Selectivity in Incorporation, Utilization and Retention of Oleic and Linoleic Acids by Human Skin Fibroblasts[1]

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ABSTRACT

Fetal human fibroblasts were grown in culture medium containing 10% fetal bovine serum supplemented with [1-^{14}C] linoleate or [1-^{14}C] oleate. At all concentrations of exogenous fatty acids, the incorporation of oleate was greater than that of linoleate. With increased medium fatty acid concentrations, linoleate in triacylglycerol (TAG) could be increased from 13 to 75% of the total incorporated; at each concentration, relatively more linoleate than oleate was in TAG. When the cells were exposed to exogenous oleate/linoleate mixtures, the composition of the mixture determined the extent of incorporation of both fatty acids. When the mixture was primarily linoleate, scarce oleate was used preferentially for phospholipids (PL); no such specificity for scarce linoleate was observed. Addition of exogenous fatty acids resulted in a shift of previously incorporated ^{14}C fatty acids from phospholipid into TAG; retention of oleate in PL was greater than that of linoleate. Incorporation of oleate into phospholipids was also higher than that of linoleate from exogenous fatty acid mixtures which were 80% saturated. It is suggested that normal human fibroblasts have adapted to the low levels of exogenous polyunsaturated fatty acids in culture media by increased use of oleate in phospholipid. Even when the cells are supplemented with linoleate, the preferential use of oleate in phospholipid groups is retained.

INTRODUCTION

Mammalian cells in culture normally obtain much of their fatty acids from serum in the growth medium (1,2), and their fatty acid composition reflects that of the medium (3). Fetal bovine serum, used in culture media for many serum-requiring cell lines, is very low in essential fatty acids (4). Cells in culture exhibit lower polyunsaturated fatty acid (PUFA) levels, principally linoleate plus arachidonate, and higher levels of oleate than do corresponding tissues in vivo (5). Growth of cells in medium without lipids results in a further decrease in PUFA and an increase in oleate (6).

Human skin fibroblasts readily incorporate exogenous fatty acids into cellular lipids (7,8), and exchange previously incorporated fatty acids with those in the culture medium (7). Like transformed cell lines, their fatty acid composition can be modified extensively by medium supplementation with fatty acids (9). Furthermore, these cells respond to increased medium concentrations of free fatty acids by synthesizing triacylglycerol droplets (10), thus providing an alternative metabolic fate for incorporated fatty acids.

This study examines the specificity of linoleate and oleate use by GM-10 fibroblasts grown in medium containing fetal bovine serum. It was hypothesized that these cells might exhibit preferential incorporation of linoleic acid, and that, especially when linoleic acid was relatively scarce, it might be incorporated into phospholipid rather than triacylglycerol. Our results indicate, however, that under a wide variety of experimental conditions, there is a small, but consistently significant preference for oleate rather than linoleate in phospholipid synthesis.

MATERIALS AND METHODS

Cell Culture and Fatty Acid Supplementation

GM-10 cells, human diploid fibroblasts derived from skin of a 3-month fetus (General Mutant Cell Repository, Camden, NJ), were propagated in Eagle's Minimum Essential Medium, supplemented with 10% noninactivated fetal bovine serum (both from Grand Island Biological Co., Grand Island, NY) at 37 C in a humidified, 5% CO_2 atmosphere. Replicate 25-cm^2 flasks were seeded with 3-4 × 10^5 cells in 4.0 ml medium; all experiments used cells between 15 and 30 generations in culture. Medium supplementation with fatty acids was done 24 hr after subculture, when the cells were actively mitotic (7).

[1-^{14}C] Linoleic acid (51.0 mCi/mmol) and [1-^{14}C] oleic acid (48.0 mCi/mmol) were obtained from New England Nuclear Corp., Boston, MA. Radiolabeled fatty acids were greater than 99% free fatty acid as determined by thin layer chromatography (TLC). Upon methylation with Boron trichloride/methanol, gas liquid chromatography (GLC) and ^{14}C detection using a Packard 804 Gas Flow Proportional Counter, the [^{14}C] oleate and [^{14}C]-

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linoleate were 94-97% pure; no labeled impurity represented more than 1.0% of the total radioactivity. Linoleic acid (Grade III) and oleic acid (Sigma grade) from Sigma (St. Louis, MO) were greater than 98% pure by GLC. Fatty acid solutions were stored in hexane under N_2 at -20 C; concentrations were confirmed by titration (11). For each experiment, aliquots of fatty acid and [^{14}C] fatty acid solutions were evaporated to dryness under N_2 and redissolved in dilute NaOH. The resulting sodium salts were filter sterilized and added to serum before dilution with culture medium. Alternatively, solutions of fatty acids in 95% ethanol were added directly to the serum; the final ethanol concentration in the culture medium was 0.2%.

