ABSTRACT: Freshly isolated rat hepatocytes were incubated for 20 min with [U-14C]glycerol in the presence or absence of unlabeled linoleic (18:2n-6), arachidonic (20:4n-6), or docosahexaenoic (22:6n-3) acid, added as albumin complex in 10% ethanol. Most of the radioactivity (~95%) recovered in hepatocyte lipids was present in phosphatidylcholine (PC), phosphatidylethanolamine (PE), and triacylglycerol (TAG). The presence of exogenous fatty acids resulted in (i) higher incorporation of [U-14C]glycerol, (ii) higher percentage of label in TAG, and (iii) enhanced formation of PC and PE molecular species bearing the exogenous fatty acid at both the sn-1 and sn-2 positions of glycerol. In each case, these molecular species contained 60 to 70% of the label in that lipid class. Further incubation of the cells for 40 and 80 min in the absence of labeled substrate and exogenous fatty acids resulted in a redistribution of label among PC and PE molecular species due to deacylation–reacylation at the sn-1 position of glycerol. 


The glycerophospholipids of mammalian cells, including rat hepatocytes, consist of specific molecular species which differ from one another in their relative amounts, physical properties, and half-life times. They most likely fulfill certain structural requirements as membrane components and serve as resources for the generation of signaling molecules (for reviews see Refs. 1–4). All cellular glycerophospholipids are continually synthesized, remodeled, transported between organelles and membranes, and degraded. The regulation of these processes is of particular importance for the major membrane lipids, phosphatidylcholine (PC) and phosphatidylethanolamine (PE) which, in rat hepatocytes, constitute over 75% of the cell’s glycerophospholipids (5). It has long been known that individual molecular species of rat hepatocyte PC and PE have drastically different half-life times ranging from 1 to 20 h (6,7). Based on both stable isotope and radioisotope-labeling experiments, we have proposed that the molecular species of both PC and PE are generated by continuous de novo synthesis and remodeling by deacylation–reacylation at both the sn-1 and sn-2 positions of glycerol.

Much evidence exists that phospholipid synthesis and turnover in hepatocytes are highly compartmentalized and that interorganelle movement (8,9) as well as lipoprotein assembly and secretion involve newly synthesized phospholipids (10–13). It is therefore logical to assume that remodeling by deacylation–reacylation also involves newly synthesized molecular species of PC and PE (14,15).

Based on acyl turnover measurements by 18O incorporation from H218O-containing media, we have proposed that de novo synthesis and remodeling of rat hepatocyte PC and PE are quantitatively similar and linked temporally and spatially. In this scheme, de novo synthesis via the Kennedy pathway (16) would result in the formation of primarily four molecular species, and the major remodeling step would be the introduction of stearic acid (18:0) and arachidonic acid (20:4) at the sn-1 and sn-2 positions, respectively, of newly formed PC and PE (5). This hypothesis is in agreement with a great variety of early data on the labeling patterns of PC and PE in liver cells and subcellular preparations (reviewed in Refs. 1,2).

Although stearic and arachidonic acids are primarily introduced into PC and PE by the remodeling pathway, small amounts of 1,2-diarachidonoyl PC exist in many cells (17–19), including rat hepatocytes where they appear to be synthesized de novo via glycerophosphate (15). If these and other highly unsaturated molecular species are continually synthesized but remain at low levels, they must be either continually degraded or remodeled into other species. Furthermore, if cells are exposed to excess fatty acids they would be expected to be taken up into glycerolipids by the de novo pathway owing to the finite amounts of precursor species available for remodeling. Indeed, we have shown that excess fatty acids, including arachidonic acid, are incorporated into PC primarily by the de novo pathway resulting in the initial formation of molecular species having the exogenous fatty acid esterified at both the sn-1 and sn-2 positions. In the case of 20:4-20:4 PC, rapid remodeling occurred at the sn-1 position resulting in the formation of the predominant molecular species 18:0-20:4 PC (15).

The purpose of the present study was to determine if other polyunsaturated fatty acids, when present in excess, are in-
corporated into glycerolipids by analogous pathways and whether newly synthesized polyunsaturated molecular species of PE are remodeled at the sn-1 position as previously shown for PC (15).

**MATERIALS AND METHODS**

**Materials.** Male Sprague-Dawley rats, 200–350 g, were obtained from Sasco, Inc. (Omaha, NE). Collagenase Type IV from *Clostridium histolyticum*, phospholipase C from *Bacillus cereus*, 4-dimethylaminopyridine, 3,5-dinitrobenzoyl chloride, and dinitrophenol PC were from Sigma (St. Louis, MO). Dianachidonoyl PC was from Serdary Research Labs (Englewood Cliffs, NJ). [U-14C]glycerol (130 Ci/mol) was from American Radiolabeled Chemicals, Inc. (St. Louis, MO); unlabeled fatty acids and tridocosahexaenoylglycerol were from Nu-Chek-Prep (Elysian, MN). 1,2-Diadosohexaenoylglycerol was prepared by the Grignard procedure (20,21). Briefly, 10 mg tridocosahexaenoylglycerol was dissolved in 1.5 mL diethyl ether. Fifty µL of ethyl magnesium bromide (3 M solution in diethyl ether; Aldrich Chemical Co., Milwaukee, WI) was added. After 30 s at room temperature, the reaction was terminated with 50 µL of glacial acetic acid followed by 1 mL of water. 1,2-Diacylglycerols (DAG) in the organic phase were purified by thin-layer chromatography (TLC) on silica gel H, developed in hexane/diethyl ether/glacial acetic acid, (50:50:1, by vol).

