Dietary Induction of Acyl Chain Desaturases Alters the Lipid Composition and Fluidity of Rat Hepatocyte Plasma Membranes†

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ABSTRACT: Rats were maintained on a regimen of intermittent starvation followed by refeeding a fat-free diet in order to induce hepatic acyl desaturase activities and other enzymes involved in lipid synthesis. The effects of the dietary regimen on the lipid composition and fluidity of isolated hepatocyte plasma membranes were compared to corresponding effects on microsomal preparations. The dietary regimen increased the content of monoenoic and polyenoic acyl chains and decreased the cholesterol/phospholipid molar ratio in the plasma membranes. Accordingly, the lipid fluidity of the plasma membranes was significantly increased as assessed by the fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene and 12-(9-anthroyloxy)stearate and the intramolecular excimer fluorescence of 1,3-di(1-pyrenyl)propane. In the microsomal membranes, substantial increases in the content of monoenoic acyl chains were offset by decreases in polyenoic acids, and no change in cholesterol/phospholipid ratio was observed. Correspondingly, the lipid fluidity of the microsomal membranes remained almost unchanged. The enhancement of lipid fluidity in the hepatocyte plasma membranes was accompanied by an increase of approximately 68% in the specific activity of the $(Na^+ + K^+)$-dependent adenosinetriphosphatase. The results demonstrate that a dietary regimen can modulate in vivo the lipid composition, fluidity, and enzyme function of the hepatocyte plasma membrane.

There is considerable evidence that the motional freedom or "lipid fluidity"[1] of the hepatocyte plasma membrane influences important activities and functions of the organelle, including the $(Na^+ + K^+)$-dependent adenosinetriphosphatase [$(Na^+ + K^+)$-ATPase][2] (Keeffe et al., 1979), the adenylate cyclase (Houslay et al., 1976a,b; Dipple & Houslay, 1978), and the process of biliary secretion (Davis et al., 1978; Keeffe et al., 1979; Simon et al., 1980; Storch & Schachter, 1983). The mechanisms which regulate the membrane fluidity, however, are relatively undefined, and the present investigation was undertaken to explore the hypothesis that the activity of hepatic acyl chain desaturases can modulate in vivo the composition and fluidity of the plasma membrane lipids. This possibility seemed reasonable, inasmuch as cis unsaturation of phospholipid acyl groups increases the molecular packing area in monolayers (Demel et al., 1967; Jain, 1972) and the fluidity in bilayers (Lentz et al., 1976; Seelig & Seelig, 1977; King & Spector, 1978; Pessin et al., 1978; Klausner et al., 1980). Moreover, recent evidence supports the more general hypothesis that plasma membrane fluidity can be regulated in vivo by modulating the biosynthesis of specific membrane lipids. In rat enterocytes, changes in cholesterol biosynthesis modulate the cholesterol content and the fluidity of the luminal, microvillus membranes (Brasitus & Schachter, 1982).

The experiments described in this paper utilized a dietary regimen of intermittent starvation followed by refeeding a fat-free diet to induce liver fatty acyl desaturase activities (Oshino & Sato, 1972; Strittmatter et al., 1974; Pugh & Kates, 1977). These enzyme activities are in the microsomal fractions of liver homgenates, and they use as substrates the coenzyme A thio esters of palmitic (16:0) and stearic (18:0) acids (Marsh & James, 1962; Holloway et al., 1963; Enoch et al., 1976; Jeffcoat & James, 1977) or the acyl groups of phospholipids (Pugh & Kates, 1977, 1979). Several investigators have explored the effects of dietary induction of hepatic acyl desaturases on the fluidity of microsomal membrane suspensions. Holloway & Holloway (1977) reported no increase in the fluidity of hepatic microsomes as assessed by the fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene (DPH), although aortic microsome fludity was enhanced. Similarly, Pugh et al. (1980) noted that a starve–refeed regimen which increased the hepatic microsomal desaturase activities failed to enhance the fluidity of these membranes as monitored by the fluorescence polarization of DPH, *cis*-parinaric acid, and *trans*-parinaric acid. In contrast to the lack of effect on the fluidity of hepatic microsomal fractions, the following results demonstrate that dietary induction of liver acyl desaturases increases significantly the lipid fludity and the acyl chain unsaturation of the hepatocyte plasma membranes. Further, the enhanced fludity is associated with increased activity of the plasma membrane $(Na^+ + K^+)$-ATPase.

