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Influence of the fatty acid composition of lipids in chylomicron remnants derived from fish or corn oil on the lipid profile of cultured rat hepatocytes

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The aim of this work was to characterise the lipid and fatty acid composition of chylomicron remnants enriched in n-3 or n-6 polyunsaturated fatty acids (PUFA) and to investigate their influence on the fatty acid profiles of the lipids of rat hepatocytes cultured in monolayers. Chylomicrons were prepared from the lymph collected from the thoracic duct of rats given an oral dose of fish or corn oil (high in n-3 and n-6 PUFA, respectively), and remnants were prepared *in vitro* from such chylomicrons using rat plasma containing lipoprotein lipase. The fatty acids predominating in the oils abounded also in their respective chylomicrons and remnants, especially in triacylglycerols. Chylomicrons as well as remnants contained small amounts of phospholipids and long-chain PUFA that were minor in, or absent from, the dietary oils, evidently provided by the intestinal epithelium. The incubation of hepatocytes for 6 h, with either n-3 or n-6 PUFA-rich remnants (0.25-0.75 mM triacylglycerol) resulted in a dose-dependent increase in the amount of triacylglycerols and phospholipids in the cells, which was not affected further by increasing the incubation time to 19 h. Whereas hepatocyte triacylglycerols mostly incorporated the PUFA predominating in each remnant type, the fatty acid profile of cell phospholipids was virtually unchanged. In addition, irrespective of whether they were enriched in n-3 or n-6 PUFA, remnants promoted a relative decrease in the amount of cholesteryl esters, a minor hepatocyte lipid class poor in PUFA. The results demonstrate that the hepatocyte fatty acid profile is modulated in a lipid-class specific way by the amount and type of dietary PUFA delivered to cells in chylomicron remnants.

Key words: Dietary n-3 PUFA, Dietary n-6 PUFA, Chylomicron remnant, Hepatocyte fatty acid profile.

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Epidemiological studies have demonstrated that the intake in the diet of oils rich in n-6 polyunsaturated fatty acids (PUFA) (1, 14) or in the n-3 PUFA eicosapentaenoic (20:5n-3) and docosahexaenoic (22:6n-3) acids decreases the risk of the development of atherosclerosis and related cardiovascular disease (10, 35). Dietary n-6 and n-3 polyunsaturated fatty acids both lower plasma lipids, but, whereas the n-6 PUFA are hypocholesterolemic through a decrease in low-density lipoprotein (LDL) cholesterol concentrations (31, 33) and have little effect on plasma triacylglycerol (TG) (32), the n-3 PUFA cause a substantial hypotriglyceridemia and have little effect on plasma cholesterol (25, 34).

Chylomicrons are TG-rich lipoproteins that carry the dietary fat absorbed from the intestine. They are secreted into lymph and enter the blood via the thoracic duct. In the vascular endothelium, triacylglycerols are then hydrolysed by lipoprotein lipase (LPL) (28). This lipolytic process progressive and partially depletes the particles of triacylglycerols and converts chylomicrons to smaller and denser chylomicron remnants. Unlike intact chylomicrons, chylomicron remnants are readily recognised and taken up by the liver parenchymal cells through receptor-mediated processes (11), and thus, they deliver the remaining dietary lipid to the liver. The fatty acid composition of chylomicrons and remnants has been shown to be determined by the type of fat consumed in the diet (19). Furthermore, in experiments with rats *in vivo* (7), with perfused rat liver (18), and with isolated rat hepatocytes (20), it has been shown that the type of fatty acid in remnants influences the removal of these particles from the plasma and their uptake and processing by the liver.

The fatty acid composition of chylomicron remnants is altered in response to a single fatty meal (19). Remnants enriched in a specific type of fatty acids can be prepared from rats *in vivo* using standardised procedures. In previous work we have shown that the fatty acid composition of chylomicron remnants prepared *in vivo* by these methods reflects that of the dietary oils from which they derive, with small variations between preparations (19). In a further study, we developed a method to prepare remnants *in vitro* which involves treatment of chylomicrons collected from the thoracic duct of rats given an oral fat dose using post-heparin rat plasma, which contains LPL (26), followed by isolation of the remnant particles by ultracentrifugation (4, 25). Clearly, this approach is technically less demanding and reduces the number of aminals used compared to the procedures required for the preparation of chylomicron remnants *in vivo*.

Although the fatty acid composition of the total lipid of remnants prepared *in vivo* was found to reproduce well that of the parent oils (19), a more detailed study of the distribution of fatty acids in their major lipid classes is still lacking. Other questions remaining unanswered are how much of the chylomicron fatty acid, from triacylglycerols and perhaps from other lipid classes, are removed by lipolysis, and what proportion of the original dietary

Abbreviations: ACAT, acyl-CoA:cholesterol acyltransferase; BSA, bovine serum albumin; CE, cholesteryl ester; CEH cholesteryl ester hydrolase; DG, diacylglycerol; FAME, fatty acid methyl esters; GC, gas chromatography; LDL, low-density lipoprotein; LPL, lipoprotein lipase; MUFA, monounsaturated fatty acids; PL, phospholipid; PUFA, polyunsaturated fatty acids; SDS, sodium dodecyl sulphate; SFA, saturated fatty acids; TG, triacylglycerol; TLC, thin layer chromatography.

fatty acids is retained, when the chylomicron particle is converted into a remnant. Dietary fatty acids administered in high concentrations for several days are known to alter the fatty acid profile of hepatocytes, but few studies have examined the acute effects caused by the delivery of dietary lipids to the liver in chylomicron remnants. Remnants derived from different types of dietary oils carry and transfer directly to the liver triacylglycerols, diacylglycerols, phospholipids, and cholesteryl esters of different fatty acid composition, and are thus potentially capable of producing short-term changes in the cells. The aim of this work was to characterise the lipid and fatty acid composition of chylomicron remnants rich in n-3 or n-6 PUFA prepared *in vitro* and to investigate whether dietary fat type influences the fatty acid profile of lipid constituents of hepatocytes when offered to the cells in the form of chylomicron remnants. The results show that the hepatocyte fatty acid profile is modulated in a lipid class-specific way by the type of dietary PUFA transported in and delivered to cells in the remnant particles.