**Preparation of cells.** Hepatocytes were prepared by collagenase perfusion of rat liver according to Krebs et al. (22) as described previously (5,14,15). Cells were counted in a hemacytometer after mixing with trypan blue in order to assess viability, which was better than 87% at the beginning and 77–81% at the end of the experiments.

**Incubations.** Fatty acids were dissolved in 95% ethanol, and 0.1 mL of this solution was added to 0.9 mL of incubation buffer (Kreb’s-Ringer Hepes containing 10 mM glucose and 0.4% fatty acid-free bovine serum albumin). The mixtures were vortexed and sonicated to obtain a homogeneous suspension. Of these suspensions, 50-µL aliquots were added for each milliliter of incubation buffer. Hepatocytes were incubated at a concentration of 4 × 10^6 cells/mL of incubation buffer which also contained 1 µCi (7.7 nmol) [U-14C]glycerol/mL and 300 nmol fatty acid/mL. Control incubations contained ethanol only. For each regimen, 2.4 × 10^7 cells (6 mL) were incubated for 20 min in a 70-mm plastic culture dish, swirled gently in an atmosphere of O2/CO2, 95:5, at 37°C. The cells were then washed and resuspended in 6 mL of fresh incubation buffer. Two 1-mL aliquots were removed and extracted immediately; and four 1-mL aliquots were transferred to 30-mm plastic culture dishes and incubated further for 40 and 80 min in the absence of fatty acid and [U-14C]glycerol. Duplicate samples were extracted and analyzed separately. All primary data represent average values from duplicate incubations.

**Lipid analysis.** Cells were harvested by centrifugation and extracted with chloroform/methanol (2:1, vol/vol) and partitioned against 0.9% aqueous NaCl (23). The lipid extracts were taken to dryness and redissolved in 1 mL of chloroform/methanol, 2:1, containing 0.01% butylated hydroxytoluene. Aliquots of the lipid extracts were fractionated by TLC on Whatman LK5 plates (Whatman, Inc., Clifton, NJ), using chloroform/methanol/ammonium hydroxide/water (65:35:5:1, by vol) as developing solvent. Distribution of radioactivity in lipid classes was determined by scanning the plate with a Berthold LB2842 TLC Linear Analyzer (Berthold Analytical Instruments, Inc., Nashua, NH). Total phospholipids and individual phospholipid classes were quantified by phosphorus assay (24).

Both PC and PE were isolated by preparative TLC on layers of Silica Gel H (Merck, EM Science, Gibbstown, NJ) using the same solvent system. They were checked for purity, hydrolyzed to DAG, and converted to 3,5-dinitrobenzoates (DNB) as described (5,14,15). DAG–DNB were separated on a 3.9 × 300 mm Waters Nova Pak C18 (Millipore Corp., Milford, MA) column, using a Beckman high-performance liquid chromatography (HPLC) system consisting of two 114 M solvent delivery modules, a Model 406 Analog Interface Module, a Model 168 Diode Array Detector, and System Gold software (Beckman Instruments, Inc., Fullerton, CA). The absorbance at 254 nm was monitored on the computer screen and the fraction collector (Frac-100, Pharmacia Biotech, Inc., Piscataway, NJ) was advanced manually at the beginning of each peak.

Separation and analysis of molecular species as DAG–DNB were done as previously described (5,14,15). Briefly, one-half of each sample was separated by reverse-phase HPLC in a solvent system of methanol/isopropanol/glacial acetic acid (80:20:0.01, by vol), and the peaks were collected in 7-mL scintillation vials. After evaporation of the solvent, 3 mL of Ecol-ume scintillation fluid (ICN Pharmaceuticals, Costa Mesa, CA) was added, and radioactivity was determined in a Beckman LS7500 scintillation counter. Radioactivity in each fraction was expressed as a percentage of radioactivity recovered in all fractions. The second half of each sample was separated in the same manner, and the peaks were collected for subsequent separation of coeluting species in a solvent of acetonitrile/isopropanol (70:30, vol/vol). Mol% of each molecular species was determined by integration of the HPLC peaks obtained with the two solvent systems. Data from the first and second separations were combined to give radioactivity percentage and mol% of all major molecular species of PC and PE.

**RESULTS**

**Incorporation of [U-14C]glycerol into hepatocyte glycerolipids in the presence and absence of exogenous fatty acids.** When primary isolates of rat hepatocytes were incubated for 20 min with [U-14C]glycerol in the presence or absence of unsaturated fatty acids, most of the radioactivity (~95%) was recovered in PC, PE, and triacylglycerol (TAG) (Table 1). Further incubation for 40 and 80 min after removal of labeled substrate led to increased levels of radioactivity in...