Incorporation of dietary [^{14}C]arachidonic acid and [^{3}H]eicosapentaenoic acid into tissue lipids during absorption of a fish oil emulsion

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Abstract A preferential incorporation of dietary arachidonic acid (20:4, n-6) into chyle lipoprotein phospholipids, a relative resistance of 20:4 esters of chyle triacylglycerol (TG) to hydrolysis by lipoprotein lipase, a preferential utilization of 20:4 for phospholipid acylation, and a low rate of oxidation of 20:4 are factors that may contribute to the differences seen in the incorporation into tissue lipids between absorbed 20:4 and the predominant dietary 16-18 carbon fatty acids. In this study we fed [^{14}C]20:4 and [^{3}H]eicosapentaenoic acid (20:5, n-3) as free fatty acids in a fish oil emulsion to rats and analyzed the radioactivity in different tissue lipids after 1, 2, and 4 h. The purpose was to examine the degree of similarity in the fate of the two major eicosanoid precursors during the absorption of a fish oil meal. The recovery after 2 and 4 h of ^{14}C exceeded that of ^{3}H in lipids of small intestine, serum, liver, heart, kidneys, and spleen. The differences increased with time, e.g., the liver contained 9.7 (±0.7)% ^{3}H and 17.9 (±1.4)% of the ^{14}C ($P < 0.001$), and the upper half of the small intestine 10.0 (±0.8)% of the ^{3}H and 22.8 (±1.1)% of the ^{14}C ($P < 0.001$) after 4 h. The ^{14}C and ^{3}H radioactivity per g tissue after 4 h ranked as follows: liver and brown adipose tissue > kidneys > heart, lungs, spleen, and serum > colon > white adipose tissue and testes, the differences between tissues being up to 50-fold. There were up to fourfold variations in the ^{14}C/^{3}H ratios between tissues after 4 h, the highest value being observed in the heart and the lowest in white adipose tissue. Of the radioactivity retained in liver and intestine, more ^{14}C and ^{3}H was in phospholipids and less in triacylglycerol (TG), the differences being largest in the liver, e.g., after 4 h 57.6 (±0.8)% of the ^{14}C and 29.9 (±0.9)% of the ^{3}H ($P < 0.001$) in the liver was in phosphatidylcholine (PC). In both intestine and liver the highest ^{14}C/^{3}H ratios were found in phosphatidylinositiol (PI). Also phosphatidylethanolamine (PE) contained more ^{14}C than ^{3}H but the quantitative differences were relatively small after 4 h. In heart the proportions of ^{3}H and ^{14}C found in PE and PI did not differ, whereas more of the ^{14}C was in PC and more of the ^{3}H was in cardiolipin and phosphatidylserine. In serum more of the ^{3}H appeared in TG, free fatty acids, and diacylglycerols and less in phospholipids as compared to ^{14}C. The relative distribution of ^{3}H and ^{14}C between TG, free fatty acids, and diacylglycerols did, however, not differ suggesting that 20:4 and 20:5 esters were metabolized similarly. Large proportions of both [^{3}H]20:5 and [^{14}C]20:4 were in cholesteryl esters, indicating that rat lecithin:cholesterol acyltransferase (LCAT) is as active against 20:5 as against 20:4. The interconversion of [^{14}C]20:4 to other fatty acids was examined by high pressure liquid chromatography of fatty acid methyl esters. The interconversion of [^{14}C]20:4 to other fatty acids was negligible. The proportion of ^{3}H in docosapentaenoic (22:5, n-3) and docosahexaenoic (22:6, n-3) acids did not exceed 7% in intestine and 11% in the liver after 4 h; the values observed at 1 h were below 4%. The values given thus reflect the distribution of [^{14}C]20:4, whereas in the case of [^{3}H]20:5 the formation of interconversion products may influence the data to a small extent. The study shows that dietary 20:5 is metabolized according to a pattern similar to that of 20:4. 20:5 does, however, exhibit a lower preference for incorporation into phospholipids, particularly PC and PI, and more appears in TG of intestine, serum, and liver. 20:4 and 20:5 esters of chyle TG and PC are similarly metabolized by lipases and by LCAT.—**Nilsson, Å., L. Hjelte, and B. Strandvik.** Incorporation of dietary [^{14}C]arachidonic acid and [^{3}H]eicosapentaenoic acid into tissue lipids during absorption of a fish oil emulsion. *J. Lipid Res.* 1992. **33:** 1295-1305.

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The 20-22 carbon fatty acids of the n-3 series exert many of their biological effects by being incorporated into positions in phospholipids from which eicosanoid precursors are mobilized (see refs. 1-8). As stressed in recent reviews on the absorption of polyunsaturated fatty acids (9, 10) the processes by which the n-3 fatty acids are transported to different tissues and the factors that control

Abbreviations: CL, cardiolipin; CE, cholesteryl esters; DG, diacylglycerol; FFA, free fatty acids; LCAT, lecithin:cholesterol acyltransferase; LPC, lysophosphatidylcholine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; SM, sphingomyelin; TG, triacylglycerols; VLDL, very low density lipoprotein; TLC, thin-layer chromatography; 18:2n-6, linoleic acid; 20:4n-6, arachidonic acid; 20:5n-3, eicosapentaenoic acid; 22:5n-3, docosapentaenoic acid; 22:6n-3, docosahexaenoic acid.

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their incorporation into tissue phospholipids are, however, relatively poorly characterized. Although the eicosapentaenoic acid (20:5, n-3) and its interconversion products may compete with arachidonic acid (20:4, n-6) for the incorporation into different phospholipids both in cell cultures and in vivo (11-16), there is limited information about the fate during lipid absorption of dietary 20:5 and docosahexaenoic acid (22:6, n-3) the predominant n-3 fatty acids in fish oil.

During lipid absorption different fatty acids vary in their partitioning between mucosal and chyle phospholipids and triacylglycerol (TG), 20:4 exhibiting the highest incorporation into phospholipids (17, 18). The distribution of orally fed [^{14}C]18:2 and [^{3}H]20:4 between chyle phospholipids and TG was, however, strongly influenced by the mass of polyunsaturated fatty acids in the fat vehicle. For instance, when [^{14}C]18:2 and [^{3}H]20:4 were fed in cream to thoracic duct-cannulated rats, much larger proportions of the labeled fatty acids were transported with chyle phospholipids and less with TG than when they were given in an 18:2-rich lipid emulsion (Intralipid) or in pure 20:4 (18). The relative affinity, and thus the competition of different polyunsaturated fatty acids for acylation reactions catalyzing their incorporation into phospholipids in the small intestine, may thus be an important determinant of their distribution between different chyle lipids and thereby of their further metabolism.

