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Comparison of short- and long-term effects of different dietary fats on the hepatic uptake and metabolism of chylomicron remnants in rats

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The uptake and metabolism of [^{14}C]oleate-labelled chylomicron remnants derived from olive oil, maize oil, palm oil, fish oil or butter fat was investigated using perfused livers from rats fed on the corresponding fat-supplemented diet (providing 40% of the dietary energy) or a low-fat diet for 21 d. The percentage of added [^{14}C]oleate-labelled remnant removed from the perfusate was similar for livers from rats fed on the fat-supplemented diets irrespective of the type of fat fed, whereas livers from rats fed on the low-fat diet removed more labelled fish oil and butter fat remnants than olive, maize or palm oil remnants. Following hepatic uptake in the fat-supplemented groups, the oxidation of [^{14}C]oleate-labelled remnant lipid from maize oil, fish oil, and butter fat remnants was greater than that of the lipids from olive and palm oil remnants, although only the oxidation of lipids from maize and palm oil remnants was increased by prior fat-supplementation of the diet. In addition, the livers from rats fed on the fish-oil-supplemented diet incorporated more [^{14}C]oleate-labelled remnant lipid into phospholipid compared with the livers from rats fed on the other fat-supplemented diets or the low-fat diets. These investigations show that both prior fat feeding and the composition of the fat fed, as well as the fatty acid composition of the chylomicron remnant particles themselves, influence the uptake and metabolism of chylomicron remnants by the liver.

Perfused rat liver: Dietary fat: Chylomicrons

Chylomicron remnants are formed by the action of lipoprotein lipase (*EC* 3.1.1.34) on triacylglycerol-rich chylomicrons, and are responsible for the delivery of dietary cholesterol and some triacylglycerol to the liver (Redgrave, 1970). Several studies have shown that the type of dietary fat from which chylomicrons and chylomicron remnants are derived influences their removal by the liver. Livers from dogs and rats are reported to remove [^{3}H]- or [^{14}C]fatty acid derived from labelled chylomicrons more rapidly when they are derived from cream as compared with maize oil (Nestel & Scow, 1964; Floren & Nilsson, 1977). Also, in human subjects, retinyl palmitate-labelled chylomicrons and chylomicron remnants derived from soyabean oil or cream are removed more rapidly from the circulation than those derived from olive oil (De Bruin *et al.* 1993). In this laboratory we have shown that [^{14}C]oleate-labelled fish oil and butter fat chylomicron remnants (labelled predominantly in triacylglycerol) are taken up by the perfused rat liver more rapidly than those derived from olive, maize or palm oils (Lambert *et al.* 1995). Taken together these studies show that chylomicron remnants derived from milk fat (cream or butter fat), soyabean oil or fish oil tend to be taken up more rapidly by the liver than those derived from olive, maize or palm oils. As fat-supplemented diets were not used in these studies, the differential uptake of the remnants can be attributed to the fatty acid composition of the particles themselves, which is largely determined by the type of fat from which they are derived (Lambert *et al.* 1996).

In the longer term the type of dietary fat consumed is likely to influence the fatty acid composition of the liver membranes (Kritchevsky *et al.* 1988; Hostmark *et al.* 1989) and the activity of hepatic lipase (*EC* 3.1.1.3) (Coiffer *et al.* 1987; Bravo *et al.* 1997). This may have additional effects

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on the rates of removal and metabolism of chylomicron remnants by the liver. Investigations with normolipidaemic human subjects have shown that retinyl palmitate-labelled apolipoprotein B48-containing lipoproteins (chylomicrons and chylomicron remnants) are removed from the circulation more rapidly when diets enriched in *n*-6 and *n*-3 polyunsaturated fatty acids are compared with saturated fatty acids (Weintraub *et al.* 1988; Demacker *et al.* 1991), and in a further investigation, the clearance of retinyl palmitate-labelled chylomicrons was reported to be similar in human subjects consuming fish oil or olive oil-supplemented diets (Harris & Muzio, 1993).

Other evidence suggests that the type of fatty acids in the remnant particle and in the diet may affect the metabolism of chylomicron remnant lipids within the liver. In our previous work we showed that perfused livers oxidized four- to sevenfold more [^{14}C]oleate-labelled lipids from chylomicron remnants derived from olive oil, fish oil and butter fat as compared with maize or palm oils. In addition, more of the hepatic [^{14}C]oleate-labelled lipid taken up from fish oil remnants was converted to labelled phospholipid when compared with that from olive oil, maize oil, palm oil, or butter fat remnants (Lambert *et al.* 1995). Furthermore, Moir *et al.* (1995) showed that rats consuming diets enriched with *n*-6 or *n*-3 polyunsaturated fatty acids oxidized more [^{14}C]oleate from VLDL and chylomicron remnants (labelled in cholesteryl-[^{14}C]oleate) and incorporated less into triacylglycerol, when compared with rats consuming diets enriched in saturated fatty acids. We believe more evidence is required to provide a better understanding of the longer-term effects of a broad range of different dietary fats on chylomicron remnant uptake and metabolism by the liver.

