ω –3 fatty acids increase the arachidonic acid content of liver cholesterol ester and plasma triacylglycerol fractions in the rat

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Recent studies have demonstrated that dietary fish oils rich in eicosapentaenoic acid ($C_{20:5,\omega3}$) lower the content of arachidonic acid and its metabolites in plasma and tissue phospholipids. The present study examined the fatty acid composition of cholesterol ester and triacylglycerol fractions from plasma and livers of rats fed diets enriched with saturated fatty acids (beef tallow), α -linolenic acid (linseed oil) or eicosapentaenoic acid (fish oil). Feeding diets containing linseed oil or fish oil for 28 days increased arachidonic acid ($C_{20:4,\omega6}$) levels in the cholesterol ester fraction of liver and in the triacylglycerol fraction of the plasma lipids. Plasma cholesterol esters were depleted of $C_{20:4,\omega6}$ after feeding of the diet containing either linseed oil or fish oil. The changes in $C_{20:4,\omega6}$ content cannot be explained by alterations in cholesterol ester or triacylglycerol pools of plasma and liver. These results suggest that the decrease in phospholipid $C_{20:4,\omega6}$ content generally observed after fish oil consumption may be partly due to a shift of $C_{20:4,\omega6}$ from phospholipid to the triacylglycerol and/or cholesterol ester pools in the same tissue. Triacylglycerols and cholesterol esters may therefore play a buffering role in the homeostatic maintenance of tissue phospholipid levels of arachidonic acid.

INTRODUCTION

Several recent studies have established that marine oils rich in eicosapentaenoic ($C_{20:5,\omega3}$) and/or docosahexaenoic ($C_{22:6,\omega3}$) acids have anti-aggregatory effects on the manifestation of thrombosis [1–5]. These anti-aggregatory effects are mediated by alterations in the pattern of eicosanoid production after the consumption of fish oil [2,6–8]. For example, fish-oil supplementation has been shown to decrease thromboxane A_2 (TxA_2) and prostacyclin (PGI_2) levels [7]. It has been proposed that $C_{20:5,\omega3}$ present in fish oils competes with arachidonic acid ($C_{20:4,\omega6}$) at the level of cyclo-oxygenase and thus results in the synthesis of TxA_3 and PGI_3 , which have weak pro-aggregatory thrombotic properties, preventing platelet aggregation [7,8]. It has also been suggested that a decreased $C_{20:4,\omega6}/C_{20:5,\omega3}$ ratio in platelet phospholipids may be responsible for decreased platelet aggregation after consumption of fish oil [9]. $C_{20:5,\omega3}$ present in fish oils may affect release of $C_{20:4,\omega6}$ from phospholipids, which provides the common precursor for synthesis of eicosanoids [10]. For tissues either dependent upon liver as a source of $C_{20:4,\omega6}$ synthesis or exhibiting similar metabolic control over $C_{20:4,\omega6}$ synthesis, it is reasonable to expect that limitation in the biosynthesis of $C_{20:4,\omega6}$ may limit production of eicosanoids in that tissue. We have recently shown that fish oil decreases the activity of Δ^6 -desaturase [11], which is a rate-limiting enzyme in the biosynthetic pathway leading to formation of $C_{20:4,\omega6}$ [12,13]. Fish oil also inhibits the rate of conversion of eicosatrienoic acid ($C_{20:3,\omega6}$) into $C_{20:4,\omega6}$ by inhibiting Δ^5 -desaturase activity [14]. In the present study we

provide evidence suggesting that the decrease in $C_{20:4,\omega6}$ content of membrane and tissue phospholipids may be at least in part due to a shift of $C_{20:4,\omega6}$ from phospholipid to cholesterol ester and/or triacylglycerol pools.

For this purpose we examined the fatty acid composition of cholesterol ester and triacylglycerol fractions in plasma and liver lipids after feeding rats diets supplemented with fish oil. To identify whether or not the specificity of fish-oil action can be attributed primarily to $C_{20:5,\omega3}$ and $C_{22:6,\omega3}$ fatty acids, a separate group of animals was also fed a diet enriched with linseed oil to provide high levels of α -linolenic acid ($C_{18:3,\omega3}$), a precursor of $C_{20:5,\omega3}$ and $C_{22:6,\omega3}$.