Materials and Methods

Animals and chemicals.– Male Sprague Dawley rats weighing ~300 g were used for chylomicron and remnant preparation and ~220 g for hepatocyte isolation. Animals were maintained as described previously (5). Unless otherwise stated, the commercial source of the chemicals and materials used is that described (5).

Preparation of chylomicron remnants.– One hour after the animals were given by stomach tube 1 ml Menhaden fish oil or corn oil, supplemented with tocopheryl acetate (4 mg/ml) as antioxidant, they were anaesthetised with sodium pentobarbital (60 mg/kg body weight), and the thoracic duct was cannulated as described previously (19). When the chyle was flowing continuously, 0.5 ml of the same oil fed initially was injected into the pyloric region of the stomach. The chyle was collected overnight in the presence of ampicillin (0.1 mg/ml), layered under 0.9% NaCl (d=1.006 g/ml), and centrifuged for $6x10^5$ g.min in a fixed angle rotor at 12 °C. Large chylomicrons (diameter >100 nm) were harvested from the top 1-1.5 cm by careful aspiration.

Chylomicron remnants were prepared *in vitro* by incubation of such chylomicrons with rat LPL-containing post-heparin plasma as detailed in a previous work (5). Remnants were assayed for total cholesterol and triacylglycerol by standard analytical methods (Roche Diagnostics, Barcelona, Spain). The LPL activity in post-heparin plasma preparations was assayed as described previously (4) using glycerol tri[1-^{14}C]oleate (Amersham Biosciences, UK) as the substrate. It was found to range from 9.7 to 10.8 µmol triolein hydrolysed/ml plasma/h.

Hepatocyte isolation and culture.– Hepatocytes were isolated and cultured as described earlier (17). Remnants were added to the culture medium at the indicated triacylglycerol concentration; the same amount of BSA in saline was added to control cultures. After the specified time of culture, the cells were harvested in phosphate buffered saline, pH 7.4, and washed. Cells from 6 plates were pooled and homogenized in 20 mM Tris-HCl buffer, pH 7.4, containing 250 mM sucrose, 0.5 mM dithiothreitol, 2 mM ethylenediaminetetra-acetic acid, 0.01 mM leupeptin, and 1 mM benzamidin.

Lipid analysis.- Lipids from chylomicrons, remants and hepatocyte homogenates were extracted with chloroform: methanol (1:2, v/v) (3) and aliquots were taken for the determination of neutral lipids, total lipid phosphorous and fatty acid composition of the total lipid. Lipids were resolved into classes by thin layer chromatography (TLC) and their fatty acids were analysed by gas chromatography (GC). Phospholipids (PL) were first resolved from neutral lipids using diethyl ether:acetic acid (99:1, v/v), eluted with chloroform:methanol:acetic acid:water (50:39:1:10, v/v/v/v) (2), and resolved into classes with chloroform:methanol:acetic acid:water (50:37.5:3.5:2, v/v/v/v) (15). Separate TLC plates were used for PL class quantitation and for PL fatty acid analysis. Neutral lipids were separated by TLC and quantified by optical densitometry using an image analysis system Bio Image and commercial software from Bio Image Corporation (Ann Arbor, MI) as described previously (30). Lipid classes were located by exposure of the plates to iodine vapours or under UV light after spraying the plates with dichlorofluorescein, respectively, for phosphorus (29) or for fatty acid analysis. Fatty acid methyl esters (FAME) were prepared from lipids, and the fatty acid analysis was performed with a Varian 3700 gas chromatograph equipped with two (2 m x 2 mm) glass columns packed with 15% SP 2330 on Chromosorb WAW 100/120 (Supelco Inc., Bellefonte, PA) 5 °C/min and a flame ionisation detector, as detailed (23).

Other analytical methods and statistical analysis.– Proteins were measured by the method of Bradford (6) using BSA as standard. Data were analysed by Student's t-test with $p<0.05$ taken as indicating a statistically significant difference.

Results

Lipid content and composition of chylomicron remnants derived from fish or corn oil.- Shown in Table I is the lipid content and composition of fish and corn oil-derived chylomicrons and of the corresponding remnants. For comparison, the data of the remnant triacylglycerol and total cholesterol mol percentage previously reported (5) have been included. In agreement with earlier studies (19), lipids in both chylomicron types were quite comparable. Remnants derived from either oil had also similar net amounts and proportions of all lipid classes. Of phospholipids, which represent about 6% of the total lipid, phosphatidylcholine was by far the most abundant, followed by lysophosphatidylcholine and sphingomyelin. It is notable that the remnants prepared *in vitro* using LPL-rich rat plasma had similar lipid composition to those prepared from rats *in vivo* (19). As expected, the net amount of triacylglycerol in fish and corn oil remnants was considerably lower than that of the corresponding chylomicrons (about one third in the present preparations). Inasmuch as remnants also contained less diacylglycerol and phospholipid than their corresponding chylomicrons, while the content of cholesterol remained similar, the lipid composition differed substantially between these two lipoprotein classes.

Fatty acid composition of the total lipid of chylomicron remnants derived from fish or corn oil.– The results (Table II) showed that both lipoprotein classes reflected the fatty acid profile of the dietary oils from which they were derived, also included in Table II for comparison. The fish oil used was rich in n-3 PUFA (38.0%), followed by saturates (27.6%) and monoenes

Table I. *Lipid content and composition of chylomicrons and chylomicron remnants derived from fish or corn oil.*

Chylomicrons and chylomicron remnants derived from fish oil or corn oil were prepared as described in Materials and Methods. Phospholipids were determined by phosphorous analysis, and the neutral lipids by glycerol or cholesterol analysis after their separation by TLC. Data are the mean values from two preparations of each of the particles.

