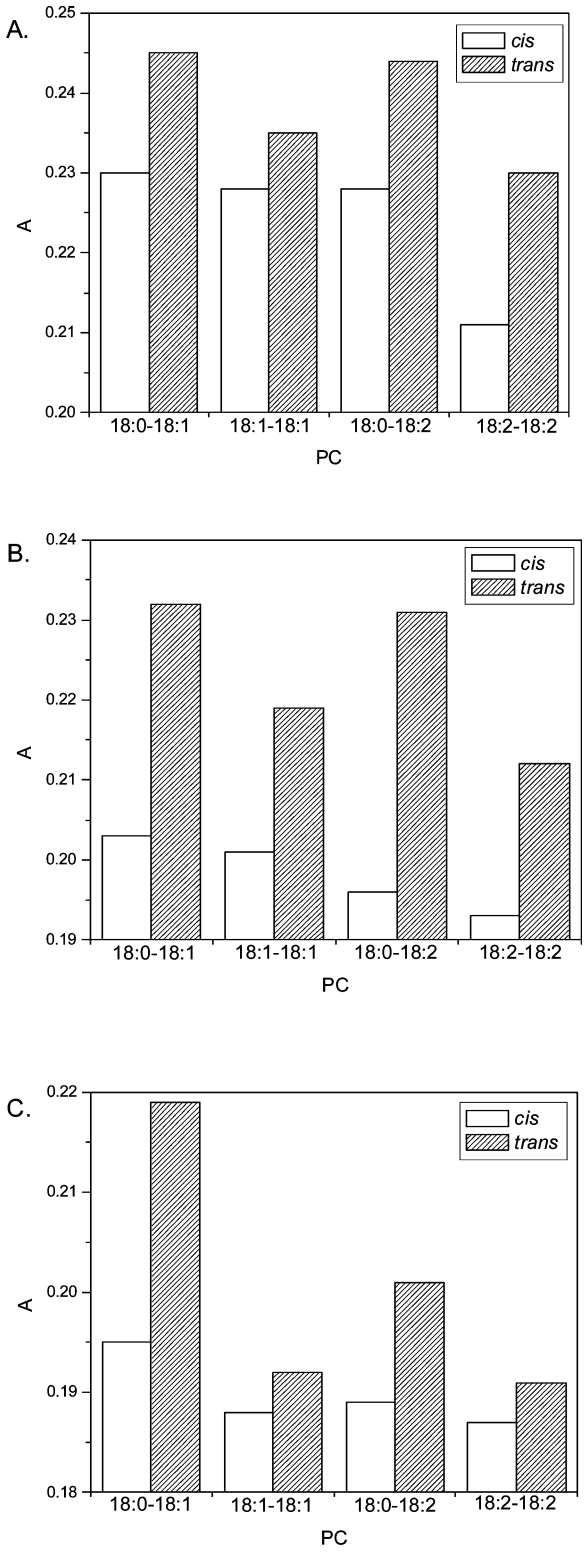

FIGURE 3: Comparative fluidity of LUVs made from *cis*- and *trans*-PCs at 37 °C determined by steady-state fluorescence polarization of the probes: (A) 2-AS, (B) 6-AS, and (C) 9-AS. Results are the average of six separate determinations, and statistical significance is as described in the Experimental Procedures.

"less reactive" than *cis* chains (*2*, *35*). For example, *trans* fatty acids are less oxidizable than their *cis* counterparts. Sargis and Subbaiah (*35*) reported that 16:0−18:$2^{\Delta 9t,12t}$ PC exhibited greater resistance to oxidation than 16:0−18:$2^{\Delta 9c,9c}$ PC. They suggested that the decreasing membrane "fluidity" with *trans* fatty acids retards the lateral propagation of lipid

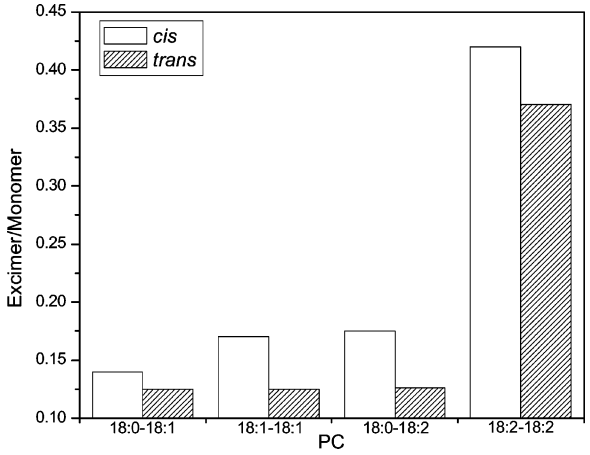

FIGURE 4: Lateral mobility of *cis*- and *trans*-PCs as determined by the excimer/monomer ratio of the fluorescent probe 1-pyrenedodecanoic acid at 37 °C. Results are the average of six separate determinations, and standard errors for the excimer/monomer ratio are ±4−8%. Statistical significance is as described in the Experimental Procedures.