MATERIALS AND METHODS

Fish oil (S-28GA) was purchased from Nissho Iwai American Corp., New York, NY, U.S.A. Beef tallow was supplied by Canada Packers, Edmonton, Alberta, Canada, and linseed oil was obtained from a local health-food store. All solvents were redistilled before use.

Animals and diets

Male Sprague–Dawley rats (50–60 g) were allotted to individual stainless-steel cages and provided with water and chow for 3 days before they were fed the experimental diets. Animals were weighed and divided into three groups of 12 rats per group so that the average body weight was similar in each group. Each group of animals was fed a semi-synthetic diet enriched with saturated fatty acids, α -linolenic acid or eicosapentaenoic + docosa-

Abbreviations used: TxA_2 , thromboxane A_2 ; PGI_2 , prostaglandin I_2 (prostacyclin); specific fatty acids, identified in the text, are expressed in the form $C_{x:y,\omega z}$; LCAT, lecithin (phosphatidylcholine) acyltransferase; ACAT, acyl-CoA:cholesterol acyltransferase.

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Table 1. Fatty acid composition of experimental diets

Fatty acids are designated by the number of carbon atoms followed by the number of double bonds. At least four random diet samples were taken for analysis. Composition of the fats added to the semi-purified diet is detailed in the Materials and methods section. The basal diet contained the following components (g/kg of diet): casein, 270; starch, 200; glucose, 207; non-nutritive cellulose, 50; vitamin mix, 10; mineral mix, 50; choline, 2.75; L-methionine, 2.5; and inositol, 6.25 (as described elsewhere [15]).

Diet fatty acid(s)	Composition (% w/w)		
	Beef tallow	Linseed oil	Fish oil
C _{14:0}	0.7	0.1	6.7
C _{16:0}	28.1	10.4	8.3
C _{16:1,ω7}	3.2	0.7	13.9
C _{17:0}	1.4	0.3	2.2
C _{18:0}	18.4	6.4	8.6
C _{18:1,ω9}	34.1	23.8	9.5
C _{18:1,ω7}	2.8	1.1	3.7
C _{18:2,ω6}	11.0	16.4	2.0
C _{18:3,ω3}	0.3	40.5	5.4
C _{20:4,ω6}	—	0.1	1.3
C _{20:5,ω3}	—	—	28.1
C _{22:5,ω3}	—	0.1	2.4
C _{22:6,ω3}	—	—	8.0
Total			
Saturated	48.6	17.2	25.8
Monounsaturated	40.1	25.6	27.1
ω-6	11.0	16.4	3.3
ω-3	0.3	40.7	43.7

hexaenoic acids. Diets were prepared by mixing either 180 g of hydrogenated beef tallow plus 20 g of safflower oil (beef-tallow diet), or 160 g of linseed oil plus 40 g of beef tallow (linseed-oil diet), or 200 g of fish oil (fish-oil diet) with 800 g of a fat-deficient, but otherwise nutritionally complete, basal diet described in detail elsewhere [15]. The animals were kept in a well-ventilated room maintained at 22 ± 2 °C and 12 h-light/12 h-dark cycle. Food and water were provided *ad libitum*. The preparation, and fatty acid composition, of experimental diets is presented in Table 1. The beef-tallow diet provided high levels of saturated and monounsaturated fatty acids (88.7%). Both diets (either linseed-oil or fish-oil) had similar amounts of ω-3 fatty acids (approx. 40%), but differed in the type of ω-3 fatty acids present. The diet containing linseed oil was enriched with C_{18:3,ω3}, whereas the diet containing fish oil had high levels of C_{20:5,ω3} and C_{22:6,ω3}.

Analytical procedures

After 28 days of feeding, animals were killed by decapitation between 08:00 and 10:00 h. Flowing trunk blood was collected in heparinized tubes and livers were quickly excised. Plasma was obtained by centrifuging the blood samples (1500 g for 10 min). Lipids from plasma and liver tissue were extracted with chloroform/methanol (2:1, v/v) [16]. Total and non-esterified cholesterol content were estimated in aliquots of plasma and liver lipid extracts by an enzymic method [17,18]. A difference between total cholesterol and unesterified cholesterol

content gave a measure for the esterified cholesterol content. Triacylglycerols from plasma and liver lipids were isolated by shaking with n-nonane/propan-2-ol (4:7, v/v) [19] and determined by the method described previously [20]. Cholesterol ester and triacylglycerol fractions were separated by t.l.c. on silica-gel G plates using a solvent system comprising hexane/diethyl ether/acetic acid (80:20:1, by vol.). Lipid fractions were saponified by heating with 0.5 M-KOH in methanol at 100 °C for 1 h, followed by methylation with 14% (w/w) methanolic BF₃ [21]. The fatty acid composition was determined by automated g.l.c. (Varian-6000) of methyl esters using a fused silica capillary column (BP20; bonded phase; Analtech, Melbourne, Australia) as reported previously [22].