Delipidized fetal bovine serum, prepared by acetone/ethanol (1:1, v/v) extraction according to Rothblat et al. (12), was used to replace fetal bovine serum in some experiments; extraction was 93% effective as determined by GLC. Protein standardization of the solvent extracted serum was done according to Lowry et al. (13). When 1.3 μg/ml α-tocopherol (phosphoric acid ester, disodium salt, ICN, Cleveland, OH) was added to the cultures, it was solubilized in pluronic F-68 (BASF Wyandotte Corp., Wyandotte, MI), with a final detergent concentration of 0.025% (14).

Lipid Extraction

After growth with supplemented fatty acids, usually for 24 hr, the cells were washed twice with calcium- and magnesium-free Earle's Basic Salt Solution and detached from the surface with 1.5 ml/flask of 0.05% trypsin in Earle's Salts (GIBCO). Each cell suspension in trypsin was transferred to a 40-ml conical tube containing 4.0 ml acetone plus 2 μg α-tocopherol; 2.5 ml of 0.025% methyl cellulose in 0.9% NaCl was used to rinse each flask. Cellular lipids were then extracted directly in a mixture of ethyl acetate/acetone/cell suspension (2:1:1 v/v) (15). Medium samples were extracted in 6 vol of ethyl acetate/acetone (2:1). Each sample was mixed, heated to 85 C, vortexed vigorously and incubated at 65 C for 40 min with a 1 min vortexing at 20 min. The solvent mixture was then centrifuged at 2000 rpm at 4 C for 10 min, and the organic phase removed and stored at -20 C under N_2 for subsequent analysis.

Thin Layer Chromatography and Scintillation Counting

Aliquots of the lipid extracts were redissolved in small volumes of chloroform/methanol (1:1, v/v) and applied to Silica Gel H coated thin layer chromatographic plates (Applied Science, State College, PA). The plates were developed by ascending chromatography in petroleum ether/diethyl ether/acetic acid (82:18:1, v/v) for separation of neutral lipid classes. After removal of phospholipids at the origin, the neutral lipid spots were visualized with iodine. Duplicate plates were developed in chloroform/methanol/acetic acid/water (50:30:8:4, v/v) for separation of phospholipids. Appropriate reference mixtures were cochromatographed with the samples.

Aqueous samples were assayed for radioactivity in 1.0 ml ACS (Amersham, Arlington Heights, IL). Samples of lipid extracts and neutral lipid spots were dissolved in 15 ml of 0.4% Omnifluor (New England Nuclear) in toluene/ethanol (2:1, v/v). Phospholipid spots were redissolved in 1.0 ml water to which ACS scintillation fluid was then added; radioactivity measurements were done with a Beckman LS-250 liquid scintillation counter.

RESULTS

Incorporation of [1-^{14}C] Linoleate and [1-^{14}C] Oleate

GM-10 fibroblasts in monolayer culture readily take up both [1-^{14}C] linoleate and [1-^{14}C] oleate from medium containing fetal bovine serum. Table I shows that, in a representative experiment, 62.5% of the [1-^{14}C]-oleate and 50.8% of the [1-^{14}C] linoleate were incorporated in 24 hr. In this system, incorporation of exogenous fatty acids is linear with time for at least 6 hr and continues to increase for over 48 hr; the apparently diminished incorporation rate after 6 hr results from turnover and release of incorporated acyl groups (10). Incorporation varied somewhat with cell density, cell generations in culture and the lot of fetal bovine serum. Uptake of 0.5-1.0 μCi/flask [1-^{14}C] linoleate was lower than that of [1-^{14}C] oleate in each of 13 independent experiments with 3 different lots of fetal bovine serum, with a significance of $p < 0.001$ (paired t-test).

The lipid extracts contained 95% of the incorporated [1-^{14}C] linoleate or [1-^{14}C] oleate; 96% of the [^{14}C] lipids were phospholipids and triacylglycerols. In the experiment shown in Table I, 21% of the [^{14}C] lipid was triacylglycerol for linoleate-fed cells, but only 14% for oleate-fed cells; the localization of relatively more of the incorporated [1-^{14}C] linoleate in triacylglycerol was significant at $p < 0.001$ for the series of 13 experiments. Neither addition to the medium of the fatty acids in ethanol rather than as aqueous soaps, nor the presence

TABLE I

Incorporation of Low Levels of [1-^{14}C] Linoleate and [1-^{14}C] Oleate by GM-10 Fetal Human Fibroblasts[a]

