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Alteration of the acyl chain composition of free fatty acids, acyl coenzyme A and other lipids by dietary polyunsaturated fats

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Abstract. Dietary alterations were used to demonstrate selective handling of fatty acids during their redistribution *in vivo.* Differences in the mol per cent of individual acyl chains in the non-esterified fatty acid, acyl-coenzyme A and phospholipid fractions reflected a result of relative precursor abundance combined with enzymic selectivities. Selective distributions were observed in the utilization of individual acyl chains between 16:0 and 18:0, 18:1 and 18:2, and among 20:3, 20:4 and 20:5, 22:6 by ligase(s), hydrolase(s) and acyl-transferases.

The variations in the mol per cent of linoleate present in the acyl-coenzyme A fraction of liver relative to that in the non-esterified fatty acids suggested an *in vivo* regulation of the level of linoleoyl-coenzyme A that influenced the synthesis of both arachidonoyl-coenzyme A and lipids.

The greater abundance of eicosapentaenoic acid in the free fatty acid fraction relative to that in the acyl-coenzyme A fraction may increase the ability of dietary 20: 5n-3 to be an effective inhibitor of the synthesis of prostaglandins derived from 20:4n-6.

Keywords. Acyl-coenzyme A; polyunsaturated fatty acids; lipids; non-esterified fatty acids.

Introduction

It has been well established that a highly selective *in vivo* handling of fatty acids results in saturated fatty acids being placed predominantly in position 1 of glycero-lipids in most tissues whereas unsaturated acids occupy position 2 (Hill and Lands, 1970; Lands and Crawford, 1975). Some selective recognition of acyl chains occurs in reactions catalyzed by liver acid: coenzyme A (CoA) ligases (Groot *et al.,* 1976; Marcel and Suzue, 1972; Lands, 1979), acyl-CoA hydrolases (Ellingson *et al.,* 1970; Jezyk and Hughes, 1971) and acyl-CoA acyltransferases (Lands and Crawford, 1975; Lands, 1979; Yamada and Okuyama, 1978; Holub, 1976; Lands *et al.,* 1982). However, until the recent work in the laboratory of Majerus (Neufeld *et al.,* 1983), little selectivity among the long-chain fatty acids had been reported for long-chain acid:CoA ligases (Hill and Lands, 1970; Groot *et al.,* 1976; Lands, 1979). Now the recognition of an arachidonate-selective ligase that can be separated from the non-specific ligase (Laposta *et al.,* 1985) opens new approaches to study the regulation of acyl chain flux. Interestingly the non-specific activity may be dominant only in liver and adipose tissue, two tissues that have major roles in general (non-selective) lipid mobilization and turnover. The acyl-CoA hydrolases apparently can discriminate between the CoA-esters of palmitate and stearate and among the CoA-esters of

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Abbreviations used: CoA, Coenzyme A; BHT, butylhydroxytoluene; t-BDMCS, t-butyldimethyl chloro-silane; SC, sucrose-casein.

oleate, linoleate and arachidonate (Jezyk and Hughes, 1971). The various acyltransferases, like acyl-CoA: glycerophosphate acyltransferase, acyl-CoA: 1-acyl glycerol phosphate acyltransferase and acyl-CoA: phospholipid acyltransferase have high selectivities that depend upon small structural details of the fatty acids (Lands, 1979). Studies on the specificity of acyltransferases have given significant amounts of information to help understand the capacity of these enzymes to control the positional placement of mono-, di- and tetra-unsaturated fatty acids in the glycerolipids. However, the accumulated *in vitro* data could not predict accurately the acyl chain composition of free fatty acids, acyl-CoA, phospholipids and triglycerides of rat liver under various dietary conditions because the actual composition of the precursor pools was unknown.