Statistical analysis

The results are shown as means ± S.D. The effect of dietary treatments was examined by analysis-of-variance procedures. Comparison between individual diets was made by using Duncan's multiple-range test [23].

RESULTS

Cholesterol ester and triacylglycerol content of plasma and liver

Feeding fish oil lowered the cholesterol ester content of rat plasma by 47.4%, whereas feeding linseed oil had no significant effect when compared with the control group fed on beef tallow (Fig. 1). In liver tissue, the cholesterol ester content was lowered to a greater extent (83.8%) than in the plasma by feeding fish oil. The liver cholesterol ester level, however, remained unchanged by feeding the linseed-oil diet (Fig. 1).

Dietary fish oil lowered triacylglycerol levels in rat plasma and liver by 53 and 68.8% respectively, whereas feeding the linseed-oil diet had no significant effect in comparison with the animals fed on beef tallow (Fig. 2).

Fatty acid composition of cholesterol esters

The arachidonic acid (C_{20:4,ω6}) content of the liver cholesterol ester fraction increased significantly in the animals fed on diets containing linseed oil or fish oil (Table 2). The increase in the C_{20:4,ω6} content of the cholesterol ester fraction after feeding diets containing

Fig. 1. Effect of dietary ω-3 fatty acids on cholesterol ester content of rat plasma and liver

Values are the means ± S.D. for six animals (*n* = 6). Values without a common superscript are significantly different (*P* < 0.05). ▨, Beef tallow; ■, linseed oil; ▩, fish oil.

fish oil was considerably greater than observed after feeding diets containing linseed oil. The monounsaturated-fatty-acid content of the liver cholesterol ester fraction decreased after feeding diets containing linseed oil or fish oil in comparison with animals fed the diet containing beef tallow. Feeding either the diet containing linseed oil or fish oil resulted in the accumulation of $\omega-3$ fatty acids ($C_{18:3}$, $C_{20:5}$, $C_{22:5}$ and $C_{22:6}$) in the liver cholesterol ester fraction.

In the plasma cholesterol ester fraction, the level of $C_{20:4,\omega6}$ was lower, whereas the $C_{20:5,\omega3}$ and $C_{22:6,\omega3}$ content was higher, in animals fed diets providing high levels of $\omega-3$ fatty acids, i.e. the linseed-oil or the fish-oil diet (Table 2). Oleic acid ($C_{18:1,\omega9}$) content of the cholesterol ester fraction in the plasma was significantly reduced by feeding diets containing linseed oil or fish oil (26 and 55 % respectively).

Fig. 2. Effect of dietary $\omega-3$ fatty acids on triacylglycerol content of rat plasma and liver

Values are means $\pm$ S.D. for six animals ($n = 6$). Values without a common superscript are significantly different ($P < 0.05$). ▨, Beef tallow; ■, linseed oil; ▤, fish oil.

Fatty acid composition of triacylglycerols

Fatty acid profiles of plasma triacylglycerols resemble the dietary fatty acid composition fed, except for the presence of $C_{20:4,\omega6}$ (Table 3). After the feeding of diets containing linseed oil or fish oil, the plasma lipid triacylglycerol fraction was enriched with $C_{20:4,\omega6}$. The enrichment was greater when feeding diets containing fish oil (approx. 6-fold) than when feeding diets containing linseed oil (approx. 2-fold) (Table 3). $C_{18:1,\omega9}$ content was significantly decreased by feeding diets containing linseed oil or fish oil.