Fatty acid added	[1-^{14}C] Linoleate			[1-^{14}C] Oleate		
	10^{-3} X dpm/flask	%	nmol/flask[c]	10^{-3} X dpm/flask	%	nmol/flask[c]
Total incorporation	1,042.3 ± 19.1	50.8	13.5	1,283.1 ± 25.4	62.5[b]	27.3
Distribution of ^{14}C in cells						
Lipids	985.8 ± 11.2	94.6	12.8	1,221.1 ± 4.3	95.2	26.0
Aqueous phase and pellet	56.0 ± 1.3	5.4	0.7	61.9 ± 1.6	4.8	1.3
Distribution of ^{14}C in lipids						
Phospholipid	738.7 ± 8.6	74.9	9.6	1,011.2 ± 11.9	82.8[b]	21.5
Diacylglycerol	13.4 ± 0.3	1.4	0.18	12.4 ± 0.5	1.0	0.26
Free fatty acid	8.6 ± 0.1	0.9	0.12	11.7 ± 0.3	1.0	0.26
Triacylglycerol	208.1 ± 3.5	21.1	2.70	172.2 ± 3.2	14.1[b]	3.67
Cholesterol esters	17.8 ± 0.2	1.8	0.23	13.6 ± 0.3	1.1[b]	0.29
Distribution of ^{14}C in phospholipids						
Lysophosphatidylcholine	4.1 ± 0.1	0.6		5.0 ± 0.2	0.5	
Sphingomyelin	16.3 ± 0.7	2.2		19.9 ± 0.6	2.0	
Phosphatidylcholine (PC)	520.8 ± 4.7	70.5		743.4 ± 2.2	73.5[b]	
Phosphatidylserine plus phosphatidylinositol	80.6 ± 1.9	11.0		99.1 ± 0.4	9.8[b]	
Phosphatidylethanolamine (PE)	52.0 ± 0.6	7.0		91.9 ± 1.2	9.1[b]	
Acidic phospholipids	65.2 ± 1.1	8.8		52.4 ± 0.3	5.2[b]	
PC/PE	10.0 ± 0.2			8.1 ± 0.2[b]		

[a]GM-10 fibroblasts were seeded at 3 X 10^5 cells/25-cm^2 flask. After 24 hr, the culture medium was replaced with 4.0 ml Eagle's Minimal Essential Medium supplemented with 10% fetal bovine serum plus either 1.0 μCi [1-14C]oleate (48.0 mCi/mmol) or 1.06 μCi [1-^{14}C]linoleate (51.0 mCi/mmol). Incubation time with radiolabeled fatty acids was 24 hr. Data are means ± SE of determinations on 3 replicate flasks.

[b]Difference between values for [1-^{14}C]oleate and [1-^{14}C]linoleate was significant at $p < 0.05$ in this representative experiment. In paired comparisons of 13 independent experiments, the differences between the 2 [^{14}C]fatty acids were significant at $p < 0.001$ for total incorporation and neutral lipid distribution. Differences in [^{14}C]fatty acid distribution between phospholipid subclasses were significant at $p < 0.01$ in paired comparisons of 4 experiments.

[c]Calculation of total uptake of free fatty acid based on 22.8 nmol oleate and 5.7 nmol linoleate/4 ml medium contributed by the fetal bovine serum. The [^{14}C]-fatty acids were added at 20.8 nmol/flask; total concentrations of [1-^{14}C]linoleate and [1-^{14}C]oleate would thus be 26.5 and 43.6 nmol/flask, respectively.

of α-tocopherol in the medium, significantly altered the results just described. The net effect of both the lower incorporation rate of [1-^{14}C]-linoleate than [1-^{14}C] oleate and the higher percentage of incorporated linoleate in triacylglycerol was that the absolute amount of supplemented linoleate incorporated into phospholipids was less than 75% that of supplemented oleate.

Fetal bovine serum contains 210-280 nmol/ml free fatty acid. The lot used for most of these experiments contributed 22.8 nmol/4 ml oleate and 5.7 nmol/4 ml linoleate when present at 10% in the culture medium. The 20.8 nmol of [1-^{14}C] oleate was thus diluted by the corresponding serum free fatty acid to a greater extent than was the 20.8 nmol [1-^{14}C]-linoleate. On a total mass basis, the greater uptake of oleate than linoleate and the more extensive use of oleate in phospholipids were even more pronounced than indicated by percentage incorporation.

Minimal Fatty Acid Supplementation

To determine if the addition of fatty acids to the medium was responsible for the observed synthesis of triacylglycerol, [1-^{14}C] oleate and [1-^{14}C] linoleate were each added to a series of flasks in decreasing amounts, so that they contributed from 10.0 to 0.9 nmol/ml fatty acid to the culture medium. The results (Fig. 1) indicated that the differences between oleate and linoleate incorporation remained with decreased concentrations of medium supplementation, and, by extrapolation, should occur with a zero level of fatty acid supplementation. Thus, at this density, GM-10 cells would incorporate 57% of the serum oleate (13.0 nmol/flask) and 50% of the serum linoleate (2.85 nmol/flask) in 24 hr.