To help interpret the selectivities that occur *in vivo,* we examined the transient accumulation of polyunsaturated fatty acids in several related lipid fractions in liver. For this purpose we chose two lipid supplements: fish oil (rich in the long-chain n-3 polyunsaturated acids) and corn oil (rich in 18:2n-6). These supplements were added to either fat-free diet or to a regular chow diet. The acyl chain compositions of the various fractions of liver lipid were used to evaluate the extent of selective recognition of acyl chains *in vivo* by enzymes of fatty acid metabolism in liver.

Materials and methods

Sodium borohydride, malate dehydrogenase, citrate synthetase, phosphotrans acetylase, NAD^+, CoA, L-maleic Acid and dithiothretiol were all obtained from Sigma Chemical Co., St. Louis, Missouri, USA. The t-butyldimethylchlorosilane-imidazole reagent was purchased from Applied Science, State College, Pennsylvania, USA. Neutral alumina and silica gel H were from E. Merck, Darmstadt, FRG. Fatty alcohols and acids were obtained from Nuchek Prep, Elysian, Minnesota, USA. All solvents were reagent grade and distilled before use. Acyl-CoA esters used for reference standards were prepared as described by Okuyama *et al.* (1969).

Sucrose-casein diets

Sprague-Dawley male weanling rats (3 days after weaning) were fed *ad libidum* for 50 days a pelleted fat-free diet based on sucrose and casein (ICN Nutritional Biochemicals, Cleveland, Ohio). Then the rats were divided into 3 groups. One group continued with the fat–free diet, and the diet of the other two groups was supplemented with 13 % (v/w) of either menhaden fish oil (kindly provided by Dr. A. P. Bimbo, Zapata Haynie Corp.; Reedville, Virginia) or commercial corn oil. The oil supplements were prepared fresh every 3 days by thorough mixing of measured amounts of the oils with the food pellets. Each oil contained 0·02% *t*-butylhydroquinone as an added antioxidant and represented about 20% of the total calories consumed.

Chow-based diets

Additional rats had been raised on a regular chow diet until they were about 2 months old and weighed 130–160 g. The chow diet was then supplemented for 14 days

with 19% (v/w) as either menhaden fish oil or corn oil (that contained 0·02% *t*-butylhydroquinone) so that about 30% of the dietary calories were derived from the supplemental oils.

Where indicated, the animals were fasted 18 h before being anesthetized with sodium phenobarbital (50 mg i.p. per kg body wt.). Their abdomen was surgically opened, and the liver was quickly removed, rinsed with ice-cold saline (0·9%, w/v) to remove blood, and then frozen at — 70°C (dry ice-acetone bath). The frozen livers were lyophilized to obtain the dry tissue for more convenient extraction of the lipids.

Analysis of acyl-CoA, fatty acids, phospholipids and triacylglycerols

The overall amount of long-chain acyl-CoA esters in the liver samples was determined essentially according to the enzymatic method described by Kamiryo *et al.* (1979). The composition of the acyl chains in the liver acyl-CoA esters was determined as described by Prasad *et al.* (1987). Fifty mg of dry tissue was suspended in 0·5 ml of 100 mM sodium acetate (pH4) with 10 mM $MgCl_2$ plus 20μl of glacial acetic acid, 3·0 nmol of either 17:0-CoA or 20:0-CoA as internal standard, 100 μl of 1% BHT in acetone, 1·5 ml of methanol and 0·7 ml of chloroform in a total volume of 2·8 ml. The tissue was homogenized at 0·4°C and further additions of chloroform and water were made to complete the extraction according to Bligh and Dyer (1959). The chloroform phase containing the major lipids was carefully removed and used for analysis of free fatty acids and phospholipids. In all subsequent steps 1% BHT in acetone was added to make a final concentration of 0·05 %. The aqueous phase was washed twice with 1·5 ml portions of chloroform, and then 2 ml of acetonitrile was added to denature the protein. After standing for 20 min at room temperature the mixture was centrifuged and the supernatant was mixed thoroughly with 40 mg Al $2^{O}3$ for 15 min at 20°C. After removing the supernatant, the alumina was washed twice with 2 ml of chloroform: methanol (1:2) to remove adhering glycerolipids, and then with 1 ml acetone to facilitate drying.