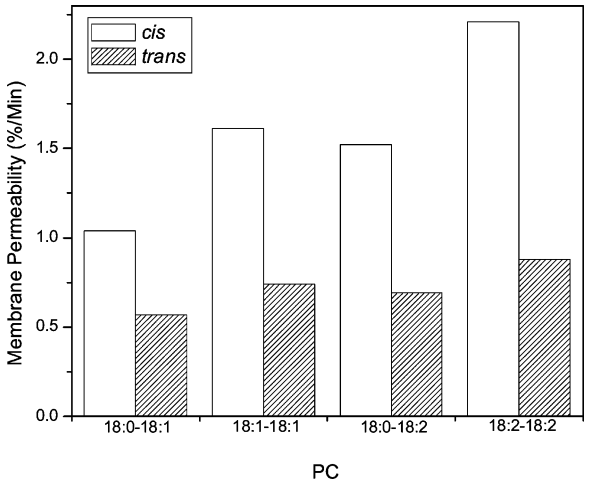

FIGURE 5: Comparative membrane permeability of LUVs made from *cis*- and *trans*-PCs as determined by the rate of carboxyfluorescein leakage. Permeability is the average of four to six determinations, and the standard error is ±0.04%/min.

radicals across the surface of the membrane, thereby limiting the rate and extent of oxidative damage. Alteration in the lateral diffusion rate should also influence the membrane microdomain composition and hence the biochemical activity. It also seems reasonable that *trans* fatty acids may significantly alter the activity of key enzymes. Although these studies are very incomplete, the activity of several proteins have been reported. Among the membrane proteins demonstrated to be effected by *trans* fatty acids are the ouabain-sensitive Na^+/K^+ ATPase (*13*), adenylate cyclase (*13*), β-adrenergic receptor (*13*), alkaline phosphatase (*14*), aniline hydroxylase (*9*) and antigen recognized by the 24.8.A iNKT cell hybridoma (*15*). It has even been suggested that *trans* fatty acids may somehow alter important intracellular signaling processes (*35*).

When incorporated into membrane phospholipids, *trans* fatty acids must replace existing saturated or *cis* unsaturated acyl chains. Larque et al. (*8*) reported that *trans* fatty acid levels in liver microsomes and mitochondria rise in parallel with dietary *trans* fat content and as the *trans* fat level increases, the saturated fatty acid levels drop. The results presented here demonstrate that *trans* fatty acids, although unsaturated, more closely resemble saturated than *cis* unsaturated fatty acids. The question then remains, if *trans* fatty acids resemble saturated fatty acids and can replace them in phospholipids, why do they have detrimental effects on human health? Are *trans* fats mistakenly identified as saturated fats and placed in the *sn*-1 chain of a phospholipid, or are they recognized as unsaturated fatty acids and placed in the *sn*-2 position, despite the fact that they do not closely resemble a *cis* acyl chain? This question has been addressed with elaidic acid. Woldseth et al. (*36*) reported that in hepatocytes elaidic acid was shown to preferentially accumulate into the *sn*-1 chain of phospholipids. Emken et al. (*37*) reported that in human erythrocytes and platelets three times more elaidic acid than oleic acid accumulated in the *sn*-1 position of PCs. In agreement, Wolf and Entressangles (*38*) showed that phospholipids from rat liver mitochondria modified *in vivo* had large quantities of elaidic acid esterified at the *sn*-1 position. The percentage of elaidic acid in the *sn*-1 position for different phospholipids was reported to be PC (31%), PE (42.5%), and PI (43%). Less than 10% of elaidic acid was found in the *sn*-2 position. On the other hand, cardiolipin, which is devoid of saturated chains, was resistant to elaidic acid incorporation. Using DPH, these authors could detect no significant alteration in "fluidity" upon elaidic acid incorporation primarily into the *sn*-1 position of membrane phospholipids. Therefore, at least for one *trans* fatty acid, we would predict that replacing *sn*-1 saturated fatty acyl chains with elaidic acid would likely have a minimal effect on the normal membrane structure and function (*38*). It is not certain if the membrane *trans* fat level could even be large enough to significantly alter the membrane structure and function. In a typical dietary study, Ibrahim and Ghafoorunissa (*14*) reported fat uptake into rat intestinal brush border membranes. Dietary hydrogenated oil (10% Indian vanaspati) was used as the *trans* source. After 10 weeks on the diet, the elaidic acid level in these membranes was only 1.9%, and this did not affect the total membrane cholesterol or phospholipid content. Why then are *trans* fatty acids considered to be toxic?

While the experiments presented here only address the relative effect of *cis* and *trans* fatty acids on the membrane structure and function, the health effects of *trans* fatty acids are undoubtedly multifaceted. Since the 1950s, it has been apparent that hydrogenated fat and *trans* fatty acids result in high blood cholesterol levels (*39*). Elaidic acid was shown to increase the total cholesterol and LDL cholesterol while decreasing the HDL cholesterol (*11*). Cuchel et al. (*40*) reported that increased LDL cholesterol levels observed after subjects consumed a diet enriched with hydrogenated fat is not attributable to decreased catabolic or increased production rates of cholesterol. Cholesterol levels are of particular interest because this sterol is a major component of membrane-signaling rafts (*41*). Also *trans* fatty acids have been linked to lower arachidonic acid levels (*14*), resulting in altered eicosanoid biosynthesis (*6*), increased calcium uptake into cells (*29*), altered phospholipid antigen binding to CD1d (*15*) and decreased lipid peroxidation (*35*).

The problem with the modern human diet however is not addressable by simple elaidic acid studies but is complicated by the abundant dietary partially hydrogenated oils that have fatty acids with a bewildering array of mixtures of *cis* and

trans double bonds. During the hydrogenation process, some *cis* bonds are converted to *trans* bonds, while other double bonds are saturated and migrate along the acyl chains (*30*). The process creates a wide range of geometric and positional fatty acid isomers. It is likely these polyunsaturated fatty acids accumulate into the *sn*-2 position of phospholipids, and once there, they may indeed significantly alter the membrane structure and function, far more than elaidic acid in primarily the *sn*-1 position. Clearly, further experiments are needed to investigate the effect of adding partially hydrogenated polyunsaturated fatty acids containing mixed *cis* and *trans* double bonds to the *sn*-2 position of membrane phospholipids.

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