In the present study the uptake and metabolism of [^{14}C]oleate-labelled chylomicron remnants derived from olive oil, maize oil, palm oil, fish oil or butter fat were compared using isolated perfused livers from rats fed on the corresponding fat-supplemented diet for 21 d before the experiments (e.g. metabolism of olive oil remnants by perfused livers from rats fed on an olive oil-supplemented diet). The results were also contrasted with those obtained using livers from rats fed on a standard low-fat diet. This experimental design enabled the effects of the longer-term adaptive changes in the liver on remnant lipid metabolism to be compared with the immediate effects of changes in the fatty acid composition of the remnants themselves.

Materials and methods

Animals and materials

Male Wistar rats were kept under constant day length (12 h) and temperature (25°) and used for chylomicron and chylomicron remnant preparation (350–370 g), and as blood perfusate donors (350–450 g). [1-^{14}C]oleic acid was obtained from Amersham International, Amersham, Bucks., UK. Sodium pentobarbital, cholesterol oxidase (*EC* 1.1.3.17), ampicillin and menhaden fish oil were obtained from Sigma Chemical Company, Poole, Dorset, UK. Triacylglycerol and cholesterol assay kits were from Boehringer Mannheim, Lewes, E. Sussex, UK. Palm oil was obtained from Rhone Poulenc, Manchester, Lancs., UK. Olive oil, maize oil, and butter were obtained from domestic suppliers. The scintillant Emulsifier 299 and the CO_2 absorber Carbo-sorb were obtained from Packard Instruments, Reading, Berks., UK. All other chemicals were obtained from BDH, Dagenham, Essex, UK.

Dietary studies

Fat-supplemented diets (40 % of the energy value of the diet as fat) were stored at 4° and prepared every 5–7 d by mixing 1 g olive oil, maize oil, palm oil, fish oil or filtered butter fat with 4·71 g of a standard low-fat rat diet (digestible energy 12·10 kJ/g; Quest Nutrition, Canterbury, Kent, UK). The standard energy value for fats was taken to be 38 kJ/g (Mills *et al.* 1986). Rats (170–188 g body weight) were placed in individual cages and were provided with 30 g/d of the fat-supplemented or the standard low-fat diets for 21 d. The rats consumed similar amounts (24–30 g/d) of the fat-supplemented or low-fat diets which were sufficient to meet the recommended daily requirements for protein and all other nutrients, and during the 21 d they maintained similar growth rates (6·0–6·8 g/d).

Preparation of [^{14}C]oleate-labelled chylomicron remnants

Olive oil, maize oil, palm oil, fish oil or filtered butter fat (0·5 ml) supplemented with α-tocopherol (4 mg/ml) was tube-fed to a rat (maintained on a standard low-fat diet). After approximately 1 h the rat was anaesthetized with sodium pentobarbital (60 mg/kg intraperitoneally), and the thoracic duct was cannulated (Bollman *et al.* 1948). When the chyle was flowing satisfactorily, [^{14}C]oleic acid (3·7 MBq) neutralized with KOH (0·1 M) and emulsified with sodium taurocholate (10 mg), plus a further 0·5 ml of the same oil or fat fed initially, was injected through the wall of the pyloric region of the stomach. The abdominal wall was then sutured and the rat was placed in a restraining cage where it had access to saline (9 g NaCl/l) for 5 h, and water 16–18 h. The chyle (containing ampicillin 0·1 mg/ml) was layered (2 ml/tube) under NaCl (density 1·006 g/ml) in 6·5 ml polyallomer tubes and ultracentrifuged at 20 000 rev./min for 21 min ($6 \times 10^5 \boldsymbol{g}$) in a fixed-angle rotor at a temperature of 12°. [^{14}C]oleate-labelled large chylomicrons (diameter > 100 nm) free from intestinal VLDL (small chylomicrons) were then removed by slicing the top 10–15 mm of the tubes using a Beckman tube slicer.

[^{14}C]oleate-labelled chylomicron remnants were prepared from these labelled large chylomicrons in functionally hepatectomized rats as described previously (Lambert *et al.* 1996). Their serum (containing labelled chylomicron remnants) was layered under NaCl (1·006 g/ml) in polyallomer tubes and ultracentrifuged for $6 \times 10^7 \boldsymbol{g}$ at 12°, and further purified by ultracentrifugation for $3{\cdot}2 \times 10^7 \boldsymbol{g}$ at 12°. Labelled chylomicron remnants were isolated from the top fraction (1 ml) by tube slicing. Contamination of the labelled remnants with VLDL and intermediate density lipoprotein (IDL) was minimized by

Table 1. Fatty acid composition (g/100 g total fatty acids) of chylomicron remnants derived from olive, maize, palm or fish oils, or butter fat in rats*

(Mean values with their standard errors for three independent preparations)

	Type of chylomicron remnant									
	Olive oil		Maize oil		Palm oil		Fish oil		Butter fat	
Fatty acid	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
14:0	0·89[a]	0·16	1·01[a]	0·09	1·01[a]	0·04	3·04[b]	0·51	4·84[b]	1·51
16:0	18·53[a]	0·23	20·25[a]	0·50	29·12[b]	0·91	20·11[a]	0·37	27·98[b]	1·66
18:0	5·12[a]	0·33	5·11[a]	0·24	4·99[a]	0·23	5·12[a]	0·53	8·30[b]	0·30
18:1	46·22[a]	3·80	22·92[b]	0·79	33·86[c]	0·63	15·69[d]	0·63	21·11[bd]	0·53
18:2	18·16[a]	1·12	33·02[b]	0·85	18·14[a]	1·50	14·89[a]	1·49	16·51[a]	1·43
20:5	0·79[a]	0·03	1·62[a]	0·31	0·74[a]	0·04	12·75[b]	1·31	1·44[a]	0·65
22:6	2·62[a]	0·46	2·57[a]	0·80	2·50[a]	0·36	7·00[b]	0·36	2·64[a]	0·26

[a,b,c,d] Mean values within a row not sharing a common superscript letter were significantly different, $P < 0·05$.