After drying the alumina carefully under a stream of N_2, it was treated with 10-12 mg of sodium borohydride for 15 min, and then with 1 N HCl. Internal standards of 15:0 and 20:0 or 15:0 and 21:0 alcohols were added and the combined alcohols were extracted with pentane and dried under N_2 in a tube and then treated with 5 μl of *t*-butyldimethysilyl (tBDMCS) reagent and 30 μl of benzene for 20 min. Methanol (0·1 ml) was added to consume the excess reagent, and then 0·5 ml of water. The tBDMCS, ethers were extracted with pentane and, after evaporating the pentane, dissolved in 10 μl of CS_2. For every analysis, solvent and reagent blanks were carried through the procedure to correct for impurities.

Samples were analyzed on a Helwett-Packard chromatograph equipped with a flame ionization detector. A 180 × 0·2 cm glass column packed with 10% Silar 5CP (Supelco, Bellefonte, Pennsylvania) was operated with a N2 flow of 30 ml/min either isothermally at 220°C or temperature programmed from 165–220°C at 16°C/min. The number of nmol of each acyl chain was calculated on the basis of its area relative to that for the added (21:0 or 20:0) alcohol internal standard. This value was then corrected for recovery of the C 17:0-CoA standard that was added to all experiments. The method consistently gave with all added CoA esters, an overall absolute recovery of about 20% in the form of the alcohol silyl ether and no selective loss of

any acyl chain was observed. Thus the internal standards provide a valid index to the amount of each endogenous acyl CoA. The free fatty acids, phospholipids and triacylglycerols in the chloroform phase of the tissue extract were chromatographically separated by thin-layer chromatography on silicic acid using petroleum ether: diethyl ether: acetic acid (60:40:1). Each fraction was converted to methyl esters in the presence of added internal standards, and the acyl chain composition was determined by gas chromatography on a Silar 5CP column maintained at 220°C.

Results

Sucrose-casein diets

The non-esterified fatty acids in the livers of rats fed the fat-free sucrose-casein (SC) diet contained high amounts of 16:1, 18:1 and 20:3 (table 1) due to high induced levels of the Δ9-desaturase. In contrast, the relatively low amounts of 18:2 and 20:4 reflected the absence of the essential fatty acid, 18: 2n-6, in the diet. Supplementation with corn oil caused large increases in the accumulated levels of 18:2 and 20:4 in cellular lipids and corresponding decreases in the endogenous acids derived from the action of the 9-desaturase, (16:1, 18:1 and 20:3). A similar compensatory decrease in these acids accompanied the addition of fish oil which increased the tissue levels of the n-3 acids, 20:5, 22:5 and 22:6.

The acyl-CoA esters contained major proportions of 18:0 (35–40%) and 20:4 (12–32%) with varied amounts of 18: ln-9, 18:2n-6, 20:3n-9 or 22:6n-3 depending on the diet supplement. The effects on the acyl-CoA by the diet alteration were quantitatively different from those for the non-esterified acids, and sometimes they were qualitatively difference. For example, feeding corn oil gave a much greater ncrease in non-esterified linoleate (18:2) than arachidonate (20:4), while the increase in linoleoyl-CoA was much less than that for arachidonoyl-CoA. Similarly, when fish oil was added, the increase in non-esterified 20:5 exceeded that for 22:6 although the reverse occurred in the acyl-CoA pool. As a result, 20: 5n-3 was poorly accumulated in the acyl-CoA fraction relative to its mol% in the diet or in the non-esterified acids.

The combined phospholipid fraction contained significant amounts of the Δ9-desaturase products, 18:1 (23%) and 20:3 (10%), and these were displaced when the n-6 or n-3 acids of corn oil or fish oil, respectively, were added to the diet. The triacylglycerols shifted similarly, but again in ways that reflected difference selectivities among the various acids involved. They had a much more dramatic increase in 18:2n-6 than did the phospholipids, whereas the mol% of 20:4n-6 was only slightly increased in triacylglycerols. Although the added n-3 polyunsaturated acids of fish oil evoked a marked drop in the amount of the elongated n-9 acid, 20: 3n-9, they did not appreciably displace the large amount of the shorter form (18: ln-9) from the triacylglycerols.