Chylomicron phospholipids are metabolized by the action of lipoprotein lipase (19, 20) and, after transfer to high density lipoproteins (21), by hepatic lipase, on the *sn*-1 ester bonds of phosphatidylcholine (PC) and phosphatidylethanolamine (22). Lecithin:cholesterol acyltransferase (LCAT) has a preference for 20:4-PC in the rat in vitro (23), and metabolizes a considerable part of the chyle 20:4 PC in vivo (24). TG 20:4 esters in very low density lipoprotein (25) and chylomicrons (26) also exhibit a relative resistance to lipoprotein lipase in comparison to the 16-18 carbon fatty acid esters. Accordingly remnants formed both in vitro (26) and in vivo (24) are enriched in TG and in diacylglycerols (DG). In vitro the 20:5 esters of chylomicrons obtained after feeding fish oil exhibited the same lipolysis pattern as the 20:4 esters (27), although when different chylomicrons were compared 20:5-rich chylomicrons were metabolized at relatively similar rates both in vivo (28) and in vitro (29). The preferential partitioning of 20:4 to chyle phospholipids as well as the relative resistance of 20:4 and 20:5 esters to lipoprotein lipase may serve the purpose to distribute these biologically potent fatty acids to tissues differently than the predominant 16-18 carbon fatty acids, with a relatively small release as free fatty acids (FFA) in blood.

These factors as well as the relative affinity of 20:4 and 20:5 for pathways that catalyze incorporation and acyl turnover in tissue phospholipids (see refs. 30 and 31), and the relative rates of oxidation of the two fatty acids (32), would be expected to be important determinants of the relative rates at which dietary 20:4 and 20:5 are channeled to different tissue phospholipid pools.

In the present study we compared the incorporation of [^{14}C]20:4 and [^{3}H]20:5 into serum and tissue lipids during the first 4 h after oral administration of the labeled fatty acids in unesterified form, dispersed in a fish oil emulsion. The purpose was to examine the degree of similarity in the fate for the two major eicosanoid precursors.

MATERIALS AND METHODS

Animals and diets

Pregnant white Sprague-Dawley rats were purchased from ALAB AB, Stockholm, Sweden. These rats and their pups were fed a semisynthetic diet containing 5.7% (w/w) soy bean lipid. The composition of the diet has been described earlier (33). The diet was given ad libitum and the rats had free access to tap water; they were kept under conditions with control of dark-light cycles (lights on 6 AM to 6 PM). The humidity was kept at 55-60% and the temperature at 22°C. Male rats (90 days old) were used for the experiments. To avoid large variations in the gastric emptying due to the presence of solid food in the stomach, the diet was withdrawn 48 h before the experiment but the rats had free access to a water solution containing 2.5% glucose, 0.5% NaCl, and 0.005% KCl (wt/vol).

Experiments with labeled fatty acids

[5, 6, 8, 9, 11, 12, 14, 15, 17, 18-^{3}H (N)]20:5 (79.0 Ci/mmol) and [1-^{14}C]20:4 (52.0 mCi/mmol) were obtained from New England Nuclear Corporation, Boston, MA. Pure nonradioactive 20:4, 20:5, and 22:6 were obtained from Nu-Chek Preparations (Elysian, MN).

The composition of the major fatty acids of the fish oil TG used (Max EPA, Nobelprodukter AB, Örebro, Sweden) was as follows: 14:0, 6.5%; 16:0, 15.3%; 16:1, 8.6%; 18:0, 3.1%; 18:1, 12.8%; 18:2, 1.1%; 18:3, 0.7%; 18:4, 2.3%; 20:1, 2.8%; 20:4, 0.8%; 20:5, 16.5%; 22:1, 2.6%; 22:4, 0.6%; 22:5, 2.2%; and 22:6, 12.3% (w/w). (Analysis by capillary gas chromatography was kindly performed by Dr. Björn Åkesson, Department of Physiological Chemistry, University of Lund.)

The radioactive emulsion was prepared as follows. A 10% (v/v) emulsion of fish oil TG was obtained by mixing 0.5 ml fish oil TG and 4.5 ml 0.9% (w/v) NaCl containing 1.0% (w/v) gum arabic. The mixture was ultrasonicated for 3 min at 30-sec intervals under a nitrogen atmosphere with cooling on ice between intervals. One hundred μCi of [^{3}H]20:5 and 20 μCi of [^{14}C]20:4 were added to a chloroform solution containing 1.0 mg egg PC which was then taken to dryness under nitrogen. It was then immediately dispersed in 2 ml 0.9% NaCl and added to 12.5 ml of the

fish oil TG emulsion. One ml of the final emulsion was given to each animal; thus each rat received 78 mg fish oil TG, containing 12 mg 20:5 and 0.6 mg 20:4, 8 μCi of [^{3}H]20:5 and 1.6 μCi of [^{14}C]20:4.

The emulsion was given intragastrically by a polyvinyl tube to rats lightly anesthetized with diethyl ether. The animals were killed 1, 2, and 4 h later (six rats at each time point). They were first anesthetized with diethyl ether and desanguinated by aortic puncture. The small intestine was removed, rinsed with cold saline, and divided into an upper and a lower half. The intestinal content and washing were collected and frozen. The other organs were thereafter rapidly removed, and all organs were immediately frozen in liquid nitrogen. They were stored at −20°C until analyzed.

Lipid analyses

Lipids were extracted from the different tissues with chloroform–methanol 1:1 (v/v) containing 0.005% butylated hydroxytoluene. The lipid extracts were then treated as described previously (17). Nonpolar lipids were separated by TLC on silica gel G plates developed in petroleum ether–diethyl ether–acetic acid 80:20:2. Phospholipids were separated on Merck silica gel 60 plates developed in chloroform–methanol–acetic acid–water 100:80:12:1.2 (v/v). The spots were identified by staining with iodine vapor, and scraped into counting vials. One ml methanol–water 1:1 (v/v) and 10 ml Instagel–toluene 1:1 (v/v) were added, and the radioactivity of the samples was determined in a Packard Tricarb 460 CD Liquid Scintillation system, using the computerized automatic external standard for quench correction.

The proportion of radioactivity in choline and ethanolamine plasmalogen was determined by examining the distribution of radioactivity on TLC before and after weak acid hydrolysis as described earlier (33).

High pressure liquid chromatography of fatty acid methyl esters

Aliquots of lipid extracts to which 100–1000 μg of fish oil TG had been added as carriers were transmethylated as described above. Methyl esters were then separated on a Nucleosil C_{18} column (4.5 × 250 mm) using Shimadzu SPD 6 A high pressure liquid chromatography equipment. The column was eluted with acetonitrile–water 90:10 (v/v) at a flow rate of 0.5 ml/min (34). The different methyl esters were detected by UV absorption at 205 mm. The different fractions were collected in liquid scintillation vials, taken to dryness under nitrogen, and counted as described above. This method provides a good separation of 20:4 (n–6), 20:5, 22:5, and 22:6 (n–3), but only a partial separation of α-linolenic acid 18:3 (n–3) and 22:6. The radioactivity migrating as 22:6 was considered to be 22:6 since the 18:3 part of the peak contained low radioactivity, and no conversion of 20:5 and 20:4 to 18:3 would be expected.