	Fish oil		Corn oil	
	Chylomicrons	Remnants	Chylomicrons	Remnants
Content (μmol/mL)				
Total lipid	27.7	10.4	33.5	11.6
Triacylglycerol	24.2	8.1	30.0	9.5
Diacylglycerol	1.7	1.0	1.8	0.7
Total colesterol	0.6	0.7	0.7	0.8
Phospholipid	1.2	0.6	1.0	0.7
Composition (mol % of the total lipid)				
Lysophosphatidylcholine	0.4	0.7	0.4	0.9
Sphingomyelin	0.1	0.2	0.1	0.3
Phosphatidylcholine	3.1	4.4	1.7	3.7
Phosphatidylserine	0.2	0.3	0.2	0.3
Phosphatidylinositol	0.4	0.3	0.3	0.3
Phosphatidylethanolamine	0.1	0.1	0.2	0.3
Diacylglycerol	6.2	9.2	5.4	6.1
Triacylglycerol	87.2	78.1	89.4	81.2
Total cholesterol	2.3	6.7	2.2	6.8

(26.2%), and contained also a small proportion of n-6 PUFA (8.2%). The corn oil preparation in turn was exceedingly rich in n-6 PUFA (62.1%, mostly 18:2n-6), followed by monoenes (25.7%) and saturates (11.7%). Also, it had some 18:3n-3, but lacked long-chain n-3 PUFA. As compared to those of the parent oils, the fatty acid profiles of chylomicrons showed some differences in the PUFA. Thus, the chylomicrons derived from corn oil acquired a small but significant proportion of long-chain n-3 PUFA whereas the chylomicrons derived from fish oil were enriched 4.4 fold in 18:2n-6 presumably during their transit through enterocytes. For corn oil, these differences in PUFA are in line with previous data (19).

The fatty acid profiles of the remnants resulting from the action of LPL-containing plasma on chylomicrons were similar to those of the parental lipoproteins (Table II). Fish oil derived remnants contained relatively large amounts of n-3 PUFA (26.8%), although the main fatty acids in both remnant types were palmitic, linoleic, and oleic acids. In comparison, corn oil derived remnants were very rich in n-6 PUFA (55.0%), especially 18:2n-6, followed by 18:1 and 16:0, these three fatty acids together representing approximately 90% of total fatty acids. The ratio of total n-3 to total n-6 PUFA, therefore, was approximately 43-fold higher in fish oil than in corn oil remnants. The fatty acid profile of corn oil remnants resembled that of their corresponding chylomicrons more closely than the fish oil remnants. It is notable that the lipid and fatty acid composition varied little between different preparations of each remnant type,

Table II. *Fatty acid composition of the total lipid from chylomicrons and chylomicron remnants derived from fish or corn oil.*

Chylomicrons and chylomicron remnants derived from fish oil or corn oil were prepared as described in Materials and Methods. The lipids were then extracted and the fatty acid composition of the total lipid was determined by GC. Data are expressed as g/100g total fatty acids and are the mean ± S.D. from 6 (remnants) or 2 (chylomicrons and oils). Differences between such duplicates were lower than 5%. The unsaturation index is defined as the sum of molar % of each fatty acid multiplied by the number of double bonds. [a] Significant differences ($p \leq 0.05$) with respect to fish oil remnants.

	Fish oil			Corn oil		
	Parent oil	Chylo-microns	Remnants	Parent oil	Chylo-microns	Remnants
14:0	7.2	6.2	2.8 ± 0.3	0.2	0.3	0.1 ± 0.04[a]
15:0	0.7	0.2	0.5 ± 0.03	0.01	0.2	0.1 ± 0.1[a]
16:0	14.8	18.8	16.8 ± 0.8	10.2	13.2	13.8 ± 0.6[a]
17:0	1.2	1.0	1.2 ± 0.3	0.1	0.2	0.2 ± 0.1[a]
18:0	3.7	4.9	5.1 ± 0.4	1.3	2.9	3.1 ± 0.1[a]
14:1	0.5	0.4	0.6 ± 0.1	0.1	0.02	0.1 ± 0.04[a]
15:1	0.4	0.7	0.2 ± 0.02	0.01	0.02	0.1 ± 0.04[a]
16:1	11.7	11.4	8.9 ± 0.8	0.5	1.1	1.1 ± 0.5[a]
17:1	2.0	1.7	1.2 ± 0.1	0.1	0.1	0.1 ± 0.04[a]
18:1	11.6	14.4	13.9 ± 0.7	25.1	22.8	24.6 ± 0.6[a]
18:2 n-6	3.2	14.2	15.8 ± 2.6	60.4	52.8	51.5 ± 1.0[a]
18:3 n-6	2.1	0.2	1.7 ± 0.3	1.7	1.9	1.4 ± 0.2
20:3 n-6	0.4	0.2	0.4 ± 0.1		0.1	0.1 ± 0.1
20:4 n-6	1.3	2.7	3.4 ± 0.8		2.1	1.8 ± 0.1[a]
22:4 n-6	0.2	0.1	0.2 ± 0.2		0.1	0.1 ± 0.02
22:5 n-6	1.1	0.5	0.5 ± 0.2		0.1	0.1 ± 0.1
18:3 n-3	1.2	1.8	1.2 ± 0.1	0.5	0.9	1.3 ± 0.5
18:4 n-3	4.3	2.7	2.7 ± 0.1		0.1	0.1 ± 0.1[a]
20:4 n-3	1.7	1.3	0.8 ± 0.1		0.2	0.1 ± 0.03[a]
20:5 n-3	14.7	7.8	12.4 ± 0.6		0.2	0.2 ± 0.1[a]
22:5 n-3	1.8	1.2	0.8 ± 0.1		0.1	0.03 ± 0.01[a]
22:6 n-3	14.4	7.7	8.8 ± 0.4		0.7	0.31 ± 0.08[a]
Unsaturation index	247.7	183.9	215.7	153.0	154.4	149.4

in agreement with our earlier work on rat remnants prepared *in vivo* (19).

Fatty acid composition of the major lipid classes of chylomicrons and chylomicron remnants derived from fish or corn oil.– Table III shows the fatty acid composition of triacylglycerols, diacylglycerols, phosphatidyl choline and cholesteryl esters from chylomicrons and remnants derived from fish or corn oil. As might be predicted, triacylglycerols had a fatty acid profile quite similar to that of the total lipid in the parent oils. The ratio of n-3 to n-6 PUFA in the acylglycerols differed markedly between fish and corn oil-derived particles. The triacylglycerols and diacylglycerols from corn oil chylomicrons and remnants contained more than 50% of the total fatty acids as n-6

Table III. *Fatty acid composition of the major lipids from fish and corn oil-derived chylomicrons and chylomicron remnants.* Chylomicrons (CM) and chylomicron remnants derived from corn oil or fish oil were prepared, lipids were extracted and separated into classes by TLC, and their fatty acids analysed by GC. Data are expressed as g/100g total fatty acids and are the mean ± S.D. from 3 separate preparations in the case of remnants and the mean of two in the case of chylomicrons. [a] Significant differences in fatty acid composition of the remnants derived from corn as compared to fish oil ($p \leq 0.05$).