Chow-based diets

Our first measurements of the lipids in unsupplemented chow-fed animals indicated much lower amounts of non-esterified fatty acids in the liver (236 nmol per g wet

Table 1. Composition of acyl chains in liver lipids of rats fed diets based on SC.

Material analyzed		*Fatty acid composition (mol%)*										
Diet fed[a]	Tissue abundance	16:0	16:1	18:0	18:1	18:2	20:3	20:4	20:5	22:4	22:5	22:6
Diets												
SC	—	—	—	—	—	—	—	—	—	—	—	—
SC+	—	13·7	0	1·6	25·6	59·0	—	—	—	—	—	—
SC+F	—	20·0	12·6	3·4	13	1·4	1·1	2·0	14·1	—	1·1	8·2
Non-esterified fatty acids[b] (FFA)												
SC	1090±90	25±2	15±2	7·2±0·8	40·±2	2·9±0·6	3·9±0·8	5±0·3	0	0·4±0·1	0	1·4±0·1
SC+C	3000±450	19±1	3±1	5·5±0·3	20·±2	33·+0·6	0·8±0·1	13±0·6	0	0·6±0·2	0·1±0·0	0·9±0·4
SC+F	1560±60	22±1	5±1	5·0±0·6	18·±1	3·7±0·1	0·2±0·0	3±0·3	25±1	0·2±0·0	3·2±0·4	13·0±0·6
Acyl-CoA[b]												
SC	141±7	10·0±0·1	2·1±0·3	38·±1	13·5±0·6	3·4±0·3	15·0±0·6	12·±1	0	1·5±0·1	0·3±0·0	3·±0·0
SC+C	141±7	10·4±0·5	0	35·±1	5·2±0·4	12·8±0·9	0·9±0·4	32·±2	0	0·8±0·2	0	2·±0·2
SC+F	186±20	11·3±0·3	2·7±0·3	40·±1	8·4±0·6	3·6±0·2	1·3±0·2	15·±1	3·3±0·2	0·5±0·1	3·3±0·2	10·8±0·4
Phospholipids[c]												
SC	20	26	—	18·	23·	4·2	10·3	12	0·3	·1	1·5	4·5
SC+C	34	23	—	23·	5·4	11·4	0·3	30	0·1	·7	1·5	4·7
SC+F	31	31	—	20·	10·8	1·5	0·4	9	10·9	·2	2·7	14·0
Triacylglycerols[c]												
SC	11	37	—	1·2	41·	2·	4·9	0·1	0·1	0·5	1·0	0·1
SC+C	9	38	—	0·9	19·	26·	0·5	2·5	0	0·6	0·6	0·3
SC+F	5	32	—	0·9	30·	6·	0·7	0·5	9·2	0·8	4·8	12·6

[a]SC indicates the fat-free SC diet; +C indicates corn oil supplementation, and +F indicates fish oil supplementation.
[b]Tissue abundance expressed as nanomoles per g wet weight of liver; mean±SEM of 4 determinations.
[c]Tissue abundance expressed as micromoles per g wet weight of liver; mean of two independent determinations.

weight) than we had observed with the SC diets (1000–3000 nmol per g) and somewhat higher amounts of acyl-CoA esters (230 *vs* 135 nmol per g). The composition of the various lipid pools differed in ways that could be related to the presence of 32 mol% 18:2 in the lipids of the chow diet. We noted that fasting the chow-fed animals overnight raised the abundance of free fatty acids in liver to about 6000 nmol per gram while diminishing the risk that they were derived directly from the dietary fat. Because we were testing for selectivities in acyl chain turnover in the liver, we elected to study the chow-fed animals with an overnight period of fasting prior to obtaining samples for analysis.

