# Variations in composition of dietary fats affect hepatic uptake and metabolism of chylomicron remnants

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The hepatic metabolism of [1-$^{14}$C]oleate- and [1,2-$^{3}$H]cholesterol-dual-labelled chylomicron remnants derived from olive, corn, palm and fish oil and butter fat was compared by adding each lipoprotein separately to the perfusate of isolated livers from rats fed on a normal diet. Labelled remnants from butter fat and fish oil were removed more rapidly from the perfusate than remnants derived from olive, corn and palm oil. The oxidation of labelled remnant fatty acid from olive oil, fish oil or butter fat was four to seven times greater than that from corn and palm oil. Labelled fatty acid in fish oil remnants was incorporated into phospholipid significantly more efficiently than the labelled fatty acid in olive, corn or palm oil remnants, with butter fat giving an intermediate value. For all the remnants, there was a significant amount of hydrolysis of labelled esterified cholesterol by the liver which was dependent on the magnitude of hepatic uptake of each type of remnant. The recovery of remnant [$^{3}$H]cholesterol label in the bile was 50 % less with palm oil remnants than with all the other remnants studied. The results indicate that the fatty acid composition of chylomicron remnants has a major impact on their uptake and metabolism by the liver.

## INTRODUCTION

It has been known for many years that the type of fat in the diet influences blood lipid concentrations. Long-chain saturated fatty acids, which predominate in palm oil and ruminant fat, increase plasma cholesterol concentrations, whereas $n-6$ polyunsaturated fatty acids have the opposite effect [1–3]. More recently oleic acid, abundant in olive oil, and long-chain $n-3$ polyunsaturated fatty acids, found in fish oil, have also been shown to be hypocholesterolaemic, although in the latter case the effects were not as marked in humans as in experimental animals [4–6].

Chylomicron remnants are formed by the action of lipoprotein lipase on triacylglycerol-rich chylomicrons [7], and are responsible for the delivery of dietary cholesterol and some triacylglycerol to the liver. The remnants, which have been shown to be atherogenic in experimental animals [8,9], are enriched in esterified cholesterol and their removal by the liver occurs mainly via a receptor specific for apolipoprotein E [10]. In humans consuming fat-enriched diets, chylomicron remnants would be expected to circulate in the plasma for much of the day. In familial type-III hyperlipoproteinaemia, the accumulation of both chylomicron and very-low-density lipoprotein (VLDL) remnants in the blood causes hypercholesterolaemia and hypertriacylglycerolaemia [11], the condition being associated with peripheral and coronary atherosclerosis. Also, there is delay in the clearance of remnant triacylglycerol from the circulation of patients with coronary artery disease [12,13].

At present, little information exists about the effects of fatty acid composition on the hepatic clearance and metabolism of cholesterol and triacylglycerol delivered to the liver in chylomicron remnants. A greater hepatic uptake of chylomicron-derived triacylglycerol fatty acids from cream compared with those from corn oil was reported in dogs and rats [14,15]. In addition, the presence of both dietary $n-6$ and $n-3$ long-chain polyunsaturated fatty acids in the diet has been reported to increase the clearance of chylomicron remnants from the plasma of humans [16,17] and rats [18]. However, these studies *in vivo* rely on the labelling of lipoproteins derived from the diet with retinyl palmitate, which does not allow unambiguous differentiation between chylomicrons, which are non-atherogenic and metabolized initially in extrahepatic tissues, and chylomicron remnants, which are atherogenic and metabolized by the liver.

Other work has suggested that dietary fat affects some of the pathways that remnant cholesterol may enter within liver. Some studies *in vivo* have shown that diets rich in polyunsaturated fatty acids increase bile acid and cholesterol excretion [19–21], although others have been unable to demonstrate any effect [22–24]. Diets supplemented with corn oil or olive oil were found to increase bile acid synthesis in rat liver [25], and hepatic acyl-CoA–cholesterol acyltransferase activity has been reported to be increased in rats fed on $n-3$ polyunsaturated fat compared with those given a saturated-fat diet [26].