Statistical analysis

Student's paired *t*-test was used for statistical analysis. Variations were indicated by standard errors of the mean (±SE).

The study was approved by the regional ethical committee of animal experimentation.

RESULTS

Recovery of radioactivity in different tissues

The recovery of ^{3}H and ^{14}C in total lipids was examined in stomach, upper small intestine, liver (**Fig. 1A**) and serum (Fig. 1B), in heart, spleen and lungs (Fig. 1C), in kidneys, brown adipose tissue and colon (Fig. 1D), and in lower small intestine, contents of the small intestine, testes, and white adipose tissue (data not shown). ^{14}C/^{3}H ratios of the lipid extracts from all organs are given in **Table 1**, and the average radioactivity per g tissue weight after 4 h is shown in **Table 2.**

The proportions of ^{3}H and ^{14}C that remained in the stomach after different time intervals were equal, the ^{14}C/^{3}H ratios thus being the same as that of the given material (Fig. 1A, Table 1).

The contents of the small intestine contained relatively small amounts of radioactivity (<2% of given dose), the ^{14}C/^{3}H ratio being slightly higher than that of the given material (Table 1).

The rates at which the two fatty acids were emptied from the stomach and absorbed by the small intestine thus did not differ. The recovery of ^{14}C in the tissue of the upper small intestine exceeded that of ^{3}H at all time intervals, the difference increasing with time, e.g., after 4 h the recovery was 22.8 (±11.1)% for ^{14}C and 10.0 (±0.8)% for ^{3}H ($P < 0.001$, Fig. 1A). Less than 2% of the radioactivity was recovered in the lower half of the small intestine (data not shown).

The recovery of ^{3}H and ^{14}C in serum did not differ after 1 h, whereas after 2 and 4 h the ^{14}C radioactivity clearly exceeded that of ^{3}H, e.g., the average recovery was 4.1 (±0.3)% for ^{14}C and 1.9 (±0.2)% for ^{3}H after 4 h ($P < 0.001$, Fig. 1B).

In the liver the recovery of ^{3}H was slightly higher than that of ^{14}C after 1 h. As in the intestine and serum, the recovery of ^{14}C clearly exceeded that of ^{3}H after 2 and 4 h, e.g., after 4 h 17.9 (±1.4)% of the given ^{14}C and 9.7 (±0.7)% of the ^{3}H was in the liver lipids ($P < 0.001$, Fig. 1A).

Also in heart, spleen, and lungs (Fig. 1C), and in kidneys (Fig. 1D) the recovery of ^{14}C after 2 and 4 h exceeded that of ^{3}H. The relative size of the differences between ^{3}H and ^{14}C varied, however, being largest in the heart, and

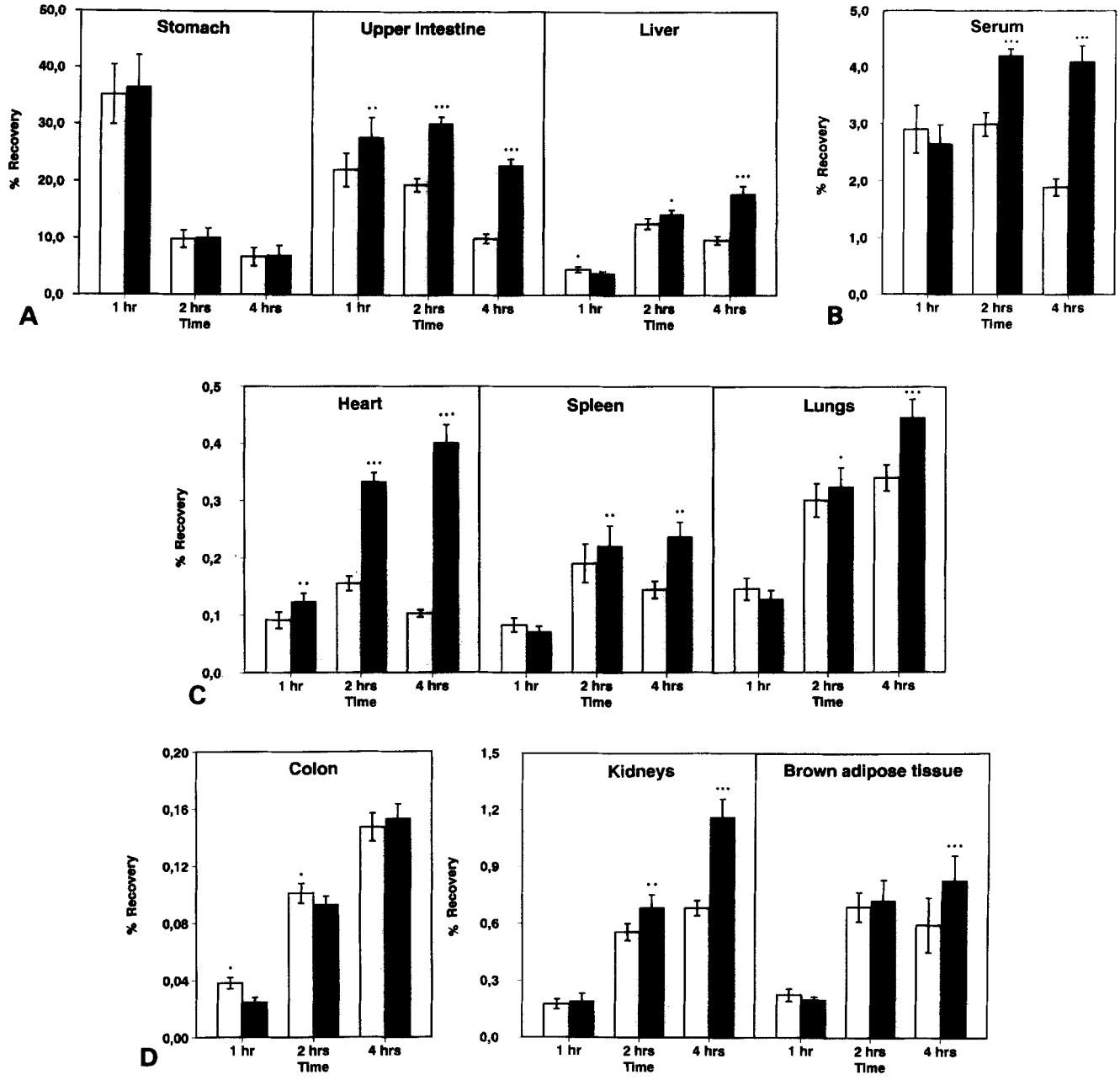

Fig. 1. A: Recovery of radioactivity in lipids of stomach, upper small intestine, and liver. The figure shows percent recovery of ^{3}H (open bars) and ^{14}C (filled bars) in total lipids at different time intervals after oral administration of [^{3}H]20:5 and [^{14}C]20:4. Values are means ± SE (n = 6). Significance of differences between recovery of ^{3}H and ^{14}C was estimated by Student's paired *t*-test (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). **B:** Recovery of radioactivity in serum lipids. Values (means ± SE, n = 6) are percent of given dose of ^{3}H (open bars) and ^{14}C (filled bars). Significances are as indicated in **A. C:** Recovery of radioactivity in lipids of heart, spleen, and lungs. Values (means ± SE, n = 6) are percent of given dose of ^{3}H (open bars) and ^{14}C (filled bars). Significance of differences as indicated in **A. D:** Recovery of radioactivity in lipids of kidneys, brown adipose tissue, and colon. Values (means ± SE, n = 6) are expressed as percent of given dose of ^{3}H (open bars) and ^{14}C (filled bars). Significance of differences as indicated in **A**.