* For details of procedures, see pp. 204–206.

using post-absorptive rats and two centrifugation steps. The fatty acid composition of the remnants was analysed as described previously (Lambert *et al.* 1996), and a summary showing the major fatty acids is provided in Table 1. No significant differences in cholesterol and triacylglycerol content were observed between the different types of remnants, and the samples added to the perfusate of isolated perfused livers were standardized to contain 1–1·5 μmol total cholesterol and 3–6 μmol triacylglycerol. The percentage distribution of [^{14}C]oleate between the major remnant lipids was as follows: triacylglycerol (80–90 %), mono- and diacylglycerols (3–5 %), non-esterified fatty acid (3–4 %), and phospholipid (2–3 %), and there were no significant differences between any of the different types of remnants prepared.

Liver perfusions

The methods for the surgical isolation of the rat liver have been described previously (Lambert *et al.* 1995). In the present experiments the blood perfusate (115 ml) was derived from rats which had been fed on a standard low-fat diet, dialysed against a Krebs and Henseleit bicarbonate buffer containing (mM): NaCl 118, KCl 4·7, $CaCl_2$ 1·32, $MgSO_4$ 1·2, Na_2HCO_3 24, KH_2PO_4 1·2, glucose 13·9, and plasma amino acids 670 mg/l. The packed cell volume was adjusted to 35 % by the addition of this buffer containing bovine serum albumin (60 g/l). The liver was perfused at a flow rate of 1·5 ml/min per g and the blood pO_2 was maintained at 100 mmHg (13·3 kPa) by gassing with a mixture of O_2–CO_2 (19 : 1, v/v) and air–CO_2 (19 : 1, v/v). At zero time, [^{14}C]oleate-labelled chylomicron remnants prepared from one of the test oils or butter fat were added to the recirculating perfusate, and the liver was perfused for a further 4 h. During the perfusion, samples were removed from the perfusate at 1, 2 and 4 h and the serum was separated from the erythrocytes by centrifugation at 3000 ***g*** for 15 min. The $^{14}CO_2$ released by the liver was collected by passing the respiratory gases through two absorbers in series containing 2 M-NaOH (30–40 ml) which were changed at 1 h intervals to ensure 100 % recovery. After 4 h the experiment was terminated, and the liver was flushed free of blood with the bicarbonate buffer containing bovine serum albumin (10 g/l).

Analytical methods

Lipid extracts were prepared from the serum and the liver using 20 volumes of chloroform–methanol (2 : 1, v/v), and partitioned with 0·4 volumes of 0·03 M-HCl. Portions of the chloroform phase were dried under N_2 and separated into lipid classes by TLC using silica gel G and hexane–diethyl ether–formic acid (40 : 10 : 1, by vol.) as the developing solvent. After location with I_2 the silica-gel bands were transferred into scintillation vials for counting. Dried lipid extracts and silica-gel bands from TLC were counted for radioactivity with a toluene-based scintillant (18 ml) containing 3 g 2,5-diphenyloxazole/l and 0·25 g 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene/l. Phospholipid bands from TLC were extracted from the silica gel G in distilled water (1 ml) and counted in the scintillant Emulsifier 299.

The triacylglycerol content in extracts of liver was determined by a semi-enzymic method which involved an initial chemical hydrolysis. Chloroform extracts of liver and tripalmitin standards were made up to a volume of 6 ml in centrifuge tubes, and phospholipids were removed by the addition of 50 mg silica gel (previously activated in an oven at 105° for 2 h). After vigorous mixing, the tubes were centrifuged at 3000 ***g*** for 10 min, and 3 ml of the supernatant fraction was dried under a stream of N_2 at 60°. Triacylglycerol hydrolysis was achieved by the addition of ethanolic KOH (1 ml; 1·25 g/l ethanol) and incubation at 60° for 30 min. H_2SO_4 (1 ml, 1·4 M) was then added to neutralize each extract, and the released fatty acids were partitioned in diethylether (4 ml). The tubes were shaken for 5–10 min and the upper diethyl ether layer was removed using a pasteur pipette. The remaining diethyl ether was evaporated under a stream of N_2. Following this procedure, portions of the aqueous liver extracts (containing glycerol released from the triacylglycerol) and aqueous serum samples could be assayed enzymically for triacylglycerol

content using the Boehringer Mannheim assay kit. Cholesterol mass was determined enzymically in aqueous samples and portions of the dried lipid extracts suspended in propan-2-ol (200 µl) according to the method of Trinder (1969). The total cholesterol was measured in these samples by the addition of cholesterol esterase (*EC* 3.1.1.13) reagent. The $^{14}CO_2$ produced by the liver was measured according to previously described methods (Lambert *et al.* 1995).

Statistical analyses

Results are expressed as means with their standard errors. Statistical significance within a group was determined by one-way ANOVA followed, where appropriate, by the Fischer's test of least difference for multiple comparisons to compare means between groups using Statview software, version 1.03, 1988 (Abacus Concepts Inc., Berkeley, CA, USA). A P value < 0.05 was considered to be statistically significant.