Experimental Procedures

Animal Experiments. Albino male rats of the Sherman-Wistar strain weighing 225–300 g were used in all the experiments. Control animals were fed a standard chow diet

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[1] The term "lipid fluidity" as applied to anisotropic bilayer membranes is used in this paper to denote the relative motional freedom of the lipid molecules or substituents thereof. A more detailed description of the sense in which we use the term has been published (Schachter et al., 1982). Briefly, as evaluated by steady-state fluorescence polarization of lipid fluorophores, we assess "fluidity" via the parameters of the modified Perrin equation described under Experimental Procedures. An increase in fluidity corresponds to a decrease in either the correlation time, τ_c, or the hindered anisotropy, r_∞, of the fluorophore. Hence, the term combines the concepts of the dynamic and static (lipid order) components of fluidity.

[2] Abbreviations: DPH, 1,6-diphenyl-1,3,5-hexatriene; 2AS, DL-2-(9-anthroyloxy)stearate; 12AS, DL-12-(9-anthroyloxy)stearate; DPP, 1,3-di(1-pyrenyl)propane; FAME, fatty acid methyl ester; $(Na^+ + K^+)$-ATPase, $(Na^+ + K^+)$-dependent adenosinetriphosphatase; Tris, tris(hydroxymethyl)aminomethane; TBS, Tris-buffered saline; 16:0, palmitic acid; 16:1, palmitoleic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 20:4, arachidonic acid.

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Table I: Relative Specific Activities of Marker Enzymes in Hepatocyte Plasma Membranes

enzyme	marker	no.[a]	sp act. ratio[b] control diet	sp act. ratio[b] starve–refeed
5′-nucleotidase	plasma membrane	8	14.8 ± 2.8	14.7 ± 1.9
succinic acid dehydrogenase	mitochondria	7	0.4 ± 0.1	0.4 ± 0.1
glucose-6-phosphatase	microsomes	5	0.3 ± 0.2	0.4 ± 0.2

[a] Number of pairs of preparations tested. [b] Enzyme specific activity ratio of purified plasma membranes/homogenate. Values are means ± SE.

(NIH Rodent Diet, Agway, Inc., Syracuse, NY) and water ad libitum and fasted overnight prior to removal of the livers. To induce acyl desaturases, the starve–refeed regimen of Strittmatter et al. (1974) was employed and consisted of the following sequence: starve, 2 days; chow ad libitum, 2 days; starve, 2 days; refeed fat-free diet (Bio-Serve, Inc., Frenchtown, NJ; mix 0848K), 1 day. Groups of four control and four starve–refeed animals were killed by exsanguination on the morning following the last day of the regimen, and the livers were perfused in situ via the inferior vena cava to remove blood prior to excision.

Membrane Preparations. Suspensions of rat hepatocyte plasma membranes were prepared by a modification of the procedure of Nigam et al. (1971), as described previously (Livingstone & Schachter, 1980). The purity of the membrane suspensions and the degree of contamination with intracellular organelles were assessed by marker enzymes. As shown in Table I, the specific activity ratio [purified plasma membrane]/[crude homogenate] for 5′-nucleotidase (Morre, 1971), the plasma membrane marker, was approximately 14.7 in both the control and starve–refeed groups. The corresponding values listed for succinic dehydrogenase (Scottocasa et al., 1967) and glucose-6-phosphatase (Morre, 1971), marker enzymes for mitochondria and intracellular microsomal membranes, respectively, were 0.3–0.4 and essentially identical in the control and experimental groups. Plasma membranes were suspended in 0.15 M NaCl at a density of 1–2 mg/mL of membrane protein and stored frozen at −20 °C. Liver homogenates were also fractionated by the method of DeDuve et al. (1975) as modified by Amar-Costesec et al. (1974) to prepare nuclear, microsomal, and mitochondrial–lysosomal fractions, which were suspended in 0.15 M NaCl at densities of 2–3 mg/mL of membrane protein and stored frozen at −20 °C. Protein concentrations were determined by the method of Lowry et al. (1951) using bovine serum albumin as the standard.