The non-esterified fatty acids in the livers of the fasted rats contained more oleate (18:1) and arachidonate (20:4) and much more linoleate (18:2n-6) than was in the non-fasted animals (table 2) while exhibiting a compensatory drop in the mol per cent of palmitate (16:0). While fasting caused the mol% of 18:2 to increase also in the acyl-CoA esters, the 18:2 decreased in the phospholipids and triacylglycerols as the mol% of 20:4 rose relative to values from the non-fasted rats. When the effects of oil-supplementation were compared among the non-esterified acids after an overight fast, the corn oil produced no appreciable change whereas the fish oil led to decreased 18:2 and 20:4 as these acids were displaced by the dietary n-3 acids, 20:5, 22:5 and 22:6.

Upon supplementing with corn oil, the acyl-CoA esters had an elevated mol% of 18:2 while 18:0 and 20:4 decreased slightly. Similarly, fish oil supplementation elevated the mol% of the dietary polyunsaturated acids, 20:5n-3 and 22:6n-3, in CoA esters while decreasing the mol% of 20:4. The phospholipids and triacylglycerols exhibited changes that qualitatively mirrored those noted for the non-esterified acids and the CoA esters. The n-3 dietary acids (or their related derivatives) tended to increase in these glycerolipids with a compensatory decrease in the amount of the (n-6) type.

Discussion

To facilitate comparisons of the relative selectivities that occurred during the changes in acyl chain composition, the results are also presented in table 3 in the form of ratios of the mol% of an acid in a product pool compared to that in the possible precursor pool. For example, the ratio for a non-esterified fatty acid to its abundance in the diet indicates in part the extent to which the acid may tend to be derived from the tissues of the animal rather than the diet. Ratios less than 1 for 18:2 during corn oil supplementation are expected because the diet is very rich in this acid, but the value of 2·6 for 18:2 during fish oil supplementation of the fat-free diet (table 3) suggested that there was a fairly vigorous displacement or mobilization of tissue reservoirs of 18:2n-6 when it was not very abundant in the diet and other polyunsaturated acids were. A similar mobilization of arachidonate, 20: 4n-6, by the fish oil supplementation may reflect competition for esterification by the 20:5n-3 (with phospholipid/acyl-CoA ratios of 2·8–3·3). A similar displacement by n-3 acids was also observed in dog plasma lipids during fish oil supplementation which increased the amount of circulating 18:2n-6 and 20:4n-6 during the first week of shifting to a diet rich in n-3 acids (B. Culp and W. Lands, unpublished results; Culp *et al.,* 1980). The triacylglycerols in plasma retained an elevated mol% of 20:4n-6 even after several weeks of fish oil supplementation (Culp *et al.,* 1980). A more direct

Table 2. Composition of acyl chains in liver lipids of rats fed chow-based diets.