Results

Serum and liver triacylglycerol concentrations in rats fed on a fat-supplemented or low-fat diet

Rats fed on the olive oil, maize oil, palm oil or butter fat-supplemented diets had serum triacylglycerol concentrations which ranged between 1·06 and 1·17 mM, while those fed on the fish oil-supplemented diet were 44 % lower at 0·60 mM (Table 2). In all cases the serum triacylglycerol concentration in rats fed on the fat-supplemented diets were lower (approximately 50 % less) than those fed on the low-fat diet. The liver weights of rats fed on the butter fat-supplemented diet were not significantly different to those from rats fed on the low-fat diet, although livers from both these dietary groups weighed significantly more than livers from rats fed on any of the other fat-supplemented diets. The livers from rats fed on olive or fish oil contained less triacylglycerol when expressed both as total µmol and µmol/g liver, than those from rats fed on the butter fat-supplemented diet, and these latter livers contained more triacylglycerol than livers from rats fed on a low-fat diet (Table 2).

Removal of [^{14}C]oleate-labelled chylomicron remnants by perfused livers from rats fed on a fat-supplemented or a low-fat diet

Livers from rats fed on the low-fat diet removed [^{14}C]oleate-labelled remnants rapidly from the perfusate over the 4 h experimental period (Fig. 1), and their rates of removal were influenced by the type of fat from which they were derived. After 4 h, butter fat remnants were removed to the greatest extent (47 %), followed by fish oil remnants (42 %), palm oil remnants (39 %), olive oil remnants (35 %) and maize oil remnants (31 %). When these values were compared with those obtained using rats fed on the corresponding fats in the diet, livers from rats fed on maize oil removed more of the [^{14}C]oleate-labelled remnants from the perfusate in 4 h (Fig. 1(b)), those from rats fed on the butter fat diet removed less (Fig. 1(e)), while those from rats fed on the olive, palm and fish oil diets showed no significant change (Fig. 1 (a,c,d)). These effects of fat-feeding eliminated the differences in the uptake of remnants of different fatty acid composition found with livers from rats fed on the low-fat diet, so that all the different types of remnants tested were removed at similar rates by the livers adapted to longer-term fat feeding.

These results were confirmed by measurements of the recovery of ^{14}C in the livers on termination of perfusion after 4 h. For the livers from rats fed on a low-fat diet, significantly more ^{14}C was recovered from labelled fish oil or butter fat remnants than from olive or maize oil remnants (Table 3). However, in the same period, the percentage of ^{14}C recovered in the livers from rats fed on each of the fat-supplemented diets was similar, reflecting their comparable disappearance from the perfusate (Fig. 1).

Metabolism of [^{14}C]oleate-labelled chylomicron remnant lipids by perfused livers from rats fed on a fat-supplemented or a low-fat diet

Overall, the effect of supplementation of the diets with fat was to decrease the difference in the oxidation of the

Table 2. Triacylglycerol content of the serum and livers of rats fed on a fat-supplemented diet or a low-fat diet‡
(Mean values with their standard errors; numbers of determinations are given in parentheses)

	Serum triacylglycerol (mmol/l)		Liver weight (g)		Liver triacylglycerol (Total µmol)		Liver triacylglycerol (µmol/g liver)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Olive oil	1·12 (6)*	0·06	11·80 (11)*†	0·32	66·52 (4)†	18·25	5·40†	1·25
Maize oil	1·10 (4)*	0·15	12·67 (12)*†	0·24	100·14 (4)*	22·41	7·80*	1·51
Palm oil	1·06 (6)*	0·09	12·02 (11)*†	0·28	89·94 (4)	35·54	7·48	2·65
Fish oil	0·60 (2)	(0·5–0·7)§	12·53 (8)*†	0·39	54·86 (4)†	14·36	4·15†	0·95
Butter fat	1·17 (5)*	0·06	14·33 (12)	0·57	214·71 (4)*	91·85	16·68*	5·01
Low-fat	2·06 (4)	0·21	13·89 (9)	0·27	44·10 (4)	4·69	3·11	0·26

* Mean values were significantly different from that for the low-fat diet, $P < 0.05$.
† Mean values were significantly different from that for the butter fat diet, $P < 0.05$.
‡ For details of procedures, see pp. 204–206.
§ Range is given due to the small number in this group.