Lipid Analyses. Total lipids were extracted from membrane preparations by the method of Folch et al. (1957). The cholesterol content was estimated by the method of Chiamori & Henry (1959) using cholesterol in 2-propanol as the standard. The total phospholipid content was quantified by estimating inorganic phosphate content after complete ashing of the lipid extracts (Chen et al., 1956; Ames & Dubin, 1960). The fatty acid composition was determined by gas chromatography. Fatty acid methyl esters (FAMEs) were prepared by using boron trifluoride (14% wt/vol, in methanol) as catalyst (Morrison & Smith, 1964), and the derivatives were analyzed in a JEOL JGX-20K gas chromatograph equipped with a flame-ionization detector and interfaced with a Hewlett-Packard 3390A integrator. The FAMEs were resolved on 6-ft glass column (o.d. 4 mm, i.d. 2 mm) packed with 10% Silar 10 C on 100/120 mesh GasChromeQ (Applied Science Laboratories, College Park, PA). The column temperature was 165 °C for 16 min followed by an increase of 2 °C/min to 195 °C. The injection temperature was 195 °C, and the carrier gas pressure was 1.8 kg/cm^2. Authentic FAME standards were purchased from Supelco, Inc. (Belefonte, PA) and sample peaks identified by retention times. Unidentified peaks comprised <2% of the total areas and were disregarded.

Fluorescence Polarization Studies. Lipid fluidity[1] was assessed by estimations of the steady-state fluorescence polarization of DPH, DL-2-(9-anthroyloxy)stearate (2AS), and DL-12-(9-anthroyloxy)stearate (12AS; Molecular Probes, Junction City, OR), as described previously (Schachter & Shinitzky, 1977; Livingstone & Schachter, 1980). The fluorescence anisotropy, r, was estimated at 24 °C by using membranes loaded with the appropriate fluorophore (Livingstone & Schachter, 1980) and suspended to a final concentration of 50–100 μg/mL membrane protein. The suspending solution, a Tris-buffered saline (TBS), consisted of 5 mM Tris buffer of pH 7.4 containing 146 mM NaCl and 4 mM KCl. Corrections for light scattering (membrane suspensions minus probe) and for fluorescence in the ambient medium (quantified by pelleting the membranes after each estimation) were made routinely, and the combined corrections were <5% of the total fluorescence intensity observed for DPH-loaded membranes and <10% of that observed for anthroyloxystearate-loaded suspensions. The results were interpreted according to the modified Perrin relationship (Heyn, 1979; Jähnig, 1979): $r = r_\infty + (r_0 - r_\infty)[\tau_c/(\tau_c + \tau_F)]$, where r_0 is the maximal limiting anisotropy, taken as 0.365 for DPH (Shinitzky & Barenholz, 1974) and 0.285 for the anthroyloxy probes (Schachter & Shinitzky, 1977), r_∞ is the limiting hindered anisotropy, τ_c is the correlation time, and τ_F is the mean lifetime of the excited state. The lifetime, τ_F, was estimated by phase fluorometry at 30 mHz (Spencer & Weber, 1969; Lakowicz et al., 1979) in an SLM 4800 subnanosecond polarization spectrophotometer (SLM-Aminco, Urbana, IL). Values of r_∞ for DPH were calculated from the fluorescence anisotropy, r, and from estimates of tan Δ obtained by differential polarized phase fluorometry as described by Lakowicz et al. (1979). The static component of membrane fluidity was assessed by an order parameter, S, where $S = (r_\infty/r_0)^{1/2}$ as described previously (Heyn, 1979; Jähnig, 1979). Fluorescence polarization results were also expressed as the anisotropy parameter, $[(r_0/r) - 1]^{-1}$ (Schachter & Shinitzky, 1977).