least pronounced in the lungs after 4 h (Fig. 1C). In testes the recovery of ^{14}C increased more than that of ^{3}H with time, although the average radioactivity of both isotopes was low (0.07 ± 0.02% ^{14}C and 0.03 ± 0.01% ^{3}H, $P < 0.01$) after 4 h. In case of brown adipose tissue the retention of ^{14}C was higher than that of ^{3}H only after 4 h (Fig. 1C). In both colon (Fig. 1D) and white adipose tissue (data not shown) the recovery of ^{3}H slightly exceeded that of ^{14}C after 1 and 2 h, and the values were similar after 4 h. The radioactivity in these organs was, however, very low, e.g., after 1, 2, and 4 h the average recoveries of ^{3}H per g white adipose tissue were 0.005, 0.011 and 0.010%,

TABLE 1. $^{14}C/^{3}H$ ratios in total lipids from different tissues at various time intervals after intragastric feeding of [^{14}C]20:4 and [^{3}H]20:5

Tissue	Ratio at 1 h	2 h	4 h
Serum	0.9 ± 0.0	1.4 ± 0.1	2.2 ± 0.1
Stomach with contents	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
Small intestine contents	1.2 ± 0.0	1.2 ± 0.0	1.5 ± 0.2
Upper small intestine	1.3 ± 0.0	1.6 ± 0.0	2.3 ± 0.1
Lower small intestine	1.2 ± 0.0	1.3 ± 0.1	1.9 ± 0.1
Colon	0.7 ± 0.1	0.9 ± 0.0	1.0 ± 0.0
Liver	0.9 ± 0.0	1.1 ± 0.1	1.8 ± 0.1
Heart	1.4 ± 0.1	2.2 ± 0.1	3.9 ± 0.2
Kidney	1.0 ± 0.1	1.2 ± 0.0	1.7 ± 0.1
Spleen	1.0 ± 0.1	1.2 ± 0.0	1.8 ± 0.1
Brown adipose tissue	1.0 ± 0.1	1.0 ± 0.1	1.6 ± 0.3
White adipose tissue	0.5 ± 0.1	0.8 ± 0.1	1.0 ± 0.1
Lungs	0.9 ± 0.0	1.1 ± 0.0	1.3 ± 0.0
Testes	0.6 ± 0.1	1.6 ± 0.1	2.6 ± 0.1

Data are means ± SE of six animals.

respectively. The recovery of ^{14}C was the same after 4 h and slightly lower after 1 and 2 h.

Thus, in all organs the $^{14}C/^{3}H$ ratios increased with time, but the values varied between 0.5 (white adipose tissue) and 1.4 (heart) after 1 h and between 1.0 (stomach, colon and white adipose tissue) and 3.9 (heart) after 4 h (Table 1).

There were considerable variations in the radioactivity per tissue weight among the different organs. Except for stomach and upper small intestine, liver and brown adipose tissue contained most radioactivity per gram tissue after 4 h (Table 2). Both the ^{14}C and the ^{3}H recoveries per gram in different tissues ranked as follows: liver and brown adipose tissue > kidney > heart, lungs and spleen, > colon, > white adipose tissue and testes (Table 2). The differences between the tissues with highest (liver and brown adipose tissue) and those with lowest radioactivity per gram tissue (white adipose tissue, testes) were about 50-fold, whereas the difference between liver and kidneys was only about two-fold (Table 2).

Distribution of radioactivity among different lipid classes

Distribution of radioactivity in neutral lipids is in **Table 3**; radioactivity in phospholipids is in **Table 4**.

In serum, percent ^{3}H in TG exceeded that of ^{14}C, whereas in phospholipids the percent ^{14}C was higher than that of ^{3}H. Percent radioactivity in TG decreased and percent in phospholipids increased with time for both isotopes. The proportions of the ^{3}H radioactivity found in FFA and DG fractions also exceeded those of ^{14}C. The relative distribution of ^{3}H and ^{14}C among TG, DG, and FFA was, however, similar. The proportions of both ^{3}H and ^{14}C in PC and CE were high and increased with time, e.g., after 4 h, 40.6 (±0.1)% of the ^{14}C and 29.5 (±1.3)% of the ^{3}H ($P < 0.01$) was in PC (Table 4) and 38.4 (±1.1)% of the ^{14}C and 41.2 (±2.0)% of the ^{3}H (ns) in CE (Table 3). The PI radioactivity increased with time and accounted for 2.4 (±0.3)% of the ^{14}C and 1.5 (±0.1)% of the ^{3}H ($P < 0.05$) after 4 h. The radioactivity in PE did not exceed 1%. Percent ^{3}H and ^{14}C in lysoPC were similar and did not exceed 4% (data not shown). The amount of radioactivity migrating as PS and CL did not exceed 0.5% (data not shown).

In upper small intestine, the proportion of ^{3}H found in TG was somewhat higher than that of ^{14}C. The proportion TG decreased with time and that in phospholipids increased correspondingly. Among the individual phospholipids, PC and PE (Table 4) contained the largest proportions of both ^{3}H and ^{14}C, although the differences between the two isotopes were relatively small, e.g., after 4 h, 22.4 (±1.0)% of the ^{3}H and 24.7 (±1.5)% of the ^{14}C ($P < 0.05$) was in PC, and 10.1 (±0.7)% of the ^{3}H and 11.3 (±0.8)% of the ^{14}C ($P < 0.05$) was in PE. In the PI fraction, the proportion of ^{14}C was higher than that of ^{3}H after 4 h (5.3 ± 0.3 and 2.7 ± 0.1%, respectively, $P < 0.001$). The fractions migrating as cardiolipin and phosphatidic acid contained more ^{3}H than ^{14}C, although only small amounts of radioactivity were found in these fractions. Percent ^{3}H and ^{14}C in phosphatidylserine (PS) did not differ (Table 4).