Triacylglycerols from the livers of rats fed the beef-tallow diet contained a relatively higher $C_{18:1,\omega9}$ content (53 %) than that observed in plasma triacylglycerols (37 %) (Table 3). Feeding three fatty-acid-enriched diets lowered the $C_{18:1,\omega9}$ content, the decrease being greater when feeding the diet containing fish oil (56 versus 42 % for the linseed-oil diet). Fatty acids of the $\omega-3$ family accumulated in liver triacylglycerols after the feeding of diets containing fish oil. Feeding linseed oil led to enrichment of liver triacylglycerols with $C_{18:3,\omega3}$ and, to a relatively smaller extent, metabolites of $C_{18:3,\omega3}$ (i.e. $C_{20:5}$, $C_{22:5}$ and $C_{22:6}$) (Table 3).

DISCUSSION

The primary function of triacylglycerols is to provide a store of chemical fuel that can be used as energy by tissue cells [24,25]. Cholesterol is chiefly transported in the blood in the form of cholesterol esters [26,27]. Cholesterol esters may also serve as stored form of cholesterol in tissues [26,27]. The present study suggests that triacylglycerols and cholesterol esters may play a dynamic role in the maintenance of lipid homeostasis under some external or internal constraints (e.g., under the influence of dietary $\omega-3$ -fatty-acid treatment in this particular study). Reports from our laboratory [11,14] and others [28–30] have recently demonstrated that

Table 2. Effect of dietary $\omega-3$ fatty acids on fatty acid composition of liver and plasma cholesterol esters

Rats were fed experimental diets for 4 weeks. Values are means $\pm$ S.D. for animals in each dietary group. Values without a common superscript are significantly different ($P < 0.05$).

Diet fatty acid	Composition (w/w)					
	Liver			Plasma		
	Beef tallow	Linseed oil	Fish oil	Beef tallow	Linseed oil	Fish oil
$C_{16:0}$	14.1 $\pm$ 1.7 ^a	7.4 $\pm$ 0.7 ^b	24.3 $\pm$ 1.0 ^c	8.1 $\pm$ 0.6 ^a	7.5 $\pm$ 0.7 ^a	10.3 $\pm$ 0.8 ^b
$C_{16:1,\omega7}$	5.9 $\pm$ 1.3 ^a	1.9 $\pm$ 0.3 ^c	8.1 $\pm$ 1.5 ^b	3.9 $\pm$ 0.5 ^a	1.4 $\pm$ 0.7 ^b	8.6 $\pm$ 0.9 ^c
$C_{18:0}$	4.0 $\pm$ 0.3	4.7 $\pm$ 0.4	4.3 $\pm$ 0.4	1.7 $\pm$ 0.5 ^a	0.8 $\pm$ 0.4 ^b	0.8 $\pm$ 0.2 ^b
$C_{18:1,\omega9}$	58.4 $\pm$ 2.5 ^a	34.4 $\pm$ 2.1 ^b	16.8 $\pm$ 1.1 ^c	16.8 $\pm$ 0.5 ^a	12.4 $\pm$ 1.0 ^b	7.6 $\pm$ 0.8 ^c
$C_{18:1,\omega7}$	0.4 $\pm$ 0.1 ^a	0.7 $\pm$ 0.2 ^a	1.5 $\pm$ 0.6 ^b	1.0 $\pm$ 0.2 ^a	0.2 $\pm$ 0.1 ^b	0.9 $\pm$ 0.1 ^a
$C_{18:2,\omega6}$	5.2 $\pm$ 0.7 ^a	13.9 $\pm$ 0.7 ^b	2.3 $\pm$ 0.8 ^c	19.8 $\pm$ 0.6 ^a	32.8 $\pm$ 2.2 ^b	2.5 $\pm$ 0.2 ^c
$C_{18:3,\omega6}$	0.3 $\pm$ 0.1 ^a	—	0.5 $\pm$ 0.1 ^b	0.2 $\pm$ 0.1 ^a	0.4 $\pm$ 0.3 ^{ab}	0.4 $\pm$ 0.1 ^b
$C_{18:3,\omega3}$	0.5 $\pm$ 0.2 ^a	15.7 $\pm$ 0.9 ^b	1.8 $\pm$ 0.3 ^c	—	4.3 $\pm$ 2.2 ^a	0.2 $\pm$ 0.1 ^b
$C_{20:3,\omega9}$	0.2 $\pm$ 0.1 ^a	—	0.7 $\pm$ 0.1 ^b	0.3 $\pm$ 0.1	0.2 $\pm$ 0.1	—
$C_{20:3,\omega6}$	3.6 $\pm$ 0.3 ^a	4.3 $\pm$ 0.6 ^a	2.4 $\pm$ 0.3 ^b	0.4 $\pm$ 0.1	0.3 $\pm$ 0.2	0.3 $\pm$ 0.1
$C_{20:4,\omega6}$	4.9 $\pm$ 0.7 ^a	9.3 $\pm$ 0.5 ^b	24.3 $\pm$ 2.4 ^c	45.5 $\pm$ 1.2 ^a	29.6 $\pm$ 2.1 ^b	18.1 $\pm$ 0.6 ^c
$C_{20:5,\omega3}$	0.2 $\pm$ 0.1 ^a	3.0 $\pm$ 0.3 ^b	7.9 $\pm$ 0.9 ^c	0.2 $\pm$ 0.1 ^a	8.6 $\pm$ 1.3 ^b	46.8 $\pm$ 1.7 ^c
$C_{20:5,\omega6}$	—	—	—	0.3 $\pm$ 0.1	—	—
$C_{22:5,\omega3}$	0.2 $\pm$ 0.1 ^a	0.6 $\pm$ 0.1 ^b	1.1 $\pm$ 0.1 ^c	—	0.1 $\pm$ 0.1	0.2 $\pm$ 0.0
$C_{22:6,\omega3}$	0.3 $\pm$ 0.2 ^a	1.3 $\pm$ 0.4 ^b	2.9 $\pm$ 1.1 ^c	0.5 $\pm$ 0.1 ^a	1.4 $\pm$ 0.2 ^b	2.6 $\pm$ 0.2 ^c