Intramolecular Excimer Fluorescence. The foregoing fluorescence polarization studies monitor rotational diffusion of lipid fluorophores as an index of lipid fluidity. The dynamic component of membrane fluidity was also assessed by estimates of the excimer fluorescence of an intramolecular excimer-forming fluorophore, 1,3-di(1-pyrenyl)propane (DPP), introduced by Zachariasse (1980) and applied to monitor short-range lateral diffusion of the pyrene substituents in erythrocyte membranes (Zachariasse et al., 1982). A solution of 0.15 mM DPP (Molecular Probes) in ethanol was prepared fresh, and 4 μL was added to 0.4 mL of TBS containing membranes equivalent to 400 μg of membrane protein and prewarmed to 37 °C. The mixture was stirred vigorously for 20 s and then incubated with shaking at 37 °C for 1 h. Three milliliters of deoxygenated TBS (TBS evacuated and flushed with Airco highly purified N_2 for 30 min) was added to each sample, and 2 mL of 20% (wt/vol) dextran 500 (Pharmacia) was layered under the membrane suspension to separate the suspension from any insoluble aggregates of DPP which can adhere to the walls of the tube in the course of the incubation. After

Table II: Effects of the Starve-Refeed Regimen on the Fatty Acid Composition of Hepatocyte Plasma Membranes[a]

component	control diet	starve-refeed	SE of difference[b]	P[b]
fatty acids (% by wt)				
16:0	30.4 ± 2.1	28.3 ± 2.8	1.8	ns[d]
16:1	8.3 ± 1.0	9.3 ± 1.6	0.7	ns
18:0	23.8 ± 2.1	19.1 ± 1.6	0.9	<0.005
18:1	21.2 ± 1.8	25.7 ± 1.3	1.6	<0.025
18:2	8.3 ± 1.1	7.7 ± 2.0	1.1	ns
20:4	6.9 ± 1.7	9.6 ± 2.0	0.9	<0.025
16:0/16:1 ratio	4.09 ± 0.70	3.60 ± 0.59	0.17	<0.025
18:0/18:1 ratio	1.14 ± 0.11	0.77 ± 0.10	0.04	<0.0005
saturated fatty acids (% of total)	54.2 ± 1.3	47.9 ± 2.0	1.0	<0.001
double-bond index[c]	0.74 ± 0.06	0.89 ± 0.09	0.05	<0.025

[a] Values are means ± SE for six pairs of membrane preparations. [b] SE of the differences and P values calculated by Student's t test of paired comparisons. [c] Double-bond index is the sum of the fraction of each fatty acid times the number of double bonds in that acid. [d] ns = not significant.

Table III: Effects of the Starve-Refeed Regimen on the Fluorescence Anisotropy Parameter of Various Fluorophores in Hepatocyte Plasma Membranes

probe	no.[a]	anisotropy parameter,[b] $[(r_0/r) - 1]^{-1}$ control diet	anisotropy parameter,[b] $[(r_0/r) - 1]^{-1}$ starve-refeed	SE of differences[c]	P[c]
DPH	6	2.29 ± 0.30	1.57 ± 0.19	0.18	<0.001
2AS	4	0.54 ± 0.10	0.40 ± 0.07	0.06	ns[d]
12AS	4	0.36 ± 0.05	0.29 ± 0.03	0.02	<0.025

[a] Number of membrane pairs. [b] Estimations were at 24 °C. Values are means ± SE. [c] Standard error differences and P values were calculated by Student's t test of paired comparisons. [d] ns = not significant.

the tubes were allowed to stand for 10 min, the upper phase containing the membranes was removed, and the membranes were pelleted and washed twice with 5 mL of deoxygenated TBS by centrifugation (40000g, 20 min, 20 °C). The washed pellet was suspended in 1.5 mL of deoxygenated TBS and allowed to stand at room temperature for at least 1 h, and fluorescence emission spectra were recorded at 25 °C with a Perkin-Elmer 650-40 fluorescence spectrophotometer by using an excitation wavelength of 345 nm. Excimer and monomer fluorescence peak intensities were estimated at 485 and 396 nm, respectively, and corrections were made for membranes carried through the procedure without DPP and for the probe added to TBS alone.