Material analyzed		*Fatty acid composition (mol%)*										
Diet fed[a]	Tissue abundance[b]	16:0	16:1	18:0	18:1	18:2	20:3	20:4	20:5	22:4	22:5	22:6
Diets												
Chow	—	21	3·3	7·1	29·8	32·6	0·4	0·2	0·2	<·1	0·1	0·3
Chow + C	—	16·6	1·3	3·7	27·5	49·6	<·1	<·1	<·1	<·1	<·1	<·01
Chow + F	—	21·0	7·8	4·7	19·3	14·0	0·2	1·5	8·3	<·1	1·2	5·8
Non-esterified fatty acids (FFA)												
Chow-NF	240 ± 30	39 ± 2	10 ± 1	5·5 ± 0·2	15 ± 2	12 ± 1	0·9 ± 0·1	7·5 ± 0·7	2·9 ± 0·2	0	2·3 ± 0·2	4·0 ± 0·6
Chow	6100 ± 1000	20 ± 1	2·0 ± 0·1	7·0 ± 0·9	23 ± 1	30 ± 2	0·6 ± 0·1	14·5 ± 0·1	0·8 ± 0·1	0·6 ± 0·1	0·5 ± 0·2	3·3 ± 0·7
Chow + C	5700 ± 1000	22 ± 2	0·9 ± 0·1	7·9 ± 1	20 ± 1	31 ± 3	1·0 ± 0·1	14· ±	0·9 ± 0·0	2·0 ± 0·3	0·1 ± 0·1	1· ± 0·1
Chow + F	6300 ± 100	22 ± 1	4·0 ± 0·5	4·7 ± 0·5	18 ± 1	18 ± 1	0·2 ± 0·0	5·5 ± 0·7	7·2 ± 0·4	0·3 ± 0·1	3·6 ± 0·1	17· ± 1
Acyl-CoA												
Chow-NF	230 ± 16	10·8 ± 0·4	0	38 ± 0·5	5·2 ± 0·1	15 ± 0·5	2·0 ± 0·2	23· ± 1	0·4 ± 0·1	2·6 ± 0·1	2·0 ± 0·2	4·2 ± 0·1
Chow	150 ± 30	5·8 ± 0·2	0·5 ± 0·1	37 ± 3	5·5 ± 1	26 ± 4	0·8 ± 0·1	20 ± 3	—	0·9 ± 0·1	0·9 ± 0·2	1·3 ± 0·4
Chow + C	130 ± 13	7·1 ± 0·4	0·2 ± 0·1	31 ± 1	6·0 ± 0·4	36 ± 2	0·9 ± 0·1	17· ± 1	—	0·4 ± 0·0	0·5 ± 0·3	1·0 ± 0·1
Chow + F	130 ± 25	9·0 ± 0·4	1·1 ± 0·1	35 ± 2	7·3 ± 2	25 ± 2	1·5 ± 0·1	12· ± 1	1·1 ± 0·4	0·9 ± 0·3	0·9 ± 0·2	4·3 ± 0·6
Phospholipids												
Chow-NF	30	25·	—	22·	5·0	14·5	0·8	20·	1·4	0·6	1·5	7·0
Chow	66	19·	—	29·	6·3	8·6	0·8	29·	0·2	0·4	0·7	5·8
Chow + C	60	18·	—	27·	6·5	12·2	0·3	28·	0·1	0·5	1·1	6·1
Chow + F	54	25	—	25·	6·0	8·5	0·5	15·	3·1	0·1	1·7	15·0
Triacylglycerols												
Chow-NF	2·4	42·	—	2·1	32·1	16·6	0·3	1·9	0·7	0·6	0·8	1·4
Chow	2·2	38·	—	8·9	36·3	3·4	0·3	7·9	—	0·6	0·5	1·5
Chow + C	7·0	27·	—	5·4	26·4	32·0	0·4	4·8	0·2	1·0	0·9	0·9
Chow + F	4·6	33·	—	4·7	28·5	11·1	0·2	2·6	3·4	0·7	3·2	10·3

[a]The diets were consumed *ad libidum* with an 18 h fast prior to killing the rats for analysis, with the exception of those designated Chow-NF which were not fasted. Supplemental fats are indicated as C for corn oil and F for fish oil.

[b]Tissue abundances are in the same terms as in table 1.

Table 3. Selectivity ratios indicating the relative enrichment of an acyl chain between two lipid pools.