The differences between ^{3}H and ^{14}C in the distribution among different lipid classes were larger in the liver than in the intestine. The percent ^{3}H in liver nonpolar lipids and TG was higher than the percent ^{14}C. Correspondingly less ^{3}H than ^{14}C was found in phospholipids (Table 4). In PC the proportion of ^{14}C was about twofold higher than that of ^{3}H. Also, the ^{14}C radioactivity in PI clearly exceeded that of ^{3}H, the difference being more than twofold at all time intervals. Also, in PE and PS the percent ^{14}C was higher than that of ^{3}H but the numerical

TABLE 2. Lipid radioactivity per g tissue at 4 h after intragastric feeding of [^{14}C]20:4 and [^{3}H]20:5

Tissue	^{14}C	^{3}H
	%	
Serum	0.33	0.15
Stomach	3.17	3.03
Colon	0.05	0.05
Liver	0.28	1.25
Heart	0.39	0.10
Lungs	0.23	0.18
Kidney	1.17	0.68
Spleen	0.35	0.20
Testes	0.04	0.02
White adipose tissue	0.02	0.02
Brown adipose tissue	2.68	1.92

Values (mean) are expressed as % of given dose.

TABLE 3. Neutral lipid radioactivity in serum, upper small intestine, and liver at various time intervals

Tissue	Time	DG 1,2	DG 1,3	FFA	TG	CE
	h			*% distribution*		
Serum						
^{14}C	1	1.3 ± 0.2	0.4 ± 0.1	2.2 ± 0.4	64.7 ± 7.0	6.2 ± 1.8
^{14}C	2	0.7 ± 0.1	0.2 ± 0.1	2.1 ± 0.3	20.2 ± 2.6	17.9 ± 2.1
^{14}C	4	0.1 ± 0.1	0.0 ± 0.0	1.3 ± 0.2	6.6 ± 0.9	38.4 ± 1.1
^{3}H	1	2.1 ± 0.3[a]	1.3 ± 0.1[b]	4.4 ± 0.7[b]	78.1 ± 4.7[b]	4.1 ± 1.5[b]
^{3}H	2	1.9 ± 0.2[c]	1.0 ± 0.0[c]	4.3 ± 0.4[c]	41.9 ± 3.5[c]	17.2 ± 2.2
^{3}H	4	1.0 ± 0.1[c]	0.6 ± 0.1[c]	3.1 ± 0.3[c]	23.6 ± 2.3[c]	41.2 ± 2.0
Upper small intestine						
^{14}C	1	3.4 ± 0.1	0.6 ± 0.2	24.1 ± 3.0	37.1 ± 3.0	0.1 ± 0.0
^{14}C	2	2.8 ± 0.2	0.3 ± 0.0	27.6 ± 2.8	24.3 ± 0.7	0.1 ± 0.0
^{14}C	4	3.2 ± 0.6	0.5 ± 0.1	27.6 ± 2.0	15.8 ± 0.7	0.1 ± 0.0
^{3}H	1	4.2 ± 0.2[b]	1.4 ± 0.1[b]	20.2 ± 1.9[a]	54.1 ± 2.8[c]	0.4 ± 0.1[c]
^{3}H	2	3.7 ± 0.1[a]	1.0 ± 0.1[c]	23.6 ± 1.9[b]	40.9 ± 0.8[c]	0.5 ± 0.0[c]
^{3}H	4	3.5 ± 0.2	1.0 ± 0.1[b]	23.9 ± 1.7[a]	30.4 ± 1.4[c]	0.6 ± 0.1[c]
Liver						
^{14}C	1	4.1 ± 0.3	0.2 ± 0.1	20.6 ± 1.9	23.0 ± 2.0	0.3 ± 0.1
^{14}C	2	3.2 ± 0.3	0.3 ± 0.1	16.2 ± 1.8	13.9 ± 1.7	0.8 ± 0.1
^{14}C	4	1.6 ± 0.1	0.2 ± 0.0	4.9 ± 0.6	6.7 ± 0.6	1.1 ± 0.0
^{3}H	1	3.6 ± 0.2	1.3 ± 0.1[c]	29.6 ± 1.2[b]	38.7 ± 1.5[c]	0.9 ± 0.1[b]
^{3}H	2	3.1 ± 0.1	1.3 ± 0.1[c]	31.0 ± 2.0[c]	31.7 ± 2.3[c]	1.3 ± 0.2[b]
^{3}H	4	2.7 ± 0.2[b]	1.1 ± 0.1[c]	20.2 ± 2.5[c]	35.3 ± 2.9[c]	2.7 ± 0.2[b]

Values represent means ± SE of six animals. DG, diacylglycerol; FFA, free fatty acids; TG, triacylglycerol; CE, cholesteryl esters.
[a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$.

TABLE 4. Phospholipid radioactivity in different tissues at various time intervals