Figure 1B shows that with decreased exogenous [^{14}C] fatty acid concentrations, the extent of use of incorporated fatty acids in triacylglycerol decreased. The percentage of incorporated [1-^{14}C] linoleate in triacylglycerol remained higher than that of [1-^{14}C] oleate. By extrapolation, 13% of the linoleate and 9% of the oleate incorporated from medium with 10% fetal bovine serum alone would be in triacylglycerol. On a mass basis, however, oleate incorporation into triacylglycerol (0.78 nmol/flask) would be greater than that of linoleate (0.37 nmol/flask). Apparently, synthesis of triacylglycerol from exogenous fatty acids is a normal occurrence for GM-10 fibroblasts grown in medium with fetal bovine serum. Furthermore, both the relatively greater incorporation of oleate than linoleate, and the use of

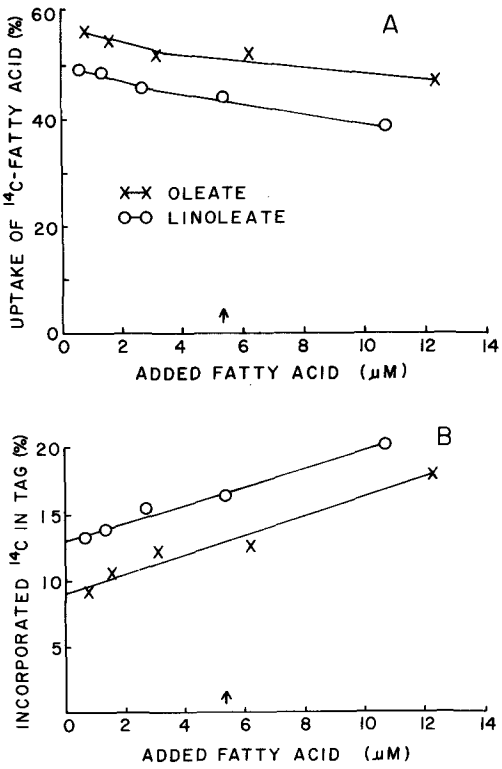

FIG. 1. Effect of minimal medium supplementation with fatty acids on incorporation of [1-^{14}C]-linoleate and [1-^{14}C] oleate. GM-10 fibroblasts were seeded at 2.5 X 10^5 cells/flask. Replicate flasks were incubated for 24 hr with 0.14-2.5 μCi [1-^{14}C] linoleate or [1-^{14}C] oleate as described in Table I. A: Uptake of [^{14}C] fatty acid in triacylglycerol (TAG). **O–O**, [1-^{14}C] linoleate; **X–X** [1-^{14}C] oleate. The arrows indicate the added [^{14}C] fatty acid concentration used in Table I.

a higher percentage of incorporated linoleate in triacylglycerol, suggest that despite the low concentration of linoleate in fetal bovine serum, there is a lack of preferential use of serum linoleate in phospholipids.

We also examined incorporation of 2.7 μM fatty acid from 4.0 ml of culture medium in which the fetal bovine serum was replaced with delipidized serum protein. Incorporation of [^{14}C] oleate was 9.19 nmol/flask (mean of 3 independent experiments); that of linoleate was 9.05 nmol/flask. Of the incorporated fatty acids, 8.72 nmol oleate and 8.40 nmol linoleate were in phospholipid, whereas 0.25 nmol oleate and 0.29 nmol linoleate were in triacylglycerol. Even with fatty acid concentrations below those of serum-supplemented media, there was greater use of oleate than linoleate in cellular phospholipids.

Effect of Saturated Fatty Acids on Incorporation of Oleate and Linoleate

Phospholipids in vivo commonly contain one saturated and one polyunsaturated fatty acid. To determine if the incorporation of linoleate into phospholipids by human fibroblasts would be enhanced by saturated fatty acids, replicate flasks were incubated with mixtures of oleate and linoleate plus either palmitate or stearate in medium containing delipidized fetal bovine serum; each fatty acid was ^{14}C-labeled in turn. As shown in Table II, the exogenous fatty acids were not incorporated into cellular lipids in quite the same proportions as supplied in the medium. For example, with increased saturation of the exogenous mixture, the percentage of oleate uptake increased and that of palmitate decreased. In all cases, however, the extent of linoleate incorporation was less than that of oleate. With 50% saturated fatty acids, the percentage use of linoleate was less than oleate at a ratio of exogenous oleate-to-linoleate of either 1:4 or 4:1. The more extensive uptake of oleate than linoleate was reflected directly in a greater incorporation, on a mass basis, of oleate into phospholipid when the two were equimolar in the medium. Thus, even when there is extensive use of saturated fatty acids, oleate is the preferred unsaturated fatty acid for phospholipid synthesis in these cells.