	Cholesteryl esters				Triacylglycerols				Diacylglycerols				Phosphatidylcholine			
	Fish oil		Corn oil		Fish oil		Corn oil		Fish oil		Corn oil		Fish oil		Corn oil	
	CM	Remnants	CM	Remnants	CM	Remnants	CM	Remnants	CM	Remnants	CM	Remnants	CM	Remnants	CM	Remnants
14:0	3.3	1.7±0.4	1.1	1.0±0.6	6.0	4.3±0.2	0.4	0.3±0.02	3.3	2.6±1.9	0.4	1.1±0.7	0.8	0.5±0.1	0.2	0.2±0.1
15:0	1.9	0.8±0.6	0.7	1.0±0.6	0.5	0.6±0.05	0.1	0.2±0.01	0.6	0.3±0.3	0.3	0.6±0.3	0.3	0.4±0.1	0.3	0.6±0.3
16:0	24.8	20.9±1.7	17.5	18.7±1.4	16.7	15.4±1.3	12.9	12.7±0.6[a]	22.2	13.6±0.4	15.4	15.0±1.8	25.4	23.6±1.2	23.0	24.7±0.7
17:0	3.7	1.7±0.3	1.8	1.4±0.3	1.0	1.2±0.1	0.2	0.2±0.02	1.3	1.4±0.3	0.2	0.5±0.2	0.9	0.8±0.1	0.8	0.7±0.1
18:0	7.3	5.7±0.3	4.8	6.2±1.2	4.1	3.6±0.3	2.4	2.5±0.2[a]	9.0	3.8±1.8	4.2	3.2±0.6	13.3	18.2±0.5	14.7	16.6±1.3
14:1	1.0	0.6±0.2	0.2	0.3±0.2	0.5	0.5±0.02	0.1	0.2±0.04	0.2	0.3±0.2	0.2	0.2±0.1	0.3	0.2±0.01	0.1	0.1±0.1
15:1	1.1	0.7±0.1	0.4	0.6±0.3	0.5	0.2±0.01	0.2	0.1±0.02	0.4	0.3±0.1	0.2	0.2±0.1	0.3	0.1±0.01	0.3	0.2±0.1
16:1	13.4	12.8±0.4	5.7	7.4±2.0	10.8	9.4±0.5	1.1	1.2±0.2	8.3	6.9±1.2	1.8	2.9±1.6[a]	3.5	1.9±0.5	1.3	2.4±1.3
17:1	1.4	1.6±0.3	0.5	0.8±0.3	1.8	1.4±0.1	0.2	0.1±0.1	1.3	1.0±0.3	0.2	0.3±0.1	0.5	0.3±0.1	0.2	0.3±0.1
18:1	18.4	20.5±1.1	24.6	24.8±2.7	14.3	13.3±0.9	23.6	25.0±1.0[a]	14.7	10.6±1.1	22.2	20.8±1.8[a]	7.2	6.8±0.4	8.1	9.0±1.1[a]
20:1	0.3	0.7±0.1	0.8	1.1±0.9	1.4	1.1±0.1	0.9	1.4±0.5	1.0	0.7±0.1	0.7	0.9±0.3	1.0	0.7±0.2	0.5	0.5±0.2
20:3 n-9	0.5	0.4±0.7	0.3	0.2±0.2	0.4	0.4±0.02	0.1	0.2±0.1	0.2	0.2±0.1	0.1	0.1±0.1	0.2	0.5±0.3	0.3	0.3±0.1
18:2 n-6	12.9	16.6±1.7	30.5	27.6±4.4[a]	12.4	11.7±1.0	52.0	50.2±0.9[a]	10.9	9.5±1.2	47.0	42.2±5.9[a]	20.4	22.5±0.8	38.1	32.3±0.6[a]
18:3 n-6	0.7	0.7±0.2	0.2	0.3±0.3	0.3	0.3±0.0	0.5	0.7±0.2	0.3	0.2±0.1	0.5	0.4±0.04	0.3	0.3±0.1	0.3	0.3±0.0
20:3 n-6	0.3	0.0±0.0	0.1	0.2±0.1	0.2	0.2±0.1	0.1	0.2±0.0	0.2	0.2±0.1	0.2	0.2±0.1	0.6	0.5±0.2	0.5	0.3±0.1

Table III (continued)