Table 3. Effect of dietary $\omega-3$ fatty acids on fatty acid composition of liver and plasma triacylglycerols

Rats were fed the lipid supplemented diets for 4 weeks. Values are means $\pm$ S.D. for six animals in each dietary group. Values without a common superscript are significantly different ($P < 0.05$).

Diet fatty acid	Composition (% w/w)					
	Liver			Plasma		
	Beef tallow	Linseed oil	Fish oil	Beef tallow	Linseed oil	Fish oil
C _{14:0}	1.4 $\pm$ 0.3 ^a	0.4 $\pm$ 0.1 ^b	1.7 $\pm$ 0.4 ^a	1.5 $\pm$ 1.0 ^a	1.2 $\pm$ 0.2 ^a	4.1 $\pm$ 0.9 ^b
C _{16:0}	24.2 $\pm$ 0.5 ^a	14.3 $\pm$ 0.8 ^b	13.5 $\pm$ 1.8 ^b	28.4 $\pm$ 1.5 ^a	14.3 $\pm$ 0.8 ^b	11.2 $\pm$ 1.0 ^c
C _{16:1,ω7}	4.4 $\pm$ 0.7 ^a	1.9 $\pm$ 0.2 ^b	7.9 $\pm$ 0.9 ^c	4.2 $\pm$ 0.4 ^a	0.8 $\pm$ 0.5 ^b	9.6 $\pm$ 1.8 ^c
C _{17:0}	0.9 $\pm$ 0.2 ^a	0.3 $\pm$ 0.1 ^b	0.6 $\pm$ 0.1 ^a	1.0 $\pm$ 0.1 ^a	0.4 $\pm$ 0.3 ^b	1.4 $\pm$ 0.2 ^c
C _{18:0}	3.6 $\pm$ 0.3 ^a	3.5 $\pm$ 0.2 ^a	2.8 $\pm$ 0.4 ^b	8.5 $\pm$ 0.3 ^a	5.0 $\pm$ 0.4 ^b	2.1 $\pm$ 0.5 ^c
C _{18:1,ω9}	52.6 $\pm$ 0.6 ^a	30.6 $\pm$ 1.2 ^b	23.1 $\pm$ 1.2 ^c	37.1 $\pm$ 2.0 ^a	23.8 $\pm$ 1.5 ^b	9.7 $\pm$ 0.4 ^c
C _{18:1,ω7}	2.4 $\pm$ 0.3 ^a	0.8 $\pm$ 0.2 ^b	1.5 $\pm$ 0.4 ^c	1.0 $\pm$ 0.3 ^a	0.5 $\pm$ 0.1 ^b	1.8 $\pm$ 0.4 ^a
C _{18:2,ω6}	7.2 $\pm$ 0.8 ^a	18.6 $\pm$ 0.2 ^b	4.4 $\pm$ 0.3 ^c	10.2 $\pm$ 1.0 ^a	17.4 $\pm$ 0.8 ^b	2.3 $\pm$ 0.1 ^c
C _{18:3,ω6}	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	0.3 $\pm$ 0.2	—	—	0.3 $\pm$ 0.1
C _{18:3,ω3}	0.2 $\pm$ 0.1 ^a	18.0 $\pm$ 1.3 ^b	2.4 $\pm$ 0.3 ^c	0.4 $\pm$ 0.1 ^a	28.3 $\pm$ 3.0 ^b	1.4 $\pm$ 0.2 ^c
C _{20:3,ω9}	—	—	—	0.2 $\pm$ 0.1 ^a	0.2 $\pm$ 0.0 ^a	0.9 $\pm$ 0.3 ^b
C _{20:3,ω6}	—	—	—	0.7 $\pm$ 0.1 ^a	0.4 $\pm$ 0.1 ^a	1.1 $\pm$ 0.1 ^b
C _{20:4,ω6}	0.4 $\pm$ 0.1 ^a	0.7 $\pm$ 0.1 ^b	1.1 $\pm$ 0.2 ^c	2.3 $\pm$ 0.5 ^a	4.1 $\pm$ 0.6 ^b	13.9 $\pm$ 1.2 ^c
C _{20:5,ω3}	0.1 $\pm$ 0.1 ^a	3.0 $\pm$ 0.4 ^b	14.6 $\pm$ 2.2 ^c	0.1 $\pm$ 0.0 ^a	1.9 $\pm$ 0.6 ^b	26.8 $\pm$ 2.8 ^c
C _{22:5,ω3}	—	3.0 $\pm$ 0.5 ^a	5.7 $\pm$ 1.0 ^b	0.2 $\pm$ 0.1 ^a	1.3 $\pm$ 0.4 ^b	2.6 $\pm$ 0.3 ^c
C _{22:6,ω3}	0.2 $\pm$ 0.1 ^a	3.3 $\pm$ 0.6 ^b	15.6 $\pm$ 2.2 ^c	0.2 $\pm$ 0.1 ^a	1.6 $\pm$ 0.6 ^b	9.7 $\pm$ 1.0 ^c