Saturated fatty acids promote less triacylglycerol synthesis than do unsaturated ones (M.D. Rosenthal, unpublished data). Although there was little accumulation of neutral lipids, small amounts of all [^{14}C]fatty acids were located in triacylglycerol. Incorporation of fatty acids on a mass basis indicated more oleate than linoleate in triacylglycerol from equimolar exogenous mixtures (data not shown). Expressed as a percentage of incorporated fatty acid in TAG, however, the two were similar at ca. 4% in 80% palmitate and 10% in 80% stearate mixtures.

Increased Medium Fatty Acid Concentrations

Higher exogenous free fatty acids concentrations resulted in increased incorporation of both oleate and linoleate; incorporation on a percentage basis decreased less than 2-fold with the 80-fold increase in concentration (Table III). Oleate incorporation was higher than that of linoleate, both in terms of the supplemented fatty acid alone (percentage basis) and total nmol/flask, including the contribution of serum free fatty acids. With increased oleate or linoleate accumulation, discrete triacylglycerol droplets became appar-

TABLE II

Incorporation from Mixtures of Saturated and Unsaturated Fatty Acids

	Exogenous fatty acids (nmol/flask)			Incorporation into lipid[b] (nmol/flask [%])			Incorporation into phospholipid		
	16:0	18:1	18:2	16:0	18:1	18:2	16:0	18:1	18:2
Expt. I									
	70.0	8.8	8.8	27.2 (38.8)	3.65 (41.5)	2.81 (31.9)	22.2	3.44	2.60
	43.8	35.2	8.8	20.1 (46.0)	12.0 (34.1)	2.44 (27.7)	17.9	10.4	2.12
	43.8	8.8	35.2	20.5 (46.8)	3.19 (36.3)	8.62 (24.5)	18.2	2.83	7.18
	18:0	18:1	18:2	18:0	18:1	18:2	18:0	18:1	18:2
Expt. II									
	70.0	8.8	8.8	24.2 (34.6)	3.77 (42.8)	2.93 (33.3)	19.0	3.24	2.54

[a]Log phase GM-10 cells were washed 2X with EBSS and incubated for 24 hr with 5.0 ml MEM with 5% delipidized serum protein in EBSS, plus 17.6 μM fatty acid mixture, composition as indicated. Each fatty acid was radiolabeled in one set of flasks. Abbreviations: 16:0, palmitate; 18:0, stearate; 18:1 oleate; 18:2, linoleate.

[b]Incorporation of each [^{14}C] fatty acid expressed as nmol/flask, and as a percentage of that supplied in the medium. Values are means, n = 3.

TABLE III

The Effect of Increased Medium Free Fatty Acid Concentrations on Incorporation of [1-^{14}C]Oleate and [1-^{14}C]Linoleate[a]

Fatty acid	Concentration		Total incorporation		Triacylglycerol		Phospholipid	
	Supplemented (nmol/flask)	Total[b] (nmol/flask)	(nmol/flask [%])		(% of incorp.)	(nmol/flask)	(% of incorp.)	(nmol/flask)
[1-^{14}C]Oleate	9.7	32.5	56.8	18.5	15.2	2.8	80.4	14.9
	44.4	67.2	53.1	36	21.8	7.8	74.2	26.7
	79.2	102	52.0	53	28.4	15	65.3	35
	149	172	47.9	82	40.0	33	55.1	45
	288	311	41.2	128	52.3	67	43.0	55
	565	588	38.7	228	62.6	143	31.9	73
	843	866	34.0	294	72.7	214	23.6	69
	1121	1144	37.2	425	79.8	340	16.6	71
	1399	1422	32.7	465	80.8	376	15.1	70
[1-^{14}C]Linoleate	9.7	15.4	46.1	7.1	18.7	1.3	76.2	5.4
	44.4	50.1	40.8	20.4	30.7	6.3	61.7	12.6
	79.2	84.9	40.3	34	40.8	14	52.5	18
	149	155	30.1	47	49.8	23	41.5	20
	288	294	24.8	73	60.0	44	33.0	24
	565	571	25.2	144	74.8	108	18.0	26
	843[c]	851	–	–	–	–	–	–

[a]Replicate flasks of log phase GM-10 cells were seeded with 2.5 X 10^6 cells. After 24 hr, the culture medium was replaced with 4.0 ml MEM plus 10% FBS supplemented with 0.5 μCi/flask [1-^{14}C]oleate or [1-^{14}C]linoleate, plus increased concentrations of the respective nonlabeled fatty acid. Values are means from 2 experiments.

[b]Total medium free fatty acid concentrations including the 22.8 nmol/flask oleate and 5.7 nmol/flask linoleate provided by the serum.