(Na^+ + K^+)-Dependent Adenosinetriphosphatase. Ouabain-inhibitable (Na^+ + K^+)-ATPase activity was estimated by quantifying the release of inorganic phosphate from adenosine triphosphate using the spectrophotometric assay of Siegel & Goodwin (1972) as described by Brasitus et al. (1980).

Results

Fatty Acid Composition of Plasma Membranes. To examine the effects of the starve–refeed regimen on the acyl chain composition of the hepatocyte plasma membrane lipids, livers were obtained from groups of control and experimental animals, the plasma membranes were isolated, and the FAME derivatives of the membrane lipids were prepared and analyzed as described previously. The results of six experiments summarized in Table II demonstrate that the starve–refeed regimen increased the double-bond index by approximately 20% ($P < 0.025$) and decreased the proportion of saturated fatty acids by approximately 12% ($P < 0.001$). Among the individual fatty acids, the major changes were increases in oleic acid (18:1; $P < 0.025$) and arachidonic acid (20:4; $P < 0.025$) and a decrease in stearic acid (18:0; $P < 0.005$). Accordingly, the ratio of 18:0/18:1 was decreased by 32% ($P < 0.001$). The corresponding ratio of 16:0/16:1 was also reduced by 12% ($P < 0.025$), although changes in the individual acids did not reach statistical significance.

Lipid Fluidity of Plasma Membranes. Hepatocyte plasma membranes isolated from groups of control and starve–refeed rats were treated with DPH, 2AS, or 12AS, and the results of fluorescence polarization studies are summarized in Table III. Corresponding to the increase in acyl chain unsaturation described above, the starve–refeed regimen increased the lipid fluidity of the membranes as indicated by a 31% reduction in the anisotropy parameter of DPH ($P < 0.001$) and a 20% decrease in that of 12AS ($P < 0.025$). A corresponding trend in the 2AS anisotropy parameter did not reach statistical significance. The values for 2AS exceeded those for 12AS, as reported previously (Livingstone & Schachter, 1980), probably because the former is localized closer to the aqueous surfaces of the membrane (Bashford et al., 1976; Cadenhead et al., 1977). Inasmuch as the chow-fed controls in these experiments were fasted overnight before excision of the livers, additional experiments were performed to determine the effects of such a fast on the DPH $[(r_0/r) - 1]^{-1}$ value of the isolated membranes. No significant effect of fasting was observed.

The excited-state lifetime (τ_F), the limiting hindered anisotropy (r_∞), and the order parameter (S) of DPH in control and starve–refeed hepatocyte plasma membranes were estimated, and the results are shown in Table IV. The dietary

Table IV: Fluorescence Parameters for Diphenylhexatriene in Hepatocyte Plasma Membranes of Control and Starve-Refeed Rats[a]

regimen	fluorescence lifetime, τ_F (ns) modulation	fluorescence lifetime, τ_F (ns) phase	limiting hindered anisotropy, r_∞	order parameter, S[b]
control diet	9.1 ± 0.4	5.8 ± 0.2	0.184 ± 0.003	0.69 ± 0.01
starve-refeed	8.8 ± 0.7	5.6 ± 0.2	0.132 ± 0.009[c]	0.58 ± 0.02[c]

[a] Estimations were at 24 °C. Values are means ± SE for three pairs of membrane preparations and six determinations. [b] Order parameter $S = (r_\infty/r_0)^{1/2}$. The r_∞ and S values were calculated from phase lifetime values. [c] $P < 0.005$ for the differences between control and starve-refeed groups.