In the present study, the uptake and metabolism of labelled fatty acid and cholesterol in chylomicron remnants derived from olive, corn, palm and fish oil and butter fat were compared in isolated perfused livers of normal rats. The experiments were designed to study the influence of the different fatty acids that predominate in these oils on both remnant clearance and the major routes of metabolism of remnant fatty acid and cholesterol in the liver.

## EXPERIMENTAL

### Animals and materials

Male Wistar rats (350–370 g) were used for chylomicron and chylomicron remnant preparation, and as liver donors; blood donors weighed 350–450 g. Rats were fed on a standard low-fat pellet diet and kept under constant day length (12 h) and temperature (25 °C). [1,2-$^{3}$H]Cholesterol and [1-$^{14}$C]oleic acid were obtained from Amersham International, Amersham, Bucks., U.K. Sodium pentobarbital, cholesterol oxidase, antibiotics and menhaden fish oil were obtained from Sigma Chemical

Abbreviations used: LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; HDL, high-density lipoprotein; CPT-I, carnitine palmitoyltransferase-I.

Co., Poole, Dorset, U.K. Palm oil was obtained from Rhone Poulenc, Manchester, Lancs., U.K. Olive oil, corn oil and butter were obtained from normal domestic suppliers. The scintillants, Emulsifier 299 and Instagel, and Carbo-sorb were obtained from Packard Instruments, Reading, Berks., U.K. All other chemicals were obtained from BDH, Dagenham, Essex, U.K. and Fisons, Loughborough, Leics., U.K.

### Preparation of labelled chylomicrons

Thoracic-duct cannulations were performed using polyethylene tubing (external diameter 1.52 mm) in rats (fed on a standard low-fat pellet diet) anaesthetized with sodium pentobarbital (60 mg/kg body wt.) [27]. The cannulations were carried out in the morning, approx. 1 h after tube-feeding of rats with either olive, corn, palm or fish oil or filtered butter fat (0.5 ml) each supplemented with $\alpha$-tocopheryl acetate (6.71 IU of vitamin E/ml) as antioxidant. When the chyle was flowing and before the body wall was sutured, [$^3$H]cholesterol (500 $\mu$Ci), suspended in another sample (0.5 ml) of the same oil or fat that was fed initially, and [$^{14}$C]oleic acid (100 $\mu$Ci), neutralized with KOH (0.1 M) and emulsified with sodium taurocholate (10 mg), were injected through the stomach wall into the pyloric region. The chyle was collected overnight in the presence of ampicillin (0.1 mg/ml), layered under NaCl (*d* 1.006) in 6.5 ml polyallomer tubes, and ultracentrifuged for $6 \times 10^5$ ***g***-min in a fixed-angle rotor at 12 °C. The top fraction (1–1.5 ml) containing labelled large chylomicrons (diameter greater than 100 nm) was harvested by tube slicing.

### Preparation of labelled chylomicron remnants

Anaesthetized post-absorptive rats that had been fed on a standard low-fat pellet diet were functionally hepatectomized by ligation of all major vessels supplying the liver and the gut. Labelled large chylomicrons, containing 30–40 $\mu$mol of triacylglycerol and 0.05 g of added glucose in NaCl (0.9%), were injected into the left ileolumbar vein. After 45 min, the rats were exsanguinated from the abdominal aorta, while an NaCl solution (0.9%) containing 1% BSA was infused into the right ileolumbar vein. The blood was allowed to clot for 15 min at 37 °C, and, after centrifugation at 3000 ***g*** for 15 min at 12 °C, the serum was layered under NaCl (*d* 1.006) in 6.5 ml polyallomer tubes and ultracentrifuged for $6 \times 10^7$ ***g***-min at 12 °C. The top fraction (1 ml) from this centrifugation containing chylomicron remnants was harvested by tube slicing and purified by further ultracentrifugation for $3.2 \times 10^7$ ***g***-min at 12 °C. After this centrifugation, the top fraction (1 ml) was isolated and used as labelled chylomicron remnants.