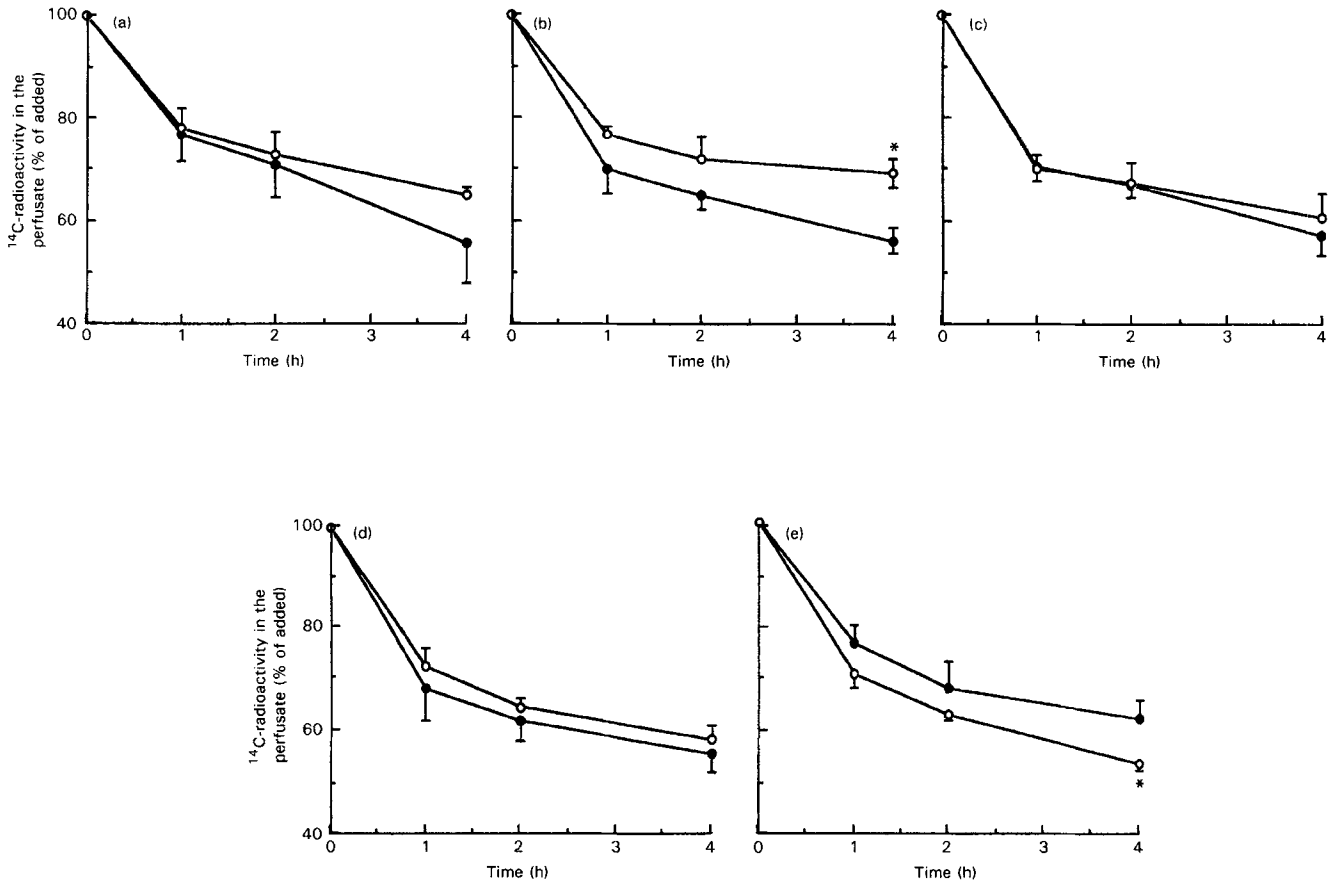

Fig. 1. Removal of [^{14}C]oleate-labelled remnants from the perfusate of isolated livers derived from rats fed on a low-fat or fat-supplemented diet. Livers from rats fed on a low-fat diet (○) or a fat-supplemented diet (●) were perfused with [^{14}C]oleate-labelled remnants derived from (a) olive oil, (b) maize oil, (c) palm oil, (d) fish oil, (e) butter fat as described on p. 205. Values are means with their standard errors represented by vertical bars for the following numbers of perfusions; low-fat diet: olive oil remnants *n* 5, maize oil remnants *n* 4, palm oil remnants *n* 6, fish oil remnants *n* 4, butter fat remnants *n* 6; fat-supplemented diets: olive oil *n* 4, maize oil *n* 6, palm oil *n* 4, fish oil *n* 4, and butter fat *n* 4. Mean values were significantly different from corresponding fat-supplemented diet, * $P < 0.05$.

Table 3. Uptake of [^{14}C]oleate-labelled chylomicron remnants by perfused livers from rats fed on a low-fat or fat-supplemented diet*

(Mean values with their standard errors after 4 h perfusion; numbers of perfusions are given in parentheses)

	Remnant [^{14}C]oleate-labelled lipids recovered in the liver (% of added)				
	Low-fat diet		Fat-supplemented diet		Percentage change (fat supplemented *v.* low-fat diet)
Source of remnants	Mean	SE	Mean	SE	
Olive oil	25·09^{a} (4)	1·39	35·91 (5)	5·83	+43·12
Maize oil	26·00^{a} (6)	1·72	30·84 (6)	2·31	+18·62
Palm oil	30·08^{ab} (4)	4·53	35·42 (6)	4·23	+17·75
Fish oil	35·90^{b} (4)	2·41	34·72 (4)	0·96	−3·39
Butter fat	37·13^{b} (4)	1·98	32·26 (6)	3·73	−15·09

^{a,b} Mean values within a column not sharing a common superscript letter were significantly different, $P < 0.05$.
* For details of procedures, see pp. 204–206.

individual [^{14}C]oleate-labelled remnant lipids to $^{14}CO_2$ observed when rats fed on the low-fat diets were used (Fig. 2). Nevertheless, the livers from rats fed on fish oil still oxidized more of their corresponding [^{14}C]oleate-labelled remnant lipids than those from rats fed on the olive oil or palm oil supplemented diets (Fig. 2(a)), and similar differences were observed when the results were expressed as a percentage of the ^{14}C in the liver (Fig. 2(b)). In addition, oxidation was also increased substantially in livers from rats fed on the maize or palm oil-supplemented diets as compared with the low-fat diets, although oxidation on the olive oil, fish oil and butter fat diets did not change significantly (Fig. 2(a,b)).