Tissue	Time	PC	PS	PI	PE	PA	CL	Nonpolar Lipids
	h				*% distribution*			
Serum								
^{14}C	1	17.5 ± 3.1	0.0 ± 0.0	0.3 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.4 ± 0.2	81.0 ± 3.3
^{14}C	2	48.0 ± 2.2	0.0 ± 0.0	1.4 ± 0.2	0.5 ± 0.1	0.0 ± 0.0	0.2 ± 0.1	47.7 ± 2.4
^{14}C	4	40.6 ± 1.0	0.0 ± 0.0	2.4 ± 0.3	0.2 ± 0.1	0.0 ± 0.0	0.2 ± 0.1	54.9 ± 1.2
^{3}H	1	8.3 ± 2.1[b]	0.2 ± 0.0[a]	0.3 ± 0.1	0.4 ± 0.1	0.2 ± 0.0	0.3 ± 0.1	89.3 ± 2.6[b]
^{3}H	2	28.1 ± 1.9[c]	0.3 ± 0.0[c]	0.7 ± 0.1[b]	0.5 ± 0.1	0.2 ± 0.0[c]	0.3 ± 0.0	68.0 ± 2.2[c]
^{3}H	4	29.5 ± 1.3[b]	0.3 ± 0.0[c]	1.5 ± 0.1[a]	0.8 ± 0.2[b]	0.2 ± 0.0[b]	0.3 ± 0.1	65.2 ± 1.1[b]
Upper small intestine								
^{14}C	1	19.6 ± 1.7	0.5 ± 0.1	2.5 ± 0.3	4.5 ± 0.2	0.2 ± 0.0	0.1 ± 0.0	72.5 ± 2.2
^{14}C	2	22.8 ± 1.4	0.9 ± 0.1	3.2 ± 0.5	6.8 ± 0.5	0.2 ± 0.0	0.3 ± 0.1	65.7 ± 2.3
^{14}C	4	24.7 ± 1.5	1.7 ± 0.1	5.3 ± 0.3	11.3 ± 0.8	0.5 ± 0.1	0.4 ± 0.1	55.8 ± 2.6
^{3}H	1	13.5 ± 1.2[a]	0.5 ± 0.1	1.0 ± 0.1[c]	2.3 ± 0.2[c]	0.2 ± 0.0[a]	0.3 ± 0.0[a]	81.7 ± 1.5[b]
^{3}H	2	19.0 ± 1.1[a]	0.8 ± 0.1	2.2 ± 0.6	4.6 ± 0.4[c]	0.4 ± 0.0[c]	0.5 ± 0.0[c]	71.8 ± 2.1[b]
^{3}H	4	22.4 ± 1.0[a]	1.7 ± 0.1	2.7 ± 0.1[c]	10.1 ± 0.7[a]	0.8 ± 0.1[c]	0.9 ± 0.1[b]	60.5 ± 1.7[a]
Liver								
^{14}C	1	29.0 ± 3.0	0.9 ± 0.1	2.1 ± 0.2	7.3 ± 1.0	0.0 ± 0.0	0.1 ± 0.1	60.1 ± 4.1
^{14}C	2	40.4 ± 3.0	1.5 ± 0.1	2.7 ± 0.2	9.8 ± 0.6	0.2 ± 0.0	0.3 ± 0.1	44.0 ± 3.7
^{14}C	4	57.6 ± 0.8	2.3 ± 0.1	5.0 ± 0.1	13.2 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	19.9 ± 0.7
^{3}H	1	15.3 ± 1.4[b]	0.7 ± 0.1[a]	1.0 ± 0.1[c]	5.2 ± 0.6[a]	0.4 ± 0.1[b]	0.9 ± 0.1[b]	75.7 ± 2.2[b]
^{3}H	2	21.1 ± 2.1[c]	0.9 ± 0.1[c]	1.0 ± 0.1[c]	6.3 ± 0.5[c]	0.3 ± 0.0[a]	0.8 ± 0.1[c]	68.7 ± 2.7[c]
^{3}H	4	29.9 ± 0.9[c]	1.6 ± 0.1[c]	1.9 ± 0.1[c]	11.2 ± 0.3[b]	0.5 ± 0.1[c]	0.9 ± 0.0[c]	53.1 ± 0.1[c]
Heart								
^{14}C	1	30.2 ± 2.1	0.4 ± 0.2	1.8 ± 0.6	2.1 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	65.6 ± 2.5
^{14}C	2	47.6 ± 1.8	0.9 ± 0.1	3.9 ± 0.3	5.4 ± 0.4	0.0 ± 0.0	0.3 ± 0.1	41.6 ± 2.4
^{14}C	4	56.2 ± 2.4	0.8 ± 0.1	4.4 ± 0.4	9.1 ± 0.5	0.1 ± 0.1	0.6 ± 0.2	28.6 ± 3.0
^{3}H	1	13.7 ± 1.5[c]	1.1 ± 0.2	1.8 ± 0.3	2.4 ± 0.4	1.1 ± 0.2[b]	1.6 ± 0.1[c]	77.4 ± 2.5[c]
^{3}H	2	27.0 ± 1.8[c]	1.3 ± 0.2[a]	2.8 ± 0.3[c]	4.7 ± 0.4	1.3 ± 0.1[c]	1.6 ± 0.2[c]	60.3 ± 2.8[c]
^{3}H	4	38.2 ± 1.7[b]	2.9 ± 0.4[b]	4.5 ± 0.3	9.2 ± 0.4	2.2 ± 0.3[c]	3.2 ± 0.4[b]	37.7 ± 1.3[a]

Values represent means ± SE of six animals. PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; PE, phosphatidylethanolamine; PA, phosphatidic acid; CL, cardiolipin.
[a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$.

differences were relatively small. In CL and PA the percent ^{3}H was higher than the percent ^{14}C. The radioactivity was low (<1%).

In the heart also there were relatively large differences in the proportions of ^{3}H and of ^{14}C in total phospholipids and in PC, e.g., 56 (±2.4)% of the ^{14}C and 38.2 (±1.7)% of the ^{3}H ($P < 0.01$) was in PC after 4 h. In contrast, the proportions of ^{3}H and ^{14}C in PE did not differ. In comparison with other organs, the proportion of ^{3}H found in cardiolipin was highest in the heart (3.2 ± 0.4%), and clearly exceeded that of the ^{14}C (0.6 ± 0.2%, $P < 0.001$) after 4 h. In contrast to the finding in other tissues, the percent ^{3}H in PS was higher than that of ^{14}C (2.9 ± 0.4 and 0.8 ± 0.1%, respectively, $P < 0.01$, after 4 h) (Table 4).

Incorporation of radioactivity into plasmalogenic phospholipids

When lipid extracts from intestines and livers of animals killed after 4 h were subjected to weak acid hydrolysis, there was no or only a marginal decrease in the proportion of radioactivity migrating as PC and PE (data not shown). There was a decrease in the PE radioactivity of the heart lipid extracts after the acid hydrolysis, which was most pronounced for ^{14}C (**Table 5**). The decrease in the cardiac PC radioactivity did not exceed 5% (data not shown).

Conversion of fatty acids

There was no measurable conversion of [^{14}C]20:4 to other fatty acids in any of the tissues examined, i.e., intestine, liver, heart, and serum. The percent conversion of [^{3}H]20:5 to [^{3}H]22:5 in the liver exceeded conversion to [^{3}H]22:6: 2.7 ± 0.3% of the ^{3}H was found in 22:6 and 7.9 ± 1.1% in 22:5 after 4 h. The percent conversion to both 22:5 and 22:6 was less in the intestine than in the liver. Also the proportion of the serum ^{3}H radioactivity found in 20:5 and 22:6 was less than that in the liver (**Table 6**). After 1 h less than 4% in the intestine and serum was in 22:5 plus 22:6. One-hour values for the liver are not given because the specific radioactivity of the lipids was too low for an adequate analysis.

TABLE 5. Percent of total phosphatidylethanolamine (PE) radioactivity present in plasmalogen PE in heart at different time intervals after administration of [^{14}C]arachidonic acid and [^{3}H]eicosapentaenoic acid

	Time	Acid Hydrolysis		% Ethanolamine Plasmalogen
		Before	After	
	h			
^{14}C	1	3.9 ± 0.8	2.7 ± 0.3	31
^{14}C	2	6.7 ± 0.4	5.0 ± 0.2	25
^{14}C	4	10.8 ± 0.4	7.5 ± 0.4	31
^{3}H	1	2.6 ± 0.4	2.5 ± 0.2	<5
^{3}H	2	4.0 ± 0.7	4.1 ± 0.4	<5
^{3}H	4	7.7 ± 0.5	6.7 ± 0.4	13

Values are given as means ± SE or means.