Remnant doses added to the perfusate of isolated livers were standardized to contain 1–1.5 $\mu$mol of total cholesterol and 3–6 $\mu$mol of triacylglycerol. The percentage distribution of the [$^3$H]cholesterol label between the esterified (70%) and non-esterified (30%) forms, and the [$^{14}$C]oleate label between triacylglycerol (80–85%), mono- and di-acylglycerols (6–10%), non-esterified fatty acid (3–4%) and phospholipid (2–3%) was similar for all the remnants studied. In addition, no differences were found in the specific radioactivities of either cholesterol or triacylglycerol fatty acid in the remnants derived from the different oils and fat.

### Liver perfusions

Livers derived from anaesthetized rats that had been fed on a standard low-fat pellet diet were perfused *in situ* with defibrinated whole rat blood (115 ml) [28]. Briefly, the procedure involved cannulation of the common bile duct followed by the portal vein to provide the inflow. The abdominal inferior vena cava was cut below the liver, and the liver was flushed free of blood with a modified Krebs–Henseleit [29] bicarbonate buffer containing glucose (250 mg/dl), plasma amino acids (67 mg/dl) and a $Ca^{2+}$ concentration of 1.32 mM, i.e. half of that originally specified. An outflow cannula was tied into the inferior vena cava in the thorax, and the abdominal vena cava and hepatic artery were ligated. The liver preparation was connected to a recirculating perfusion system in a thermostatically controlled cabinet at 37 °C. The perfusate was prepared by dialysis of the rat blood against the modified Krebs–Henseleit bicarbonate buffer, and the haematocrit was adjusted to 35% by the addition of this buffer containing BSA and ampicillin (0.1 mg/ml).

Over the first 10–15 min of perfusion, the flow rate was slowly increased to 1.5 ml/min per g, the blood $pO_2$ was stabilized at 100 mmHg (13.3 kPa) by gassing with a mixture of $O_2/CO_2$ (19:1) and air/$CO_2$ (19:1) and the liver temperature was adjusted to 37 °C. At zero time, the dual-labelled chylomicron remnants were added to the recirculating perfusate, and the liver was perfused for a further 4 h. The exit of the outflow cannula was positioned 2.5 cm below the liver, which ensured a standard pressure in the hepatic vein and therefore a constant fractional uptake in the space of Disse [30]. Blood samples were taken at 1 h intervals, and the serum was separated from erythrocytes by centrifugation at 3000 ***g*** for 15 min. Bile was collected throughout the perfusion. $^{14}CO_2$ released by the liver was collected by passing the respiratory gases through two absorbers in series containing NaOH (2 M) which ensured 100% recovery. Both the NaOH and bile-collection tubes were changed at 1 h intervals. After 4 h, the liver was flushed free of blood with the modified Krebs–Henseleit bicarbonate buffer containing 1% BSA.

### Analytical methods

Fatty acid methyl esters were prepared from the parent oils and remnant preparations using a transmethylation method [31]. The esters were separated by gas chromatography (Shimadzu BC 9A; Shimadzu Seizakusho Co., Kyoto, Japan) using a glass column (1.7 m × 2.6 mm) packed with GP 3% SP-2310/2% SP-2300 on 100/120 Chromosorb WAW (Supelco U.K., Poole, Dorset, U.K.), and using a temperature program of 190 °C for 2 min, increasing from 190 to 220 °C at a rate of 2 °C/min, and then maintained at 220 °C for a further 20 min. Peak areas were quantified by a Shimadzu CR2AX electronic data processor and the results were expressed as the percentage of the total fatty acid mass measured.