Ratio[a]	Diet fed	16:0 C[b] F[b]	18:0 C F	18:1 C F	18:2 C F	20:4 C F	20:5 C F	22:6 C F
FFA	Chow-NF[c]	1·8	0·8	0·5	0·4	37	14·5	13·
Diet	Chow	1·0	1·0	0·8	0·9	72	4·	11·
	Chow + Suppl	1·3 1·0	1·1 1·0	0·7 0·9	0·6 1·3	3·7	— 0·9	10 2·9
	SC[d] + Suppl	0·5 1·1	3·4 1·5	0·8 2·6	0·5 2·6	1·5	— 1·8	— 1·6
Acyl-CoA	SC	0·4	5·2	0·3	1·2	2·4	—	2·1
FFA	Chow-NF	0·3	6·9	0·3	1·3	3·0	0·13	1·0
	Chow	0·3	5·3	0·3	0·9	1·4	—	0·4
	Chow + Suppl	0·3 0·4	3·9 7·4	0·3 0·4	1·1 1·4	1·3 2·1	— 0·15	1·0 0·25
	SC + Suppl	0·5 0·5	6·4 7·9	0·3 0·5	0·4 1·0	2·5 4·7	— 0·13	1·8 0·8
Phospholipid	SC	2·6	0·5	1·7	1·2	1·0	—	1·5
Acyl-CoA	Chow-NF	2·3	0·6	1·0	1·0	0·9	3·5	1·7
	Chow	3·3	0·8	1·1	0·3	1·4	—	4·5
	Chow + Suppl	2·6 2·7	0·9 0·7	1·1 0·8	0·3 0·3	1·6 1·3	— 2·8	6·1 3·5
	SC + Suppl	2·2 2·7	0·7 0·5	1·0 1·3	0·9 —	0·9 0·6	— 3·3	2·4 1·3

[a]The ratios indicated were derived from the mol% values given in tables 1 and 2.
[b]C indicates corn oil supplementation, and F indicates fish oil supplementation.
[c]Chow-NF indicates the non-fasted animals receiving unsupplemented chow diets as noted in table 2.
[d]SC indicates the sucrose-casein diet noted in table 1.

study of competition between 20:5n-3-CoA and 20:4n-6-CoA (Lands *et al.,* 1982) showed a preference by the l-acyl-GPC : acyl-CoA acyltransferase of rat liver for transfer of the 20: 5n-3. The present results confirm that $_{\text{selectivity}}$, with ratios for phospholipid/acyl-CoA that were consistently higher for 20:5n-3 (3·5, 2·8, 3·3) than for 20:4n-6 (0·9, 1·2, 0·6).

High selectivity ratios for acyl-CoA/non-esterified acid may indicate that an acyl group enters the acyl-CoA pool with more facility or speed than it leaves. The particularly high value for 18:0 (4–8) contrasts sharply with the low values for 16:0 and 18:1 (0·3–0·5). Induction of hepatic fatty acid synthetase (up to 50-fold) by dietary variations (Flick *et al.,* 1977; Bernert and Sprecher, 1977) was much greater than that for microsomal elongation of 16:0-CoA to 18:0-CoA (2·5-fold). Since the synthetase produces mainly palmitate (Burton *et al.,* 1968), a higher mol% of 16:0 compared to 18:0 might be expected. The very high mol% of 18:0 in the acyl-CoA fraction strongly suggests that this acid enters the acyl-CoA pool more readily than it exits. The microsomal acyl-CoA hydrolase activity is apparently two times higher for 16:0-CoA tha 18:0 CoA (Jezyk and Hughes, 1971), but no difference between these two was obtained for phosphatidate synthesis (Possmayer *et al.,* 1969). A further look at the exit of these two acids from the acyl-CoA pool seems desirable. It will be especially important to examine whether the relatively higher content of 18:0 CoA and 20:4 CoA influences the formation of selected species of phosphatidyl inositol.

The low ratios of acyl-CoA to non-esterified acid for 20:5 suggest that this acid tends to leave the thiol ester pool more readily than it enters. Put in another way, the 20:5n-3 appears to accumulate more in the non-esterified form than as the acyl-CoA, whereas the 20: 4n-6 appears to accumulate more as acyl-CoA than as the non-esterified form. These combined tendencies could have an interesting impact upon the ability of the eicosanoid-forming oxygenases to convert the nonesterilied 20:4n-6 into biologically active materials (Bergstrom *et al.,* 1964; Murphy *et al.,* 1979). Those oxygenases act on the non-esterified acids, and 20: 5n-3 can antagonize that process (Lands *et al.,* 1973; Lands and Byrnes, 1982).