	Cholesteryl esters				Triacylglycerols				Diacylglycerols				Phosphatidylcholine			
	Fish oil		Corn oil		Fish oil		Corn oil		Fish oil		Corn oil		Fish oil		Corn oil	
	CM	Remnants	CM	Remnants	CM	Remnants	CM	Remnants	CM	Remnants	CM	Remnants	CM	Remnants	CM	Remnants
20:4 n-6	2.5	5.2±0.6	7.9	3.4±0.7	2.2	2.8±0.1	1.7	2.1±0.2	2.5	4.6±0.5	2.0	5.7±0.4	11.6	12.1±1.0	9.2	8.6±1.5[a]
22:4 n-6	0.9	0.7±0.8	0.2	0.5±0.0	0.7	0.8±0.1	0.1	0.1±0.1	0.7	0.8±0.1	0.1	0.2±0.1	0.2	0.7±0.3	0.2	0.3±0.3
22:5 n-6	0.8	0.3±0.4	0.0	0.5±0.1	0.5	0.5±0.1	0.1	0.3±0.01	0.4	0.4±0.02	0.1	0.2±0.1	0.2	0.2±0.2	0.1	0.1±0.04
18:3 n-3	0.8	1.9±0.1	1.5	1.0±0.2	2.2	1.7±0.1	1.9	1.0±0.1[a]	1.6	1.1±0.1	1.3	1.0±0.2	0.3	0.2±0.1	0.2	0.2±0.0
18:4 n-3	0.4	0.7±0.2	0.1	0.2±0.0	3.2	2.9±0.2	0.2	0.1±0.1[a]	2.5	2.4±0.6	0.3	0.3±0.1[a]	0.5	0.3±0.1	0.1	0.03±0.01[a]
20:4 n-3	0.2	0.3±0.0	0.1	0.6±0.7	1.6	1.2±0.2	0.2	0.2±0.1[a]	0.9	0.7±0.1	0.2	0.3±0.02[a]	0.5	0.3±0.1	0.1	0.1±0.1
20:5 n-3	2.5	3.4±1.6	0.7	1.6±1.2[a]	8.4	13.4±2.1	0.3	0.4±0.1[a]	7.5	24.2±2.4	1.3	1.8±1.4[a]	9.4	6.1±0.4	0.3	0.7±0.3[a]
22:5 n-3	0.1	0.3±0.3	0.0	0.1±0.1	1.5	1.6±0.1	0.1	0.1±0.02[a]	1.2	1.2±0.03	0.1	0.1±0.1 [a]	0.3	0.5±0.3	0.1	0.2±0.1[a]
22:6 n-3	0.8	1.5±0.8	0.4	0.2±0.1	8.8	11.5±1.4	0.6	0.8±0.1[a]	8.5	12.9±0.9	0.6	1.7±0.8 [a]	1.8	2.2±0.4	1.0	1.1±0.4[a]
SFA	41.1	30.8±1.9	25.9	28.1±3.7	28.3	25.1±1.7	16.0	15.7±0.8[a]	36.3	21.7±0.4	20.6	17.8±1.2[a]	40.7	43.5±1.0	39.1	42.8±0.5
MUFA	35.3	36.3±0.9	31.4	33.9±0.2	27.9	24.8±1.5	25.1	26.4±0.8	25.0	19.0±0.4	24.6	23.8±1.2[a]	11.7	9.2±1.1	9.9	12.0±2.8
n-6 PUFA	18.0	23.3±1.5	38.9	32.5±4.3[a]	16.3	16.3±1.0	54.5	53.6±0.5[a]	15.0	15.6±1.6	49.9	52.2±1.6[a]	33.3	36.3±1.4	48.3	41.9±2.1[a]
n-3 PUFA	4.7	6.5±0.7	2.7	4.4±0.5[a]	25.6	32.3±3.7	3.3	2.6±0.1[a]	22.2	42.7±1.9	3.7	5.2±2.3[a]	11.1	7.3±0.7	0.8	1.2±0.2[a]
n-3/n-6	0.26	0.28	0.07	0.14	1.57	1.98	0.06	0.05	1.48	2.74	0.07	0.10	0.33	0.20	0.02	0.03
Unsat. index	104	135	137	127	198	234	153	153	178	284	147	161	170	160	137	128

PUFA, mainly 18:2n-6, followed by MUFA and SFA with long-chain n-3 polyenes as minor components, while these lipids in fish oil particles were rich in n-3 PUFA, but SFA, MFA and even n-6 PUFA were also main groups of fatty acids.

Another notable aspect was that although the fatty acid profile of the total lipid showed little variation between chylomicrons and remnants (Table II), differences were observed in fatty acids of diacylglycerols and to a lesser extent, triacylglycerols, between these particles (Table III). For example, 20:5n-3 amounted to 8.4 and 7.5 g/100g total fatty acids in the triacylglycerols and diacylglycerols of fish oil chylomicrons, respectively, while the corresponding values in remnants were 13.4 and 24.2. One possible explanation for this is that the original chylomicron triacylglycerols, when acted upon by LPL, produced not only diacylglycerols but also monoacylglycerols (about 7.7% of the total acylglycerols in postheparinized plasma (13)) and free fatty acids, which remained in the remnants. These products were separated by the chromatographic procedures used here, but remained as components of the total lipid, resulting in a similar total fatty acid profile in spite of the changed lipid class composition.

The cholesteryl esters, minor constituents of chylomicrons and remnants (Table I), had fatty acid profiles that differed slightly between these two particle types, but markedly with the dietary oil from which they derived (Table III). In cholesteryl esters derived from corn oil, SFA, MUFA and n-6 PUFA were present in approximately equal proportions (28-34 g/100 g total fatty acids), whereas saturates and monounsaturates were more predominant in cholesteryl esters of fish oil-derived particles. In any case, 18:1, 18:2n-6 and 16:1 were the most abundant fatty acyl moieties of cholesteryl esters in chylomicrons and remnants. Interestingly, the proportion of n-3 PUFA in cholesteryl esters was low in particles derived from both oils, with the ratio of n-3 to n-6 PUFA in cholesteryl esters from fish oil particles being just 2-3 fold higher than those derived from corn oil.

Phospholipids in chylomicrons and remnants of either origin had phosphatidylcholine as the most abundant constituent (Table I). Although the percentages of n-3 fatty acids in phosphatidylcholine from fish oil derived particles were higher than those occurring in corn oil remnants (Table III), it was interesting that, irrespective of the fatty acid type predominating in the dietary oil, phosphatidylcholine from both lipoproteins had saturates and n-6 PUFA, mainly 18:2n-6 and 20:4n-6, as the dominant constituents.

Effect of chylomicron remnants derived from fish or corn oil on the fatty acid composition of rat hepatocytes in culture.– Fish or corn oil remnants were incubated with rat hepatocytes in culture, and the effects on the fatty acid profile of the cells were determined. Confirming previous findings (5), we observed that 6 h of incubation with either fish or corn oil remnants (0.25 mM or 0.75 mM TG) results in a significant dose-dependent increase in cellular total lipid, which is mainly due to an increase in the amount of triacylglycerol and phospholipid, whereas the content of unesterified cholesterol in the cells remained virtually unchanged and that of cholesteryl esters tended to be reduced (data not shown). When the cells were exposed to the remnants for a longer time period (19 h), similar results were

obtained, with no further changes apparent (data not shown). The remnants thus affected the lipid content of the hepatocytes, but no differences were found that could be ascribed to the type of fatty acids predominating in remnants.