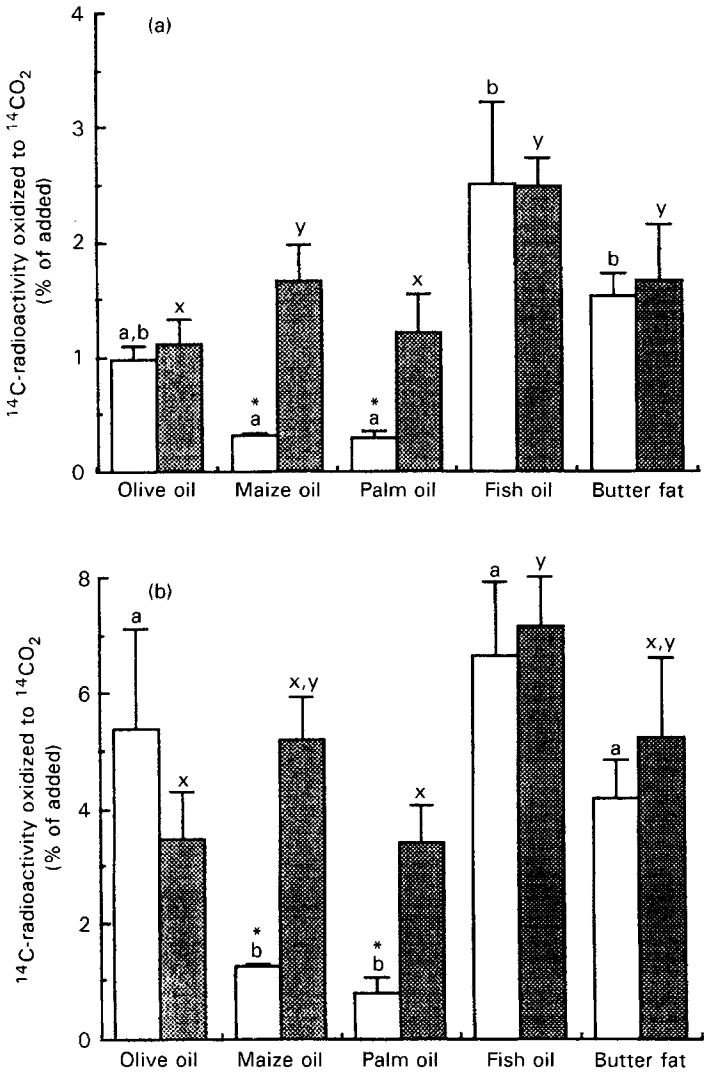

Fig. 2. Oxidation of remnant [^{14}C]oleate-labelled lipids to $^{14}CO_2$. Livers were derived from rats fed on a low-fat diet (□) or a fat-supplemented diet (▨) and were perfused with the corresponding [^{14}C]oleate-labelled remnants as described on p. 205. Values are means with their standard errors represented by vertical bars for the following numbers of perfusions; low-fat diets: *n* 4 except maize oil remnants *n* 6; fat-supplemented diet groups: olive oil *n* 5, maize oil *n* 6, palm oil *n* 6, fish oil *n* 4, and butter fat *n* 6. Mean values were significantly different from the corresponding fat-supplemented diet: * $P < 0.05$. ^{a,b} Mean values within the low-fat diet category not sharing a common letter were significantly different, $P < 0.05$. ^{x,y} Mean values within the fat-supplemented diet category not sharing a common letter were significantly different, $P < 0.05$.

The proportion of [^{14}C]oleate-labelled remnant lipid added to the perfusate that was converted to phospholipid in 4 h in livers from fish oil-fed rats was markedly greater than that found in experiments with animals fed on all the other fat diets (Fig. 3(a)). Furthermore, incorporation of radioactivity into phospholipid was increased in livers from rats fed on the fish oil diet as compared with the low-fat diet, but was unchanged on the other fat-supplemented diets. The percentage of the ^{14}C recovered in the liver in phospholipid also showed similar changes (Fig. 3(b)).

Discussion

The uptake and metabolism of [^{14}C]oleate-labelled chylomicron remnants derived from a range of different dietary fats has been investigated using perfused livers from rats fed on the corresponding fat-supplemented diets. The results have been compared with those obtained with livers of rats fed on a standard low-fat diet. This experimental design has enabled the longer-term effects of incorporating different fats into the diet to be studied, and compared with the immediate effects of changes to the fatty acid composition of the chylomicron remnant particles themselves. The dietary fats were added to the standard low-fat rat diet to provide 40% of the energy content in order to mimic the proportion of fat in the typical Western diet. However, since the addition of fat decreased the content of protein and carbohydrate in the diet by only 17·5% (by weight), and the allowance of these nutrients in the diet more than compensated for this reduction in terms of the daily requirements for the rat, the supply of essential nutrients in these fat-supplemented diets was considered to be adequate over the period of the experiment (Chwalibog, 1994).