The relatively low ratio for acyl-CoA/nonesterified acid observed for 18:2 when the sucrose-casein diet was supplemented with corn oil (0·4) needs to be interpreted in terms of competition for the limited CoASH thiol group. The competition by 30–33 mol% non-esterified 18:2 (tables 1 and 2) with 13–15 mol% non-esterified 20:4 led to a greater mol% of 20:4-CoA with the sucrose-casein diet, but to relatively more 18:2-CoA with the chow diet. Apparently other regulatory features occur during the alteration in diet, which might include altered cellular amounts of the arachidonate-specific ligase (Laposta *et al.,* 1985) or different amounts of lipid acceptors that permit facile transfer of linoleate from the acyl-CoA pool (*e.g.* transfer to diacylglycerol to form triacylglycerol). The decreases in the mol% of 18:1 and 20:3 when the diets were supplemented with added polyunsaturated fat is consistent with a decreased induction (Prasad and Joshi, 1979) or inhibition (Jeffcoat and James, 1978) of the 9-desaturase as well as with competitive displacement of the 18:1 and its products from the finite cellular lipid pools by the polyunsaturated acids. The greater effect on the elongated form, 20:3, suggests that a particularly effective competition in the acyl-CoA pool by the polyunsaturated acids may prevent the conversion of 18:1-CoA to 20:3-CoA.

The relatively high selectivity ratios for phospholipid/acyl-CoA for 22: 6n-3 with chow-fed animals (3 ·5, 4·5, 6·1) suggest that this acid may move more readily from the

acyl-CoA pool into phospholipid under fasting than non-fasting conditions (1·5, 1·7, 1·3, 2·4). Again the results suggest the possibility of additional factors controlling the selective action of the acyltransferases during fasting. Such an action could result in vigorous salvage of this highly unsaturated fatty acid during times of limited supply, and it may be involved in the apparent ability of tissues to retain 22: 6n-3 more effectively than any other polyunsaturated acid. The selectivity ratios of 10, 11 and 13 for 22:6 in non-esterified acids relative to the diet indicate very active mobilization of the endogenous acid. If this fatty acid does indeed have an important role in membrane function (Neuringer *et al.,* 1984) its selective retention would clearly have survival value.

Many years ago we approached the question of selective acyl transfer by measuring the abundances of the molecular species of phosphatidylcholine in rat liver during fasting followed by feeding a fat-free (SC) diet (Lands and Hart, 1966). The results in that study are confirmed here in that during fasting the chow-fed rats accumulated higher mol% of the tetraene *04** and hexaene *06* molecular species of lecithin in the liver. Then the subsequent fat-free diet induced high levels of fatty acid synthetase and Δ9-desaturase to produce large amounts of 16: ln-7 and 18: ln-9 and their corresponding *01* and *11* molecular species with a relative loss from the liver of the *02, 04* and *06* molecular species in the absence of dietary polyunsaturated acids. The dietary shift apparently led to the export of many polyunsaturated acids from the liver and their replacement by monoenoic acids in a way that created an apparent local deficiency of essential fatty acid. That selective export apparently does not have this consequence during fasting when the monoenoic acids are not as abundant.

The use of the sensitive subnanomole assay for determining the acyl chains in the acyl-CoA pool has allowed us to see shafts in composition due to diet changes. Our results have shown that it is possible to discern selective movements of acyl chains into and out of the acyl-CoA pool of liver. The application of the method to a primary culture of hepatocytes could permit frequent sampling at closely spaced time intervals so that more precisely defined selective flux rates could be determined. We expect to see continued study of this very small pool of dynamically metabolized thiolesters as biochemists examine the important contributions of polyunsaturated fatty acids to human health.

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* The designations *04, 06, 01, 11,* etc. indicate molecular species of lecithin for which the number of double bonds in fatty acids at postions 1 and 2 are noted (Lands and Hart, 1966).

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