[c]Linoleate concentrations above 200 μM were toxic in this series.

ent in the cytoplasm; similar lipid droplets have been described in L cells (6). The GM-10 cells retained their characteristic morphology except at the highest medium fatty acid concentrations. The accumulation of lipid droplets was accompanied by esterification of a greater percentage of the incorporated fatty acids into triacylglycerol; at comparable exogenous fatty acid concentrations, relatively more of the incorporated linoleate than oleate was esterified into triacylglycerol. Thus, at these higher fatty acid concentrations as well, GM-10 cells use oleate in phospholipids to a far greater extent than linoleate.

Use of Oleate/Linoleate Mixtures

To extend the observation that linoleate and oleate were used differently from free fatty acid mixtures such as that of fetal bovine serum, GM-10 cells were incubated with mixtures of oleate and linoleate, with a total supplemented fatty acid concentration of 140 μM, resulting in substantial triacylglycerol accumulation while maintaining viable, actively mitotic cells. As in Table III, when a single fatty acid was added, incorporation of [1-^{14}C]-oleate was greater than that of [1-^{14}C] linoleate. From any given fatty acid mixture, however, the percentage incorporation of oleate and linoleate were identical (Fig. 2A). Thus, the incorporation of [1-^{14}C] oleate was depressed when it was diluted with linoleate rather than oleate.

Figure 2B shows that in oleate/linoleate mixtures which were 95-97.5% oleate, distribution of incorporated [1-^{14}C] oleate and [1-^{14}C] linoleate between phospholipid and triacylglycerol was similar. With increased linoleate in the fatty acid mixture, the percentage of incorporated [1-^{14}C] linoleate in phospholipid declined whereas that of [1-^{14}C]-oleate did not. Thus, when exposed to excess linoleate, these cells preferentially used scarce oleate for phospholipid and stored relatively more of the incorporated linoleate in triacylglycerol. Similar results (not shown) were obtained when the oleate/linoleate mixtures were added to the culture medium with delipidized serum protein rather than fetal bovine serum. The ratio of [1-^{14}C] oleate in phosphatidylcholine to that in phosphatidylethanolamine (PC/PE) decreased from 6.4 in 100% oleate to 5.0 when the [1-^{14}C] oleate was diluted with linoleate. The PC/PE ratio of the incorporated [1-^{14}C] linoleate remained 7.1-7.2 throughout the range of mixtures. These data suggest that when oleate is relatively scarce, it is used preferentially for PE and also for phosphatidylinositol plus phosphatidylserine.

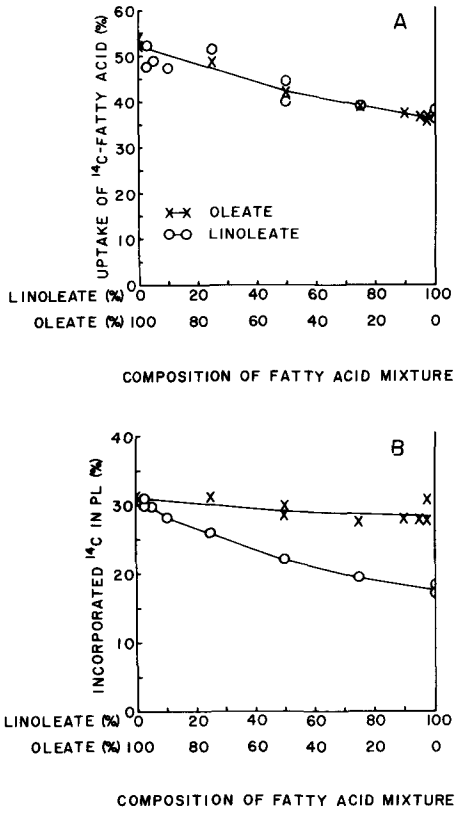

FIG. 2. Effect of different oleate/linoleate mixtures on incorporation of [1-^{14}C] linoleate and [1-^{14}C]-oleate. Replicate flasks were incubated for 24 hr in MEM plus 10% FBS plus 0.5 μCi [1-^{14}C] linoleate or [1-^{14}C] oleate, plus nonradiolabeled fatty acids to result in the indicated oleate/linoleate mixtures. Total supplemented fatty acid concentration was 140 μM. Uptake of [^{14}C] fatty acid as a percentage of that added to the medium. B: Percentage of incorporated [^{14}C] fatty acids in phospholipids (PL). O--O, [1-^{14}C]-linoleate; X--X, [1-^{14}C] oleate.