TABLE 6. Percent distribution of [^{3}H]fatty acid between 20:5, 22:5, and 22:6 in various tissues

Tissue	Time	20:5	22:5	22:6
	h			
Upper small intestine	1	97.0 ± 0.4	1.3 ± 0.3	1.8 ± 0.2
	4	93.1 ± 0.7	4.9 ± 0.6	2.0 ± 0.2
Liver	1			
	4	89.5 ± 1.3	7.9 ± 1.1	2.7 ± 0.3
Serum	1	94.2 ± 1.4	3.6 ± 1.0	2.2 ± 0.6
	4	92.2 ± 0.9	5.2 ± 0.7	2.5 ± 0.5

Values are means ± SE of six animals.

DISCUSSION

This study compares the absorption and incorporation into tissue lipids of [^{3}H]20:5 and [^{14}C]20:4 fed as unesterified fatty acids in an emulsion of fish oil TG. There were considerable similarities in the patterns of incorporation of the two labeled fatty acids into tissue lipids, but also important differences. More ^{14}C than ^{3}H was retained in lipids of several tissues, and a larger proportion of the recovered ^{14}C was in phospholipids. In particular, more ^{14}C than ^{3}H was found in PC and PI, whereas the retention of the two isotopes in PE was more similar.

The partitioning of the labeled fatty acids between mucosal and chyle phospholipids and TG may be an important determinant of their further metabolism, and will be influenced both by their relative affinity for the different acylation pathways and by the mass of different polyunsaturated fatty acids that compete for incorporation into phospholipids (17, 18). In this study the administered lipid emulsion contained 78 mg fish oil TG (12 mg 20:5). Using the same fish oil TG preparation (max EPA), Chernenko et al. (35) found a lipolysis pattern with pancreatic lipase indicating that 55% of the 20:5 was located at primary *sn* 1 and 3 positions. Forty five percent was at the 2 position, and might thus be incorporated into chyle TG by the monoacylglycerol pathway (36). If a similar course of lipolysis occurred in our in vivo experiments, about 7 mg would be released by pancreatic lipase and mixed with labeled fatty acids. Rat bile PC contains about 20% 20:4 at position 2 (37) and the output of PC in bile during the first 1.5 h after application of a bile fistula is about 6 μmol/h (38). About 1.2 μmol 20:4 (approximately 0.4 mg) would then reach the intestine as bile phospholipid every hour. Some 20:4 would also be released from phospholipids and reabsorbed during the normal turnover of the mucosal cells, but the amount is hard to estimate.

More ^{14}C than ^{3}H was retained in intestinal phospholipids after 4 h and more of the ^{3}H in both intestine and

serum was in TG (Table 3). This indicates that more of the [^{3}H]20:5 was incorporated into TG and secreted in chyle lipoproteins, and that more of the [^{14}C]20:4 was incorporated into phospholipids of the intestinal mucosal cells, i.e., the differences were similar to those seen between [^{14}C]18:2 and [^{3}H]20:4 in earlier studies (17, 33). In the present study more of the 20:4 was, however, in TG and less in phospholipids both in serum and intestine, indicating that the fish oil fatty acids competed more efficiently with 20:4 for incorporation into phospholipids, than the fatty acids of other lipid vehicles used in the other studies (triolein, Intralipid, cream).

After 1 h, 78.1 ± 4.7% of the ^{3}H and 64.7 ± 7.0% of the ^{14}C ($P < 0.01$) in serum was in TG. At the longer time intervals the differences in recovery between ^{14}C and ^{3}H, as well as the proportions of both ^{3}H and ^{14}C in PC, increased and the proportions in TG decreased (Table 3). These findings indicate that a major portion of both labeled fatty acids was incorporated into chyle TG, although more [^{14}C]20:4 than [^{3}H]20:5 was partitioned into chyle PC. Since the labeled PC is cleared from blood at a slower rate than the labeled TG (24), this may explain the increase in the ^{14}C to ^{3}H ratio of serum with time.

Eicosapentaenoic acid (20:5) may decrease hepatic and intestinal lipoprotein secretion (39–41) by suppressing the action of acyl-CoA 1,2 diacylglycerol acyltransferase (42) and apoB synthesis (40). In other studies most of the 20:5 was, however, secreted in chyle lipoproteins after a fish oil meal (43, 44), and in the present study the TG radioactivity in intestine and in serum exhibited similar time courses as in earlier studies in which [^{3}H]20:4 was fed in Intralipid or triolein (17). It is unlikely that a fish oil-induced suppression of chylomicron secretion influenced the present data significantly.

In vitro chyle 20:4 and 20:5 esters were hydrolyzed by lipoprotein lipase at a slower rate than the 16–18 carbon fatty acid esters (26, 27), thereby being enriched in TG and DG of chylomicron remnants. Accordingly, more [^{14}C]18:2 than [^{3}H]20:4 appeared in FFA in serum and less in DG after intravenous injection of doubly labeled chylomicrons (24). Similar differences were seen after oral feeding of the two fatty acids (17). In this study the relative distribution among serum TG, DG, and FFA was similar for [^{3}H]20:5 and [^{14}C]20:4, although a larger proportion of the serum ^{3}H was found in all three fractions (Table 3). The data thus indicate that, as in vitro (27), 20:4 and 20:5 esters of the chylomicrons exhibit similar lipolysis patterns. The partitioning between lipolysis by lipoprotein lipase and retention in the chylomicron remnants during the metabolism of chylomicron TG would then be expected to be similar for 20:4 and 20:5.

When [^{14}C]18:2 and [^{3}H]20:4 were given orally (17, 33) or injected as doubly labeled chylomicrons (24), much more [^{3}H]20:4 than [^{14}C]18:2 appeared in serum CE. In the present study large proportions of both [^{3}H]20:5 and [^{14}C]20:4 appeared in serum CE, and the [^{3}H]CE/[^{3}H]PC ratio even exceeded the [^{14}C]CE/[^{14}C]PC ratio (Tables 3 and 4). Since only small amounts of the labeled fatty acids were incorporated into CE in intestine and liver, it is unlikely that they were preferentially secreted in chylomicrons or VLDL. The data rather indicate that LCAT in the rat has a high preference not only for 20:4- (23) but also for 20:5-PC. Detailed in vitro studies on the activity of rat LCAT towards 20:5 are lacking. In fish oil-fed monkeys the reactivity of plasma phospholipids with LCAT was decreased due to the content of n–3 fatty acids in the plasma phospholipids (45). In contrast, Holub, Bakker, and Skeaff (46) using whole human plasma from fish oil-supplemented subjects as substrate concluded that the relative order of reactivity of polyenoic fatty acids with LCAT was 20:5 > 20:4 > 22:6. The activity of LCAT towards 20:5 PC may thus differ with the animal species and with the form of the substrate used.

We thus conclude that [^{3}H]20:5 and [^{14}C]20:4 were partitioned differently between phospholipids and chyle TG in the intestinal mucosal cells, but when appearing in chyle TG and PC the two fatty acids were similarly metabolized by lipases and by LCAT.