dietary $\omega-3$ fatty acids decrease the C_{20:4, ω 6} levels in plasma and tissue phospholipids. Mechanisms underlying decreased C_{20:4, ω 6} levels in membrane and tissue phospholipids of animals fed $\omega-3$ fatty acids are not clearly understood. It has been proposed that C_{20:5, ω 3} present in fish oil competes with C_{20:4, ω 6} for incorporation into phospholipids [31]. C_{20:5, ω 3} is a preferred substrate to C_{20:4, ω 6} for lysophosphatidylcholine acyltransferase, leading to the formation of phosphatidylcholine [31]. We have recently demonstrated that dietary $\omega-3$ fatty acids of linseed or fish oil inhibit the pathway for chain elongation-desaturation leading to biosynthesis of C_{20:4, ω 6} [11,14]. The results presented here suggest the existence of another mechanism involving a shift of phospholipid C_{20:4, ω 6} to cholesterol ester and/or triacylglycerol pools. Accumulation of C_{20:4, ω 6} in liver cholesterol esters and plasma triacylglycerols was observed after feeding $\omega-3$ -fatty-acid-enriched diets (Tables 2 and 3). These changes in C_{20:4, ω 6} content cannot be explained entirely by changes in cholesterol ester and triacylglycerol pool sizes resulting from feeding dietary $\omega-3$ fatty acids. For example, feeding the linseed-oil diet had no significant effect on cholesterol ester or triacylglycerol content of plasma and liver, but is associated with an increase in C_{20:4, ω 6}-fatty-acid content in liver cholesterol ester and plasma triacylglycerol fractions.

Under normal circumstances, phosphatidylcholine containing C_{20:4, ω 6} at the *sn*-2 position and arising from the liver synthesis serves as a substrate for cholesterol esterification in plasma [32]. This reaction, catalysed by lecithin (phosphatidylcholine):cholesterol acyltransferase (LCAT), acquires free cholesterol from plasma and results in the formation of cholesterol arachidonate and lysophosphatidylcholine. Cholesterol arachidonate and lysophosphatidylcholine are carried to the liver where phosphatidylcholine ('lecithin') is again synthesized [33] and secreted into plasma to esterify more free cholesterol.