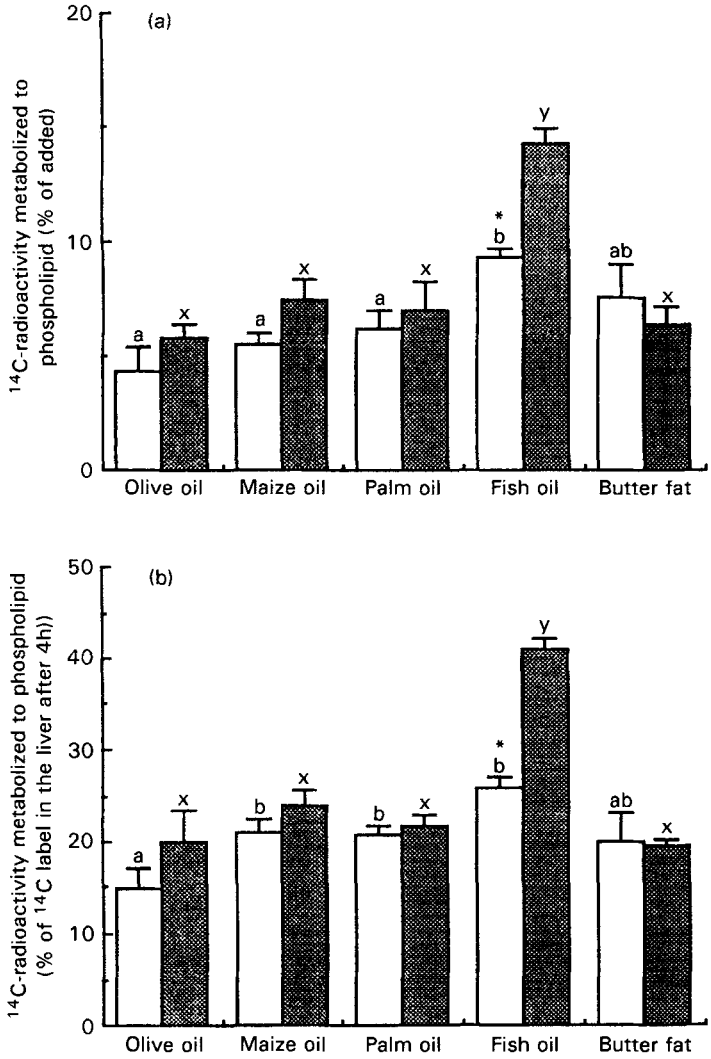

Fig. 3. Net conversion of remnant [^{14}C]oleate-labelled lipids into labelled phospholipids. Livers were derived from rats fed on a low-fat diet (□) or a diet supplemented with olive oil, maize oil, palm oil, fish oil, or butter fat (▩) and were perfused with the corresponding [^{14}C]oleate-labelled remnants as described on p. 205. Values are means with their standard errors represented by vertical bars for the following numbers of perfusions; low-fat diets: *n* 4 except for maize oil remnants *n* 6; fat-supplemented diet groups: olive oil *n* 3, maize oil *n* 6, palm oil *n* 4, bütter fat *n* 6, and fish oil *n* 4. Mean values were significantly different from the corresponding fat-supplemented diet: $^{*}P < 0.05$. ^{a,b} Mean values within the low-fat diet category not sharing a common letter were significantly different, $P < 0.05$. ^{x,y} Mean values within the fat-supplemented diet category not sharing a common letter were significantly different, $P < 0.05$.

Rats fed on all of the fat-supplemented diets had a reduced non-fasting serum triacylglycerol concentration when compared with rats fed on the standard low-fat diet, although the fish oil diet reduced serum triacylglycerol concentrations to a greater extent than the other fats tested (Table 2). The mechanism for the hypotriacylglycerolaemic effect of the fat-supplemented diets is not clear at present, although the results with the fish oil diet are in agreement with previous work (Rustan *et al.* 1992). The amount of triacylglycerol in the liver of rats fed on each of the fat-supplemented diets was increased when compared with those fed on a low-fat diet, although the increase was much smaller for rats fed on the fish oil as compared with the other fat-supplemented diets (Table 2). The mechanism for the reduced accumulation of triacylglycerol in livers from rats fed on the fish oil as compared with the other fat-supplemented diets could be due to the stimulatory effects of eicosapentaenoic acid (*n*-3 polyunsaturated fatty acid present in fish oil) on mitochondrial and peroxisomal fatty acid oxidation (Rustan *et al.* 1992; Willumsen *et al.* 1993), and the inhibitory effects of this fatty acid on acyl-CoA: diacylglycerol acyltransferase (*EC* 2.3.1.20) activity which catalyses an essential step in the pathway of triacylglycerol synthesis (Rustan *et al.* 1988). In addition, diets high in fish oil have been found to reduce serum non-esterified fatty acid concentrations when compared with other polyunsaturated fats, which would also be expected to decrease their availability for hepatic triacylglycerol synthesis (Singer *et al.* 1990; Rustan *et al.* 1992).