Pulse-chase Studies

In order to examine the metabolism of linoleate and oleate after incorporation into cellular lipids, cells were labeled with the respective [^{14}C] fatty acids and then grown in medium without radioactivity. Table IV shows that medium supplementation with 100 μM fatty acid greatly enhanced release of previously incorporated [^{14}C] fatty acids. Nearly all of this increase was in the form of free fatty acids; more [^{14}C] fatty acids were released with linoleate supplementation than with oleate. In the absence of fatty acid supplementation, [1-^{14}C] oleate and [1-^{14}C] linoleate were released to a similar extent. With addition of 100 μM fatty acid, release of [1-^{14}C] oleate was greater than that of [1-^{14}C] linoleate. Although

TABLE IV

Effect of Added Oleate and Linoleate on Release of Previously Incorporated [1-^{14}C] Fatty Acid

Released fatty acid (10^{-3} × dpm/flask	Total[b]	Phospholipid[e]	Free fatty acid[e]	Aqueous phase[e]
[1-^{14}C] Linoleate:				
Control	76.4 ± 0.3	13.7	17.1	42.5
Added oleate	170.3 ± 2.3	13.1	110.2	38.3
Added linoleate	228.3 ± 1.1[d]	14.4	162.1	43.1
[1-^{14}C] Oleate:				
Control	64.4 ± 1.4	11.0	17.0	33.8
Added oleate	205.6 ± 1.7[c]	14.4	156.8	29.6
Added linoleate	264.5 ± 3.5[c,d]	15.4	208.9	33.9

[a]Replicate flasks of GM-10 cells were incubated for 24 hr with 0.65 μCi/flask [1-^{14}C] linoleate or [1-^{14}C]-oleate as described in Table I; initial corporation was 778.2 × 10^3 and 798.3 × 10^3 dpm/flask for [1-^{14}C]-linoleate and [1-^{14}C] oleate, respectively. Each flask was then rinsed twice with sterile EBSS and the cells incubated for a second 24 hr in MEM plus 10% FBS (control) with 100 μM fatty acid added as indicated.

[b]Total medium radioactivity; means ± SE, n = 3.

[c]Difference between values for [1-^{14}C] linoleate and [1-^{14}C] oleate, with released fatty acid expressed as a percentage of initial incorporation, has $p < 0.05$.

[d]Difference between effects of added oleate and added linoleate has $p < 0.05$.

[e]2.0 ml of medium from each flask was extracted with 4.0 ml acetone and 8.0 ml ethylacetate as described in Materials and Methods. The aqueous phase was separated and the lipids analyzed by TLC.

absolute levels of [^{14}C] fatty acid release varied with cell density and age of the culture, the differences observed in Table IV, and Table V were significant in each of 3 independent experiments.

Medium supplementation with fatty acids resulted in transfer of previously incorporated [1-^{14}C] linoleate and [1-^{14}C] oleate from phospholipid to triacylglycerol (Table V). With either fatty acid in the chase medium, relatively more [1-^{14}C] linoleate was transferred than was [1-^{14}C] oleate. Although more [1-^{14}C]-oleate was released as free fatty acid, retention of [1-^{14}C] oleate in phospholipids was greater than that of [1-^{14}C] linoleate. This difference was quite pronounced with the linoleate chase; 44.0% of the initially incorporated [1-^{14}C] oleate but only 35.1% of the [1-^{14}C] linoleate remained in phospholipid. The losses of both [^{14}C] fatty acids from phospholipids occurred disproportionately from PC. These results, like those obtained with use of oleate/linoleate mixtures, indicate the preferential use of oleate for phospholipids when diploid human fibroblasts are challenged with excess exogenous linoleic acid.

DISCUSSION

These studies have demonstrated that GM-10 normal human skin fibroblasts take up and esterify oleate somewhat more readily than linoleate from medium containing either fetal bovine serum or delipidized serum protein. Of the incorporated fatty acids, relatively more linoleate is esterified in triacylglycerol; especially when it is limited, oleate is used preferentially for phospholipid. By contrast, Yoshida sarcoma cells preferentially incorporate linoleate rather than oleate from a variety of exogenous fatty acid mixtures (16). The rate of esterification of linoleate into phospholipids by Ehrlich ascites tumor cells is greater than that of oleate (17). Although L 1210 murine leukemia cells incorporate oleate more extensively than linoleate (18), the excess oleate is in triacylglycerol, with similar amounts of the 2 fatty acids incorporated into phospholipid. Since the studies on tumor cells used fatty-acid-poor albumin in place of serum, our results may result from differences in growth conditions and/or those between normal and transformed cells.

Both exogenous fatty acids (6) and hypertriglyceridemic serum (19) stimulate triacylglycerol accumulation in culture. Spector et al. (9) have demonstrated that exogenous fatty acids stimulate triacylglycerol synthesis whereas cellular phospholipid and cholesterol content is unchanged. Supplementation of medium containing fetal bovine serum with up to 100 nmol/ml oleate or linoleate does not affect the growth rate of these cells. Although exogenous fatty acids modify the acyl profile of cellular triacylglycerol to a greater extent than that of phospholipid (9), this study indicates that at any concentration of exogenous fatty acid, relatively more of the incorporated linoleate than oleate is esterified into triacylglycerol. Furthermore, the data suggest that, of those