This cycle results in the net transfer of cholesterol from plasma to liver for further utilization. The existence of such a cycle has been previously demonstrated [34,35]. When the diet contains fish oil, C_{20:5, ω 3} present in fish oil replaces some of the arachidonic acid in the phosphatidylcholine of the liver and lowers C_{20:4, ω 6} content in phosphatidylcholine. In plasma, LCAT has greater specificity for C_{20:5, ω 3} than C_{20:4, ω 6} [36]. Therefore the phosphatidylcholine containing C_{20:5, ω 3} in the *sn*-2 position will be utilized for synthesis of cholesterol esters in preference to that containing C_{20:4, ω 6} in the *sn*-2 position. This is consistent with the accumulation of C_{20:5, ω 3} in the cholesterol ester fraction of the plasma. In liver, the acyltransferase responsible for esterification of lysophosphatidylcholine has a higher specificity for C_{20:5, ω 3} than for C_{20:4, ω 6} [31]. Thus C_{20:4, ω 6} originating from plasma cholesterol arachidonate or from synthesis *de novo* stays with cholesterol, raising the C_{20:4, ω 6} content of liver cholesterol esters compared with the group fed no C_{20:5, ω 3} (i.e. the group fed beef tallow). Some of the C_{20:4, ω 6} from either plasma cholesterol arachidonate or from synthesis *de novo* may be incorporated into triacylglycerols and secreted into the plasma, thereby raising the C_{20:4, ω 6} level when animals are fed a fish-oil-enriched diet.

On the other hand, liver acyl-CoA:cholesterol acyltransferase (ACAT) has higher specificity for monounsaturated fatty acids than for polyunsaturated fatty acids under normal circumstances [37]. Thus the liver cholesterol ester fraction contained approx. 65% of monounsaturated fatty acids in rats fed on the diet containing beef tallow. When the diet was supplemented with either linseed oil or fish oil, the Δ^9 -desaturase activity was inhibited and decreased monounsaturated fatty acids content in phospholipids [38], cholesterol esters and triacylglycerol fractions (Tables 2 and 3). As monounsaturated fatty acids are not readily available for cholesterol esterification by ACAT after linseed-oil or fish-oil supplementation, and C_{20:4, ω 6} originating from

plasma cholesterol arachidonate or from synthesis *de novo* is not readily incorporated into phospholipid in the presence of $C_{20:5,\omega3}$; free cholesterol may acquire available $C_{20:4,\omega6}$ to synthesize cholesterol esters and raise the $C_{20:4,\omega6}$ content of cholesterol esters in liver tissue.

For animals fed linseed oil enriched with $C_{18:3,\omega3}$, similar, but smaller, changes in fatty acid composition of the cholesterol ester and triacylglycerol fractions were exhibited in comparison to animals fed the fish-oil diet. Therefore the phenomenon of shifting $C_{20:4,\omega6}$ from phospholipids to cholesterol ester and triacylglycerol pools is not specific to animals fed fish oil but appears to be a general property of $\omega-3$ fatty acids. A similar phenomena has been observed in streptozotocin-induced diabetes mellitus, in which $C_{20:4,\omega6}$ shifts from the phospholipid to the triacylglycerol fraction [39]. The physiological significance of this shift of $C_{20:4,\omega6}$ from polar to non-polar lipid fractions under diet and disease conditions remains to be explained. Whether or not this role is solely as an acceptor of phospholipid $C_{20:4,\omega6}$ or whether it may also supply to, and serve as a buffer for, $C_{20:4,\omega6}$ in tissue phospholipid pools is a provocative notion that remains to be examined.