The fatty acid composition of phospholipids and triacylglycerols from rat hepatocytes exposed to n-3 or n-6 chylomicron remnants (0.25 mM or 0.75 mM TG) for 6 h is compared in Table IV. The fatty acid profile of the total phospholipid remained virtually constant irrespective of the amount of remnants added, the type of oil from which they derived (Table IV), and the time period of exposure (6 h, Table IV; 19 h, data not shown). Only minor incorporation of n-3 PUFA of fish oil remnants in cell phospholipids was seen. In contrast, hepatocyte triacylglycerols became enriched in the type of PUFA (n-3 or n-6) that was being supplied in remnants in a dose-dependent manner (Table IV). n-3 PUFA were minor constituents of triacylglycerols in untreated hepatocytes, the main fatty acids being saturates, monounsaturates and polyunsaturates of the n-6 series (mainly 18:2 and 20:4n-6). After incubation of hepatocytes with corn oil remnants, the aforementioned n-6 PUFA increased to a larger extent in triacylglycerols than other fatty acids, as reflected by the decrease in the proportions of saturates and n-3 PUFA. After incubation with fish oil remnants, the percentage of n-3 PUFA (mainly 20:5n-3, 22:5n-3 and 22:6n-3) increased dose-dependently (50 and 85% at 0.25 and 0.75 mM remnant concentration, respectively), but was not affected significantly by extending the incubation time from 6 to 19 h (data not shown).

Free cholesterol and cholesteryl esters are lipid classes whose amount was not increased after 6 h of culture with remnants (5). Given that triacylglycerols and phospholipids increased, it can be deduced that the percentage of cholesteryl ester, which is a very minor hepatocyte component, decreased significantly. Even though the small amount of cholesteryl esters in the present preparations prevented individual fatty acid analysis, pooled samples showed that these lipids from control cells were rich in SFA (especially 16:0 and 18:0) and MUFA (mainly 18:1) and poor in PUFA (approximately 10% of each n-3 and n-6 polyunsaturates) (data not shown). Moreover, only minor incorporation of remnant n-3 or n-6 PUFA in cholesteryl esters was found in cells after treatment with either remnant type, whereas the level of 18:1 enhanced markedly (data not shown).

Discussion

In the first part of this work we characterised the lipid and the fatty acid compositions of major lipid classes of chylomicron remnants prepared *in vitro* from chylomicrons collected from rats fed fish or corn oil. Then, we investigated the effects of dietary n-3 and n-6 polyunsaturated fatty acids delivered in remnants on the fatty acid profile of major lipid classes of rat hepatocytes. The results showed that the fatty acid profile of hepatocytes is modulated by the amount and the type of PUFA delivered to cells and that the changes induced are lipid class-specific and relatively rapid since they were achieved in the first 6 hours of cell exposure to remnants.

The fatty acids predominating in each of the dietary oils also prevailed in their corresponding chylomicrons and remnants. This was particularly so for the triacylglycerols, the small proportions of phosphatidylcholine and cholesteryl

Table IV. *Fatty acid composition of phospholipids and triacylglycerols in cultured rat hepatocytes treated with chylomicron remnants enriched in n-3 or n-6 polyunsaturated fatty acids.*

Rat hepatocytes cultured in monolayers were incubated with or without (control) chylomicron remnants derived from fish (n-3 remnants) or corn oil (n-6 remnants) at a 0.25 or 0.75 mM of TG for 6 h. The cells were then washed, harvested and homogenized to extract the lipids. These were then separated, and the fatty acid composition of triacylglycerols and phospholipids was determined by GC. Data are expressed as g/100g total fatty acids and are the mean±S.D. from 3 separate preparations. Differences between 0.25 and 0.75 mM were scarcely or not significant. [a] Significant differences ($p \leq 0.05$) with respect to controls. [b]Significant differences ($p \leq 0.05$) associated with the remnants derived from corn oil as compared to fish oil.

	Phospholipids					Triacylglycerols				
		n-3 remnants		n-6 remnants			n-3 remnants		n-6 remnants	
	Control	0.25 mM	0.75 mM	0.25 mM	0.75 mM	Control	0.25 mM	0.75 mM	0.25 mM	0.75 mM
14:0	0.5±0.2	0.4±0.1	0.5±0.1	0.4±0.1	0.3±0.1	1.5±0.4	1.2±0.4	1.3±1.0	0.7±0.4	1.1±0.7
15:0	0.4±0.1	0.3±0.1	0.3±0.02	0.3±0.1	0.3±0.1	1.1±0.7	0.7±0.3	0.7±0.5	0.6±0.3	0.6±0.3
16:0	19.7±0.4	18.5±0.7	19.0±0.9	18.4±0.5	18.8±0.2	24.5±1.3	21.9±1.6	20.4±2.1	19.3±1.2 [a]	17.7±0.7[a]
17:0	0.7±0.1	0.5±0.2	0.6±0.2	0.5±0.2	0.5±0.3	0.7±0.1	0.5±0.2	0.6±0.2	0.6±0.2	0.4±0.3
18:0	19.7±0.3	19.8±0.5	20.7±1.2	20.9±0.8	20.6±0.8	5.9±1.0	4.6±1.0	4.3±1.2	4.9±1.4	3.1±0.7[a]
14:1	0.1±0.1	0.1±0.03	0.1±0.04	0.1±0.04	0.1±0.04	0.2±0.1	0.1±0.1	0.2±0.1	0.1±0.1	0.1±0.04
15:1	0.1±0.1	0.1±0.02	0.1±0.03	0.1±0.03	0.1±0.02	0.3±0.3	0.3±0.2	0.3±0.2	0.1±0.04	0.2±0.1
16:1	2.7±0.4	2.5±0.3	2.7±0.6	2.4±0.3	2.2±0.1	8.1±0.9	8.5±1.8	8.4±0.4	6.6±1.2	6.2±2.2
17:1	0.3±0.1	0.3±0.1	0.2±0.1	0.2±0.1	0.2±0.1	0.6±0.02	0.5±0.1	0.6±0.1	0.5±0.1	0.4±0.2
18:1	10.8±0.1	11.2±0.9	10.2±1.4	11.2±1.5	11.2±1.2	26.6±3.2	27.4±6.5	27.6±7.5	29.7±5.6	30.8±3.7
20:1	0.3±0.2	0.3±0.2	0.1±0.04	0.2±0.03	0.2±0.04	0.3±0.1	0.3±0.1	0.3±0.1	0.4±0.1	0.4±0.2
20:3 n-9	0.7±0.7	1.0±0.1	0.9±0.1	0.9±0.1	0.6±0.3	1.1±0.2	1.3±0.7	1.2±0.2	2.1±0.7	1.6±0.3

Table IV (continued)