Table V: Effects of the Starve-Refeed Regimen on the Fatty Acid Composition of Hepatocyte Microsomal Membranes[a]

component	control diet	starve-refeed	SE of difference[b]	P[b]
fatty acids (% by wt)				
16:0	27.3 ± 1.6	31.2 ± 1.3	1.6	<0.05
16:1	1.3 ± 0.4	7.8 ± 0.6	0.4	<0.0005
18:0	27.9 ± 3.3	14.5 ± 1.2	3.6	<0.025
18:1	13.6 ± 1.7	28.8 ± 1.6	0.6	<0.0005
18:2	10.8 ± 1.7	3.5 ± 0.5	1.4	<0.01
20:4	14.8 ± 3.7	10.2 ± 1.5	3.1	ns[d]
22:6	4.0 ± 0.3	3.4 ± 0.6	0.9	ns
16:0/16:1 ratio	27.4 ± 6.6	4.0 ± 0.2	7.4	<0.05
18:0/18:1 ratio	2.09 ± 0.21	0.51 ± 0.06	0.17	<0.005
saturated fatty acids (% of total)	55.2 ± 4.6	45.7 ± 2.0	4.1	ns
double-bond index[c]	1.14 ± 0.23	1.00 ± 0.12	0.17	ns

[a] Values are means ± SE for four pairs of membrane preparations. [b] SE of differences and P values calculated by Student's t test of paired comparisons. [c] Double-bond index is defined in Table II. [d] ns = not significant.

Table VI: Effects of the Starve-Refeed Regimen on the Fluorescence Anisotropy Parameter of Diphenylhexatriene in Hepatocyte Intracellular Organelles[a]

fraction	no.[b]	anisotropy parameter, $[(r_0/r) - 1]^{-1}$ control diet	anisotropy parameter, $[(r_0/r) - 1]^{-1}$ starve-refeed
microsomal	4	0.92 ± 0.05	0.88 ± 0.05[c]
mitochondrial-lysosomal	2	1.03, 1.00	0.99, 0.91
nuclear	2	1.02, 0.83	0.98, 0.96

[a] Estimations were at 24 °C. Values are means ± SE. [b] Number of membrane pairs tested. [c] For the paired t test, the standard error of the difference from the controls is 0.008 and $P < 0.01$.

Table VII: Effects of the Starve-Refeed Regimen on the Molar Ratio of Cholesterol/Phospholipid in Hepatocyte Membranes[a]

fraction	no.[b]	cholesterol/phospholipid molar ratio control diet	cholesterol/phospholipid molar ratio starve-refeed	SE of difference[c]	P[c]
plasma membranes	6	0.58 ± 0.07	0.44 ± 0.06	0.04	<0.01
microsomes	4	0.43 ± 0.07	0.37 ± 0.05	0.05	ns[d]

[a] Values are means ± SE. [b] Number of pairs of membranes tested. [c] SE of differences and P calculated by Student's t test of paired comparisons. [d] ns = not significant.

regimen did not affect the lifetimes but did lower r_∞ by 28% ($P < 0.005$) and S by 15% ($P < 0.005$), in accord with an increase in fluidity (van Blitterswijk et al., 1981).

Lastly, the effect of the starve–refeed regimen on the dynamic component of membrane fluidity was explored further by estimates of the excimer fluorescence of DPP. The values of the excimer/monomer fluorescence intensity ratios at 37 °C for the control and starve–refeed preparations, respectively, were 0.35 ± 0.02 and 0.44 ± 0.04 (mean ± SE; five determinations on three sets of membrane preparations; $P < 0.025$ by paired t test). The results confirm an increase in fluidity owing to the starve–refeed regimen.

Hepatocyte Microsomal Suspensions. To compare the foregoing effects of the starve–refeed regimen on purified plasma membranes with its effects on intracellular membranes, microsomal fractions were prepared, and the fatty acid composition was determined. As shown in Table V, the lipids of the starve–refeed microsomal suspensions contained increased 16:1 ($P < 0.001$) and 18:1 ($P < 0.001$) and decreased 16:0 and 18:0. Despite the resulting significant decreases in the ratios of 16:0/16:1 and 18:0/18:1, however, the overall double-bond index and the percentage of saturated fatty acids were not altered significantly, owing to offsetting, lower values for the polyenoic acids. Similar findings have been reported by others (Allman et al., 1965; Peluffo et al., 1976; Kates & Pugh, 1980), and the lack of net change in the double-bond index probably explains the small decrease of only 4% ($P < 0.005$) in the microsomal DPH anisotropy parameter shown in Table VI. The starve–refeed regimen, moreover, did not change significantly the DPH $[(r_0/r) - 1]^{-1}$ values of the nuclear or mitochondrial–lysosomal fractions (Table VI). Thus, the increase in lipid fluidity of the plasma membranes owing to the starve–refeed regimen cannot be ascribed to contamination of the suspension with intracellular organelles.