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REFERENCES

- Croft, K. D., Beilin, L. J., Vandongen, R. & Mathews, E. (1984) *Biochim. Biophys. Acta* **795**, 196–207
- Dyerberg, J. & Jorgensen, K. A. (1982) *Prog. Lipid Res.* **21**, 255–269
- Lokesh, B. R., Hsieh, H. L. & Kinsella, J. E. (1986) *Ann. Nutr. Metab.* **30**, 357–364
- Ahmed, A. A. & Holub, B. J. (1984) *Lipids* **19**, 617–624
- Carroll, K. K. (1986) *Lipids* **21**, 731–732
- Dyerberg, J. (1986) *Nutr. Rev.* **44**, 125–134
- Herold, P. M. & Kinsella, J. E. (1986) *Am. J. Clin. Nutr.* **43**, 566–598
- Kinsella, J. E. (1986) *Food Technol.* **40**, 80–97
- Sanders, T. A. B., Vickers, M. & Haines, A. P. (1981) *Clin. Sci.* **61**, 317–324
- Needleman, P., Raz, A., Minkes, M. S., Farrendelli, J. A. & Sprecher, H. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 944–948
- Garg, M. L., Sebokova, E., Thomson, A. B. R. & Clandinin, M. T. (1988) *Biochem. J.* **249**, 351–356
- Holloway, P. W., Peluffo, R. & Wakil, S. J. (1963) *Biochem. Biophys. Res. Commun.* **12**, 300–304
- Stoffel, W. (1961) *Biochem. Biophys. Res. Commun.* **6**, 270–273
- Garg, M. L., Thomson, A. B. R. & Clandinin, M. T. (1988) *J. Nutr.* **118**, 661–668
- Clandinin, M. T. & Yamashiro, S. (1982) *J. Nutr.* **112**, 825–828
- Folch, J., Lees, M. & Sloan-Stanley, G. H. (1957) *J. Biol. Chem.* **226**, 497–509
- Trinder, P. (1969) *Ann. Clin. Biochem.* **6**, 24–27
- Siedel, J., Schlumberger, H., Klose, S., Ziegenhorn, J. & Wahlefeld, A. W. (1981) *J. Clin. Chem. Clin. Biochem.* **19**, 838–839
- Giegel, J. L., Ham, A. B. & Clema, W. (1975) *Clin. Chem.* **21**, 1575–1581
- Van Handel, E. & Zilversmit, D. B. (1957) *J. Lab. Clin. Med.* **50**, 152–157
- Metcalf, L. D. & Schmitz, A. A. (1961) *Anal. Chem.* **33**, 363–364
- Hargreaves, K. & Clandinin, M. T. (1987) *Biochim. Biophys. Acta* **918**, 97–105
- Steel, A. G. D. & Torrie, J. H. (1960) *Principles and Procedures of Statistics*, McGraw-Hill Book Co., New York
- Marinetti, G. V. (1970) *Compr. Biochem.* **18**, 117–155
- Brindley, D. N. (1985) in *Biochemistry of Lipids and Membranes* (Vance, D. E. & Vance, J. E., eds.), pp. 213–241, The Benjamin/Cummings Publishing Co.
- Spector, A. A., Mathur, S. N. & Kaduce, T. L. (1979) *Prog. Lipid Res.* **18**, 31–53
- Krause, B. R. & Hartman, A. D. (1984) *J. Lipid Res.* **25**, 97–110
- Swanson, J. E., Black, J. M. & Kinsella, J. E. (1987) *J. Nutr.* **117**, 824–832
- Iritani, N. & Narita, R. (1984) *Biochim. Biophys. Acta* **793**, 441–447
- Charnock, J. S., Abeywardena, M. Y. & McLennan, P. L. (1986) *Ann. Nutr. Metab.* **30**, 393–406
- Iritani, N. & Fujikawa, S. (1982) *J. Nutr. Sci. Vitaminol.* **28**, 621–629
- Glomset, J. A. (1968) *J. Lipid Res.* **9**, 155–167
- Holub, B. J., Breckenridge, W. C. & Kuksis, A. (1971) *Lipids* **6**, 307–313
- Holub, B. J. & Kuksis, A. (1971) *Can. J. Biochem.* **49**, 1005–1011
- Stein, Y. & Stein, O. (1966) *Biochim. Biophys. Acta* **116**, 95–107
- Holub, B. J., Bakker, D. J. & Skaeff, C. M. (1987) *Atherosclerosis* **66**, 11–18
- Gidez, L. I., Roheim, P. S. & Eder, H. A. (1965) *J. Lipid Res.* **6**, 377–382
- Garg, M. L., Wierzbicki, A. A., Thomson, A. B. R. & Clandinin, M. T. (1988) *Biochim. Biophys. Acta* **962**, 330–336
- Takahashi, R., Horrobin, D. F., Watanabe, Y., Kyte, V. & Billard, V. (1987) *Biochim. Biophys. Acta* **921**, 151–153

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