	Phospholipids					Triacylglycerols				
		n-3 remnants		n-6 remnants			n-3 remnants		n-6 remnants	
	Control	0.25 mM	0.75 mM	0.25 mM	0.75 mM	Control	0.25 mM	0.75 mM	0.25 mM	0.75 mM
18:2 n-6	12.1±0.6	11.1±0.7	10.8±1.1	13.2±1.0	13.8±1.4	15.2±0.2	14.7±3.2	14.6±2.3	22.7±1.9[a,b]	24.8±1.6[a,b]
18:3 n-6	0.2±0.1	0.2±0.1	0.2±0.1	0.1±0.0	0.1±0.1	0.1±0.03	0.3±0.3	0.3±0.3	0.2±0.1	0.3±0.3
20:3 n-6	0.9±0.3	1.2±0.2	1.0±0.1	1.2±0.2	1.2±0.2	0.9±0.1	0.8±0.4	0.9±0.3	1.0±0.2	0.9±0.4
20:4 n-6	20.5±0.3	20.7±1.4	20.8±2.5	20.4±1.2	20.2±0.9	5.1±1.3	4.8±0.3	4.6±0.2	4.1±1.0	4.4±0.1
22:4 n-6	0.8±0.1	0.7±0.1	0.7±0.1	0.7±0.2	0.7±0.1	0.2±0.1	0.4±0.5	0.3±0.4	0.3±0.2	0.3±0.2
22:5 n-6	1.0±0.1	0.9±0.1	0.8±0.1	0.6±0.2	0.5±0.2	1.2±0.5	1.2±0.3	1.1±0.2	1.1±0.3	1.0±0.2
18:3 n-3	0.3±0.2	0.3±0.1	0.2±0.04	0.1±0.02	0.2±0.02	0.2±0.01	0.5±0.2	0.6±0.3[a]	0.5±0.2	0.7±0.2
18:4 n-3	0.2±0.1	0.3±0.1	0.5±0.1	0.2±0.0	0.2±0.1	0.2±0.1	0.5±0.1[a]	0.6±0.1[a]	0.3±0.1	0.4±0.1
20:4 n-3	0.4±0.3	0.3±0.1	0.3±0.1	0.3±0.1	0.4±0.1	0.3±0.04	0.4±0.3	0.7±0.2	0.2±0.3	0.9±0.4
20:5 n-3	0.5±0.01	1.3±0.7	1.1±0.1	0.6±0.1	0.3±0.1	2.1±0.5	3.4±1.0	3.9±0.7[a]	0.7±0.5[a,b]	0.6±0.2[a,b]
22:5 n-3	0.8±0.02	1.0±0.2	0.9±0.1	0.8±0.1	0.9±0.1	0.8±0.2	1.9±0.9	1.6±0.4[a]	1.0±0.3	0.8±0.3
22:6 n-3	6.3±0.05	7.0±0.9	7.1±1.4	6.4±1.1	6.5±0.6	2.7±0.7	3.7±0.6	5.0±0.6[a]	2.5±0.2	2.3±0.2
SFA	41.0±1.0	39.1±1.3	40.9±2.2	40.5±0.5	40.4±0.7	34.0±3.4	30.5±3.5	27.8±4.9	26.0±1.6[a]	21.9±3.3[a]
MUFA	14.0±0.5	13.4±1.3	13.3±2.2	13.9±1.7	13.8±1.3	35.7±1.9	37.1±8.4	36.8±7.6	36.5±6.5	36.7±4.5
n-6 PUFA	35.5±0.1	34.7±1.8	34.2±3.3	36.0±1.1	36.4±1.1	22.5±1.6	20.2±5.7	20.7±4.6	28.8±2.7[a]	29.6±4.0[a]
n-3 PUFA	8.6±0.5	10.2±0.5	10.0±1.7	8.3±1.3	8.4±0.7	6.4±0.3	9.8±0.7[a]	11.8±0.3[a]	4.7±0.4	5.6±0.4
n-3/n-6	0.24	0.29	0.29	0.23	0.23	0.28	0.49	0.57	0.16	0.19
Unsat. index	181.6	193.8	187.3	181.3	180.6	133.2	152.9	162.2	141.8	147.4

esters both lipoprotein particles contained being acquired by the chylomicron particles during the process of their assembly in the intestinal epithelial cells (16). The finding that the particles contain phospholipids such as phosphatidylcholine is consistent with the fact that chylomicrons also gain some new fatty acids in the process, as is the case with 22:6n-3 after corn oil, or with n-6 PUFA with fish oil-derived lipoproteins. These fatty acids were evidently elaborated in, and mostly provided by, the enterocytes suggesting that they are required for the formation, the stability, and/or other aspects of the function of this type of lipoprotein particle. Human enterocytes have been found to elicit the capacity to convert 18C precursors into 20:5n-3 and 22:6n-3 (8). A similar conclusion may be reached by observing the fatty acids of the chylomicrons formed after the administration of dietary fats rich in SFA, MUFA or PUFA (19). Differentiated CaCo-2 cells have been reported to use nascent triacylglycerols and preformed phospholipids for chylomicron assembly (22). Although in the postprandial state, newly absorbed fatty acids are used for the synthesis of triacylglycerols, which are targeted for secretion as part of chylomicrons, preformed cellular phospholipids are preferentially used for chylomicron assembly and thus, do not contain dietary fatty acids (22). This is in line with our observation in rats, that the fatty acids absorbed and incorporated into the triacylglycerols of secreted chylomicrons reflect the composition of dietary fat, but those of phospholipids diverge to a great extent, even though some effects are apparent.

As seen in remnants prepared in rats *in vivo* (19), the *in vitro* conversion of chylomicrons to remnants enhanced the differences in fatty acid composition of the lipids of remnants. The fatty acid profile of cholesteryl esters and phosphatidylcholine in the particles did not change by the action of LPL, a result consistent with the fact that these lipids are not preferential substrates for the deacylating activity of this enzyme. The fatty acid profile of triacylglycerols and diacylglycerols, however, showed a number of differences between chylomicrons and remnants, particularly evident when they derived from fish oil. Both glycerolipids in fish oil remnants had a higher proportion of total n-3 PUFA than those present in chylomicrons, suggesting that LPL had less affinity for triacylglycerols and diacylglycerols molecular species containing n-3 PUFA than for species containing other fatty acids, especially n-6 PUFA such as 18:2n-6. This preference was not evident with corn oil, because in this case the particle lipids are poor in long-chain n-3 PUFA, and because the triacylglycerols derived from corn oil are more homogeneous than those derived from fish oil with respect to molecular species composition. This is in agreement with previous reports showing that the *in vitro* activity of LPL from cardiac (21) and other tissues (24) is highest on chylomicron triacylglycerols containing 18:0, 18:2n-6 or 18:3n-3 and lowest when this lipid class contains 20:4n-6, 20:5n-3 or 22:6n-3.