The differences in radioactivity per tissue weight between various organs were large, the values being highest in liver and brown adipose tissue (Table 2). These large differences cannot be fully explained. The enrichment of [^{14}C]20:4 and [^{3}H]20:5 esters in remnant TG and DG (27), the metabolism of chyle PE and PC by hepatic lipase (22), and the metabolism of HDL CE formed by LCAT would be expected to favor the uptake of labeled fatty acids by the liver. Brown adipose tissue contains high levels of lipoprotein lipase (47). A high uptake by brown adipose tissue was also a general feature in autoradiographic studies after intravenous injection of long-chain radioactive fatty acids as FFA, including 20:4 and 20:5 (48), and was seen also after oral feeding of [^{14}C]18:2 (49). A high incorporation into liver phospholipids was also seen after intravenous injection of [^{14}C]20:4 as FFA (50). Furthermore there may be a net transfer or exchange of intact radioactive chyle phospholipids that may vary between different tissues. The quantitative roles of the different transport pathways in the distribution of dietary 20:4 and 20:5 to various tissues are therefore difficult to estimate and need further study.

[^{3}H]20:5 may be oxidized faster than [^{14}C]20:4 (32). This could be an important explanation as to why the ^{14}C to ^{3}H ratios in several tissues increased with time. Further information about the rates at which the different eicosanoid precursor fatty acids are oxidized by different tissues is needed. Clarification is also needed as to whether the higher rate of oxidation of 20:5 than of 20:4 is due to a higher activity of the oxidizing enzymes towards this fatty acid, or whether 20:5-CoA is more available for oxidation due to differences in the turnover rate between 20:5- and

20:4-containing molecular species of the glycerophospholipids.

[^{3}H]20:5 and [^{14}C]20:4 may be incorporated into tissue phospholipids either by the pathways for de novo synthesis or by reacylation of lysophospholipids. In general, the preferential location of 20:5 and other polyunsaturated fatty acids to the *sn* 2-position of glycerophospholipids cannot be explained by a preferential utilization of certain DG species for the reaction catalyzing de novo synthesis of PC, PE, or PI (30, 31). In this study the ^{3}H/^{14}C ratio of phosphatidic acid was much higher than those of PC, PE, and PI, suggesting that a major part of the labeling of these glycerophospholipids did not occur via de novo synthesis. In vitro the 1-lysoPC acyl-CoA acyltransferase has a preference for long-chain polyunsaturated fatty acids (51), with 20:4 being a preferred substrate at low acyl-CoA concentrations (52). In microsomal preparations the enzyme uses both n-3 and n-6 fatty acids similarly (53). In vivo, intravenously injected 1-palmitoyl-lysoPC and -lysoPE were both preferentially reacylated with 20:4 after their uptake by the liver (54), and in intestine the 1-lysoPC acyl-CoA acyl transferase catalyzes the formation of large amounts of PC from absorbed 1-lysoPC, thereby contributing a considerable part of the chylomicron PC (55). Newly synthesized PI species reflect the fatty acid composition of available DG species, but the newly synthesized PI undergoes rapid deacylation-reacylation cycles during which stearic acid is preferentially incorporated at position 1 and 20:4 at position 2 (56). With microsomal preparations 1-lysoPI is preferentially acylated with 20:4-CoA in vitro (57), whereas the relative affinity of 20:5-CoA for this enzyme has not been characterized. In view of these observations, it has to be assumed that the 1-lyso-phospholipid acyl-CoA acyl transferases have important roles in the incorporation of dietary 20:4 and 20:5 into phospholipids of both the intestine and other tissues, although their quantitative importance cannot yet be defined.

In liver, intestine, and heart the percent ^{3}H and ^{14}C in PE increased with time, the increase being most pronounced for ^{3}H (Table 4). The reason for this is not clear. Enzymes catalyzing the preferential transfer of 20:4-CoA from PC to lysoPE have, however, been described in several tissues, including heart, lymphocytes, and lungs (58-61) (see also references in 30). Such transacylase activity, which was also active with lysoPI as acceptor, was originally described in the liver (62). The role of these enzymes in the transfer of eicosanoid precursors to PE and PI may well be important, but their activity against n-3 fatty acids has not yet been characterized. Further studies of these enzymes and of the acyl turnover of individual molecular species of the different lysophospholipids, such as those reported in isolated hepatocytes (58), are needed.

In feeding experiments with fish oil, the time courses for the increase in tissue levels of individual n-3 fatty acids vary between organs and phospholipid classes (63-65). For example, in mice the levels of 22:6 but not of 20:5 and 22:5 increased markedly in heart cardiolipin, which normally contains very high levels of 18:2 and little 20:4 (64). In this study the amount of ^{3}H in cardiolipin exceeded that of ^{14}C, particularly in the heart (Table 4). Further studies of the utilization of 20:5 and its interconversion products by the enzymes catalyzing de novo synthesis and reacylation of cardiolipin are necessary to explain this finding.

The patterns for distribution of ^{3}H and ^{14}C may be influenced if a significant interconversion to other labeled fatty acids occurs. In this study the proportions of the [^{3}H]20:5 present in intestine and serum that were converted to 22:5 and 22:6 were relatively small. The distribution of ^{3}H radioactivity in serum and intestine can thus be approximated to reflect the distribution of [^{3}H]20:5. There was little conversion of 20:5 to other fatty acids in the intestine. The proportion of ^{3}H in 22:5 and 22:6 was higher in the liver, but did not exceed 8% in 22:5 and 4% in 22:6. As 22:6 is preferentially incorporated into PE, the interconversion reactions may contribute to the increase in ^{3}H in PE with time. The increase was, however, too large to be explained by fatty acid interconversions only, and was also seen for [^{14}C]20:4.

In summary, the following picture emerges of the fate of the dietary 20:5 in comparison to 20:4. The preference of 20:5 for incorporation into the intestinal and chylomicron phospholipids is less pronounced than for 20:4, but the fish oil fatty acids may compete with 20:4 for incorporation into these phospholipids and thereby increase the proportion of 20:4 that is transported by chyle TG. The action of lipoprotein lipase and LCAT on 20:5 and 20:4 esters during the metabolism of chylomicrons is similar, the TG esters of both fatty acids exhibiting a relative resistance to lipoprotein lipase, and the PC esters a preference for rat LCAT. After the uptake by the tissues, 20:4 is more efficiently retained in PC and PI, whereas the difference in retention in PE is less pronounced. In the case of PI and PE, the differences between [^{3}H]20:5 and [^{14}C]20:4 are smaller than those observed earlier between dietary [^{14}C]18:2 and [^{3}H]20:4 (17, 33). The reasons for the differences in retention between [^{14}C]20:4 and [^{3}H]20:5 and its interconversion products are not clear. The possibility must be considered that small differences in the preference of the various acyl-CoAs for the acylation pathways may be fortified through complex series of reactions, and thereby lead to large differences in the retention of different polyunsaturated fatty acids in phospholipids.

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