[^{14}C]oleate-labelled fish oil or butter fat remnants were removed more rapidly from the perfusate than olive, maize, or palm oil remnants by livers from rats fed on a low-fat diet (Fig. 1, Table 3). As the remnants did not differ in any other respect, their differential removal must have been due

to variations in their fatty acid composition (Lambert *et al.* 1995). However, fat-supplementation of the diet abolished the differential hepatic removal of these labelled remnants so that all types of remnants were removed to the same extent (Fig. 1). This was largely due to maize oil diets increasing, and butter fat diets decreasing, the removal of their respective remnants (Fig. 1, Table 3). In the longer-term, adaptive effects of dietary fats may alter the activity of LDL receptors and/or hepatic lipase which have been implicated in hepatic remnant removal (Daggy & Bensadoun, 1986; Sultan *et al.* 1990; Choi *et al.* 1991). Diets rich in both *n*-6 and *n*-3 polyunsaturated fatty acids have been shown to increase hepatic LDL-receptor activity in rats, hamsters and baboons (*Papio cynocephalus*), while dietary saturated fatty acids have a suppressive effect (Spady & Dietschy, 1985; Fox *et al.* 1987; Ventura *et al.* 1989; Spady & Woollett, 1990). Since it is believed that this receptor plays a part in the hepatic uptake of chylomicron remnants via the recognition of apoliproprotein E (Choi *et al.* 1991), this may explain their increased uptake by perfused livers from rats fed on maize oil (enriched in *n*-6 polyunsaturated fatty acids) or the decrease in the livers from rats fed on butter fat (enriched in saturated fatty acids). Hepatic lipase has been shown to promote the uptake of chylomicron remnants by livers from human subjects (Brekenridge *et al.* 1982) and rats (Daggy & Bensadoun, 1986). However, as both the activity (Coiffer *et al.* 1987) and the hepatic expression of mRNA (Bravo *et al.* 1997) for this enzyme have been found to be increased to a greater extent in rats fed on saturated as compared with *n*-6 polyunsaturated fats, it seems unlikely to be involved in the changes in remnant uptake observed in the present work.

Livers from rats fed on the low-fat diet oxidized more of the [^{14}C]oleate-labelled lipids from olive oil, fish oil and butter fat remnants to $^{14}CO_2$ than those from maize and palm oil remnants (Fig. 2). The presence of eicosapentaenoic acid and medium-chain fatty acids (such as myristic acid) in the diet has been shown to increase carnitine palmitoyl transferase-I activity (Pegorier *et al.* 1988; Surette *et al.* 1992), although it is not clear whether this can explain the increased oxidation of [^{14}C]oleate-labelled lipids from fish oil and butter fat remnants in the relatively short 4 h period of perfusion. In the longer-term, the increase in oxidation of remnant lipids found with the maize and palm oil supplemented diets in comparison with the low-fat diet (Fig. 2) may be related to adaptive changes in the carnitine palmitoyl transferase-I protein, which has been reported to be markedly less sensitive to malonyl-CoA inhibition in hepatocytes from rats fed on a diet enriched in soyabean oil as compared with a low-fat diet (Pegorier *et al.* 1988). Comparing the different fat-supplemented diets, it is clear that our results are consistent with previous work suggesting that *n*-3 polyunsaturated fatty acids in fish oil diets are most effective stimulants of mitochondrial and peroxisomal oxidation (Rustan *et al.* 1992; Willumsen *et al.* 1993), and this also supports the likelihood of reduced availability of fatty acids for esterification and diminished accumulation of triacylglycerol in the livers of rats fed on this diet (Table 2).

An important observation from the current study is the significantly greater conversion of [^{14}C]oleate-labelled lipids into phospholipid from fish oil remnants as compared with the other types of remnants tested in the livers from rats fed on either fat-supplemented or the low-fat diet (Fig. 3). Moir *et al.* (1995) have previously shown that more [^{14}C]oleate from cholesteryl [1-^{14}C]oleate-labelled chylomicron remnants was incorporated into hepatic phospholipids when rats were fed on a diet supplemented with fish oil as compared with lard, maize oil or safflower oil. Taken together, these studies demonstrate that fish oil-supplemented diets increase the general rate of phospholipid synthesis in the rat when compared with other dietary fats. These observations could be explained by evidence which shows that eicosapentaenoic acid (present in the fish oil remnants used; Table 1) can directly inhibit diacylglycerol acyltransferase activity *in vivo* (Coleman & Bell, 1976; Rustan *et al.* 1992), and in isolated liver parenchymal cells (Rustan *et al.* 1988). This would markedly reduce the flux of [^{14}C]oleate-labelled lipids towards triacylglycerol synthesis, and as a consequence, significantly increase their utilization for the synthesis of phospholipids.

In summary, the results reported here show that different types of fat given in the diet over a period of 21 d have differential effects on the hepatic uptake and metabolism of lipids carried in chylomicron remnants of the corresponding fatty acid composition. Compared with the results obtained with perfused livers from rats fed on a low-fat diet, uptake was increased by the maize oil and decreased by the butter fat diet. These changes eliminated the differences in the uptake of remnants of different fatty acid composition found with livers from rats fed on a low-fat diet, so that all the different types of remnants tested were removed at similar rates by the livers of rats adapted to longer-term fat feeding. The hepatic oxidation of [^{14}C]oleate-labelled lipids from maize and palm oil remnants was increased by the corresponding fat-supplemented diet. Conversion of the label to phospholipid was markedly increased when the diet was supplemented with fish oil, but not with the other fats tested. These findings clearly indicate that adaptive changes which occur in the rat liver on long-term feeding of different types of fat have effects on the uptake and metabolism of chylomicron remnants which may alter or modify the acute effects of variations in the fatty acid composition of remnant particles.

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