In spite of the noted differences in fatty acids, the overall lipid distribution did not differ significantly between fish or corn oil remnants as indicated by the total glycerol:total cholesterol ratios. Consequently, the amount of lipid added to the culture within the two remmnant types was equivalent at each of the concentrations tested. For the lipids in remnants to affect the fatty acids of lipids in hepatocytes, the particles must have interacted with the cells, and their components internalised,

metabolised and incorporated. It was recently observed that remnants from fish oil are internalised more rapidly than corn oil remnants at low, but not at high, remnant concentrations (20). Based on the uptake rates given in (20), at the concentrations of remnants used in the present study, it is likely that after six hours both the fish and the corn oil particles were processed completely by the hepatocytes. In our treated cells, triacylglycerol and phospholipid levels increased in a dose-dependent manner, but while triacylglycerols incorporated the PUFA predominating in each remnant type, the fatty acid profile of phospholipids was virtually unaffected. Given that triacylglycerol formation in hepatocytes resembles that in enterocytes in that fatty acyl groups are readily transferred to acyltransferases for incorporation into triacylglycerols, we conclude that the differences observed in the fatty acid composition of hepatocyte lipids can be attributed mainly to the different types of fatty acids entering into cells from the triacylglycerols carried by chylomicron remnants.

Our interest in cholesteryl esters lies in that n-3 and n-6 PUFA delivered to hepatocytes in remnants did not alter substantially the fatty acid profile of this lipid, but instead they did cause a relative decrease in its cellular content (data not shown) and affected the activity and the expression at the mRNA and post-transcriptional levels of key enzymes controlling hepatocyte cholesteryl ester metabolism (5). In particular, we reported that addition of either type of remnants led to a decreased activity of ACAT and microsomal and cytosolic CEH (5). The findings here are consistent with the fact that PUFA are not preferential substrates for ACAT (9) but may have a regulatory role on liver cholesteryl ester metabolism, as reported in the macrophage J774 cell line (12). The findings also suggest a possible correlation between the relative decrease in the level of cholesteryl ester observed in hepatocytes cultured for 6 h and the low activity of these enzymes, as ACAT activity was reduced to a greater extent than CEH activity, as a result of interaction with remnants. This possibility remains to be investigated, as the interpretation may be more complex. Hepatocyte levels of the mRNA encoding for one of the forms of ACAT, ACAT2, were down-regulated by fish oil remnants and not by corn oil remnants (as seen also in J774 cells (12)), while the cytosolic CEH mRNA was down-regulated by corn oil but not fish oil remnants, and both remnant types induced the expression of the microsomal CEH protein (5). The possibility that the CEH isoforms involved could act on diverse molecular species of cholesteryl esters is open.

In conclusion, the results presented here demonstrate that, although the fatty acid composition of the total lipid of chylomicrons and remnants prepared *in vitro* resembles that of the dietary oil from which they were derived, there are significant differences in the fatty acid profile of the various lipid classes, with triacylglycerols showing the closest similarities to the bulk of the parent oil and phospholipids and cholesteryl esters being the most divergent. The fatty acid analysis of these components show that, although most of the lipid secreted in chylomicrons originates from the diet, an indispensable part is contributed by the enterocytes. Our findings also show that when hepatocytes are exposed to chylomicron remnants, significant changes in the fatty acid of the cellular triacylglycerols, but not of the phospholipids, result after a few hours of exposure. The fact that dietary fats car-

ried in chylomicron remnants are able to alter the fatty acid profile of cellular triacylglycerols suggests a potential role for dietary fats as modulators of aspects of liver function involving triacylglycerols, such as the secretion of very low density lipoproteins.

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E. N. MALDONADO, Y. CHICO, K. M. BOTHAM, M. I. AVELDAÑO y B. OCHOA. *Influencia de la composición en ácidos grasos de los remanentes de quilomicrones derivados de aceite de pescado o de maíz en el perfil lipídico de los hepatocitos de rata en cultivo.* J. Physiol. Biochem., **59** (2), 85-100, 2003.

El objetivo de este estudio es caracterizar la composición en ácidos grasos y lípidos de remanentes de quilomicrones enriquecidos en ácidos grasos poliinsaturados (PUFA) n-3 o n-6 e investigar su influencia en los perfiles acílicos de los lípidos mayoritarios de hepatocitos de rata cultivados en monocapa. Los quilomicrones se obtuvieron de la linfa recogida del conducto torácico de ratas a las que se había administrado una dosis de aceite de pescado (rica en PUFA n-3) o de maíz (rica en PUFA n-6) por vía oral. Los remanentes se prepararon *in vitro* tratando dichos quilomicrones con plasma de rata conteniendo lipoproteína lipasa. Los ácidos grasos que abundaban en el aceite también abundaban en los respectivos remanentes, especialmente en los triglicéridos. Tanto quilomicrones como remanentes contenían pequeñas cantidades de fosfolípidos y PUFA de cadena larga ausentes en los aceites. La incubación de los hepatocitos con remanentes ricos en PUFA n-3 o n-6 (con 0.25-0.75 mM de triglicéridos) durante 6 h produjo un aumento del contenido celular de triglicéridos y fosfolípidos, pero mientras los triglicéridos celulares incorporaron los PUFA dominantes en cada tipo de remanente, el perfil de ácidos grasos de los fosfolípidos prácticamente no se modificó. Además, e independientemente de que estuvieran enriquecidos en PUFA n-3 o n-6, los remanentes redujeron el nivel celular de ésteres de colesterol, un lípido minoritario en el hepatocito en el que se incorporan cantidades muy bajas de PUFA. Los resultados demuestran que el perfil de ácidos grasos de los hepatocitos es modulado por la cantidad y el tipo de PUFA suministrado a las células en remanentes de quilomicrones de modo específico del tipo de lípido.

Palabras clave: Ácido graso poliinsaturado n-3, Ácido graso poliinsaturado n-6, Dieta, Remanente de quilomicrón, Ácidos grasos de hepatocito.

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