Cholesterol/Phospholipid Ratios. The effects of the starve–refeed regimen on the cholesterol/phospholipid molar ratios of the purified plasma membranes and the microsomal fractions were examined, in view of the important influence of the ratio on the fluidity of lipid bilayers (Ladbrooke & Chapman, 1969). As shown in Table VII, the dietary regimen lowered the cholesterol/phospholipid ratio of the plasma membranes by 24% ($P < 0.001$), with no significant effect on the microsomal suspensions. Thus, the increase in fluidity of the plasma membranes owing to the starve–refeed regimen is due to both an increased unsaturation of acyl chains and a decreased cholesterol/phospholipid ratio.

($Na^+ + K^+$)-Dependent Adenosinetriphosphatase. Inasmuch as prior studies indicate that ($Na^+ + K^+$)-ATPase activity is related to membrane fluidity in model liposomes (Kimelberg & Paphadjopoulos, 1974), human erythrocyte ghosts (Giraud et al., 1981), and rat hepatocyte plasma membranes (Davis et al., 1978; Keeffe et al., 1979), the effects of the starve–refeed regimen on the ouabain-inhibitable ($Na^+ + K^+$)-ATPase activity of isolated plasma membranes were examined. Values (mean ± SE) of the specific activity (nanomoles of phosphate per milligram of protein per minute) of the control and starve–refeed preparations, respectively, were 28.7 ± 3.5 and 48.3 ± 6.4 ($P < 0.025$; five control and four starve–refeed determinations).

Discussion

The foregoing results demonstrate that a starve–refeed regimen which stimulates hepatic fatty acyl and phospholipid desaturase activities (Oshino & Sato, 1972; Strittmatter et al., 1974; Pugh & Kates, 1977) alters the lipid composition and increases the fluidity of the hepatocyte plasma membrane. By contrast, the microsomal fraction of the liver homogenate, which contains much of the acyl desaturase activity and undergoes a substantial change in lipid composition owing to the

regimen (Table VI), exhibits almost no change in fluidity (Table VII), in accord with the observations of others (Holloway & Holloway, 1977; Kates & Pugh, 1980; Pugh et al., 1980). Further, no alterations in fluidity of the mitochondrial–lysosomal or nuclear fractions were observed (Table VI), indicating that the plasma membrane change is relatively specific and not due to contamination with intracellular organelles. The physiological significance of this increase in fluidity of the plasma membrane is unknown. It is reasonable to suspect, however, that it might facilitate the response of the hepatocyte to the lipid deprivation imposed by the dietary regimen.

The changes in lipid composition which underlie the increase in fluidity of the plasma membranes in contrast to the microsomal membranes are clarified by comparing the acyl chain compositions in Tables II (plasma membranes) and V (microsomes). In the plasma membranes, the starve–refeed regimen increased the content of monoenoic (largely 18:1) and polyenoic (arachidonic, 20:4) acids, with an increase in the double-bond index and significant reductions in the percentage of saturated fatty acids and the ratios of 16:0/16:1 and 18:0/18:1. Thus, net unsaturation per mole of fatty acid was increased and the lipid fluidity enhanced. In the microsomal membranes, by contrast, the dietary regimen did not alter the double-bond index or the percentage of saturated fatty acids (Table V). Although the content of monenoic acids (16:1 and 18:1) rose considerably, this change was offset by a reduction in polyenoic acids (largely 18:2), as reported previously (Allmann et al., 1965; Peluffo et al., 1976; Kates & Pugh, 1980).