TABLE V

Effect of Added Oleate and Linoleate on Redistribution of Previously Incorporated [^{14}C] Fatty Acids[a]

Lipid class	[1-^{14}C] Linoleate				[1-^{14}C] Oleate			
	Initial incorporation	After 24 hr chase in:			Initial incorporation	After 24 hr chase in:		
		Control	Oleate	Linoleate		Control	Oleate	Linoleate
Neutral lipid (NL)	112.4 ± 0.2	52.5 ± 0.7	280.3 ± 2.8	266.2 ± 0.8	83.8 ± 1.0	34.5 ± 1.4	238.0 ± 0.8	182.6 ± 3.3
Phospholipid (PL)	665.8 ± 0.9	649.3 ± 1.8	327.1 ± 3.9	273.2 ± 0.3	714.5 ± 8.8	699.4 ± 2.8	354.7 ± 2.5	351.1 ± 0.2
PL (% of PL + NL)	85.6	92.5	53.9	50.6[c]	89.5[b]	95.3[b]	59.8[b]	65.8[b,c]
PL (% of initial PL + NL)	85.6	83.4	42.0	35.1[c]	89.5[b]	87.6[b]	44.4	44.0[b]
Phosphatidylcholine (PC)	520.0 ± 4.1	458.1 ± 9.6	217.6 ± 11.1	171.1 ± 0.2	557.9 ± 1.9	503.9 ± 5.2	265.5 ± 3.7	223.0 ± 2.2
Phosphatidylserine plus phosphatidylinositol	76.9 ± 1.6	92.3 ± 3.7	49.2 ± 1.1	37.0 ± 0.4	69.7 ± 2.8	79.0 ± 0.3	39.7 ± 1.0	48.8 ± 3.3
Phosphatidylethanolamine (PE)	50.6 ± 0.3	77.6 ± 7.0	49.4 ± 1.9	45.8 ± 40.1	68.9 ± 0.5	92.0 ± 1.4	49.3 ± 3.2	60.0 ± 1.0
PC/PE	10.3	5.9	4.4	3.7[c]	8.1[b]	5.5	5.2[b]	3.7[c]

[a]Experimental conditions as in Table IV.

[b]Difference between values for [1-^{14}C] linoleate and [1-^{14}C] oleate has $p < 0.05$.

[c]Difference between effects of added oleate and added linoleate has $p < 0.05$.

free fatty acids normally present in fetal bovine serum, a higher percentage of the linoleate incorporated into cellular lipids should be in triacylglycerols.

Stern and Pullman (5) have shown that adaptation of hepatocytes to culture involves loss of mitochondrial acyl-CoA: glycerol-3-phosphate acyl transferase. Reliance on the microsomal enzyme enables those cells to synthesize dimonounsaturated phospholipids and thus maintain membrane fluidity despite a scarcity of polyunsaturated fatty acids. The hepatocytes continue to esterify oleate instead of saturated fatty acids in the 1-position of phosphoglycerides even when the culture medium is supplemented with linoleate. A similar enzymatic adaptation in human skin fibroblasts might explain the preferential use of scarce oleate in phospholipid. The greater incorporation of oleate than linoleate from mixtures of 80% palmitate or stearate would suggest that these cells also synthesize 1-saturated, 2-monounsaturated phospholipids in preference to the 1-saturated, 2-polyunsaturated species common in vivo.

This study indicates that the preferential use of oleate relative to linoleate in phospholipids is more pronounced in PE than in PC. A similar exclusion of excess polyenoic fatty acids from PE has been reported in L cells (20). Supplementation of L cells with linoleic acid perturbs the unsaturation of phospholipids of the cell homogenate more than those of the plasma membrane; this suggests a cellular mechanism for minimizing possible increases in cell membrane fluidity.

The localization of relatively more incorporated linoleate than oleate in triacylglycerol by GM-10 cells may represent a similar homeostatic mechanism.

A number of relatively normal cell lines apparently require polyunsaturated fatty acids (21-23); studies in which serum is completely replaced with hormones use media which contain linoleic acid (24). No requirement for linoleate has, however, been demonstrated in WI-38 human lung fibroblasts, even at serum protein concentrations of 100 μg/ml (25). Diploid human fibroblasts retain the ability to elongate and further desaturate both linoleate and linolenate (26,27) and to convert arachidonic acid to prostaglandins (28). About 10-15% of incorporated linoleate is modified in the first 24 hr (26); the longer chain polyunsaturated fatty acids are found in both phospholipid and triacylglycerol (Rosenthal, unpublished data). While substantial levels of polyunsaturated fatty acids apparently are not required for growth of these cells in culture, trace amounts of linoleate may be required for prostaglandin synthesis and possibly optimal cell growth. Further investigation is required to determine if cells depleted in polyunsaturated fatty acids might exhibit preferential use of linoleate.

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