The starve–refeed regimen also increased the content of 16:0 in the microsomal but not in the plasma membranes. This increment may result from the induction of the fatty acid synthetase complex (Flick et al., 1977; Jeffcoat, 1979). Indeed, the starve–refeed regimen is known to stimulate many enzymes required for lipid synthesis, including acetyl coenzyme A carboxylase (Allmann et al., 1965; Volpe & Vagelos, 1976), fatty acid synthetase (Gibson et al., 1966), Δ^9-acyl desaturases (Oshino & Sato, 1972), and phospholipid desaturases (Pugh & Kates, 1977). The widespread effects of the dietary regimen may account for the decrease in the cholesterol/phospholipid molar ratio of the plasma membranes (Table VII). It is conceivable that the utilization of precursor acetyl coenzyme A for the enhanced synthesis of fatty acids reduces the quantity available for cholesterol biosynthesis. Starvation, moreover, is known to decrease the activity of the hepatic hydroxymethylglutaryl coenzyme A reductase, the controlling enzyme of the cholesterol synthetic pathway (Bucher et al., 1960; Dietschy & Wilson, 1970). Irrespective of the precise explanation, however, the results demonstrate that the starve–refeed regimen enhances plasma membrane fluidity by both increasing acyl chain unsaturation and decreasing the cholesterol/phospholipid ratio.

The experimental results provide three kinds of evidence to demonstrate the increase in plasma membrane fluidity owing to the starve–refeed regimen. First, the static component of membrane fluidity, i.e., the degree of order of the lipids, was examined by estimating r_∞ and the order parameter S for DPH (Heyn, 1979; Jähnig, 1979). The values in Table IV indicate a decrease in lipid order due to the regimen. Second, the dynamic component of membrane fluidity was assessed by estimates of the anisotropy parameter of 12AS (Table III). In parallel studies (Storch & Schachter, 1983), we have demonstrated that the fluorescence lifetime of 12AS is not affected by the dietary regimen. Further, since r_∞ values for 12AS are relatively small (Schachter et al., 1982; Vincent et al., 1982), the decrease in the anisotropy parameter (Table III) implies a corresponding reduction in the correlation time and hence an increase in fluidity. Lastly, the excimer/monomer fluorescence intensity ratio of DPP was used to assess short-range lateral diffusion in the plasma membrane. The increase in this ratio owing to the dietary regimen signifies enhanced fluidity, in agreement with the preceding methods.

The increase in lipid fluidity of the plasma membranes owing to the starve–refeed regimen was accompanied by an increase of 68% in the specific activity of the $(Na^+ + K^+)$-ATPase. This membrane enzyme was studied because of its functional importance and well-documented lipid dependence in a variety of membranes (Tanaka & Abood, 1969; Kimelberg & Papahadjopoulos, 1974; Wheeler & Walker, 1975; Sinensky et al., 1979; Giraud et al., 1981; Abeywardena et al., 1983; Yeagle, 1983), including rat hepatocyte plasma membranes (Keeffe et al., 1979, 1980). Although the present results are in accord with the observations of Keeffe et al. (1979, 1980) that the $(Na^+ + K^+)$-ATPase activity varies concordantly with the membrane fluidity, it bears emphasis that the number of enzyme units was not monitored in these hepatocyte membrane studies. Hence, the effects of a particular procedure, such as the starve–refeed regimen, could result from changes in the number of enzyme units as well as from alterations in the function of each unit. Regardless of the mechanism, the possibility of modulating the activity of this important enzyme by dietary means in vivo deserves emphasis.

In view of prior studies, which indicate that the modulation of cholesterol biosynthesis in the rat enterocyte in vivo can alter the composition and fluidity of the intestinal microvillus membrane (Brasitus & Schachter, 1982), and of the present experiments, which demonstrate diet-induced alterations in the hepatocyte plasma membrane, it is reasonable to conclude that the lipid fluidity in vivo of these plasma membranes is not fixed but is regulated to meet functional needs.

Registry No. Acyl desaturase, 9014-34-0; cholesterol, 57-88-5; ATPase, 9000-83-3.

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