Lipids were extracted from the serum and the liver using a minimum of 20 vol. of chloroform/methanol (2:1, v/v). The extracts prepared were partitioned with 0.4 vol. 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 iodine, the bands were transferred into scintillation vials for counting. Erythrocyte samples were washed three times with NaCl (0.9%), and haemolysed with distilled water. The lipids were extracted into 20 vol. of propan-2-ol/chloroform (2:1, v/v) [32]. Dried lipid extracts and silica-gel bands from TLC were counted for radioactivity with a toluene-based scintillant (18 ml) containing 3 g of 2,5-diphenyloxazole/l and 0.25 g of 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 $^3$H-labelled bile acids and non-esterified [$^3$H]cholesterol in the bile were extracted with chloroform/methanol (2:1, v/v) and partitioned with 0.4 vol. of distilled water. Portions of the aqueous phase containing bile acids were counted for radioactivity using Instagel scintillant. The conjugated $\beta$-muricholic, cholic and chenodeoxycholic acids were separated by TLC using silica gel G and ethyl acetate/methanol/acetic acid/water (35:12:2:2, by vol.) as the developing solvent. After location with iodine, silica gel bands from TLC were counted for radioactivity using the scintillant Emulsifier 299. The non-esterified cholesterol mass in the chloroform extracts of bile was determined enzymically using cholesterol oxidase [33]. The masses of conjugated $\beta$-muricholic, cholic and chenodeoxycholic acids in the bile were determined directly by radioimmunoassay [34]. The total amount of $^{14}CO_2$ produced by the liver was measured by modification of the method of Mayes and Felts [28]. The $^{14}CO_2$ collected in NaOH (2 M) was made up to 100 ml with distilled water. Samples of volume 10 ml were removed from each 1 h collection and mixed together, producing a total volume of 40 ml. Then 15 ml of this radioactive NaOH/carbonate solution was added to the outer compartment of a 50 ml Erlenmeyer flask fitted with a centre well containing 1.5 ml of Carbo-sorb. The flask was stoppered with a Subba-Seal bung and 15 ml of air was withdrawn with a syringe and needle, and 2.3 ml of $H_2SO_4$ (5 M) was injected into the outer compartment. After incubation in a shaking water bath for 90 min at 25 °C, the contents of the centre well were transferred with washings of industrial methylated spirits (4.5 ml) into a counting vial. The samples were counted for radioactivity with the toluene-based scintillant described above. Results are expressed as means ± S.E.M. Statistical analyses were performed using the unpaired Student's $t$ test.

## RESULTS

### Fatty acid composition of the oils and fats, and the chylomicron remnants derived from them

Analysis of fatty acids in the oils and fats used produced results similar to those reported previously [35]. Thus the predominant fatty acids identified were, in olive oil, oleic acid ($C_{18:1}$, $n-9$; 75 % of the total fatty acid), in corn oil, linoleic acid ($C_{18:2}$, $n-6$; 58 %), and in butter fat, palmitic acid ($C_{16:0}$; 35 %). Palm oil was rich in palmitic acid (44 %), and contained a relatively high proportion of oleic acid (38.7 %). Fish oil contained a preponderance of $n-3$ polyunsaturated fatty acids comprising eicosapentaenoic (20:5, $n-3$; 15.3 %) and docosahexaenoic acid (22:6, $n-3$; 12.1 %), with a significant distribution of palmitic acid (19.7 %) and oleic acid (13.3 %).

The fatty acid composition of the corresponding remnants (Table 1) reflected that of the oils and fats from which they were derived. Remnants derived from palm oil and butter fat contained a high percentage of saturated fatty acids, whereas those derived from corn and fish oil contained polyunsaturated fatty acids in the highest proportion. In the case of corn oil remnants this was due to an increased linoleic acid content, whereas fish oil remnants contained more of the $n-3$ polyunsaturated fatty acids. Olive oil remnants had the highest content of the monounsaturated fatty acid, oleic acid, although palm oil remnants also contained a relatively high proportion, reflecting its presence in the parent oil. Nevertheless, all the remnants were modified in composition compared with their parent oils or fat by incorporation of endogenous fatty acids during their formation, leading to diminution of the content of the predominating fatty acid(s). Of particular significance was the finding of 3–4 % $n-3$ $C_{20:5}$ and $C_{22:6}$ in chylomicron remnants derived from corn, palm and olive oil and butter fat, in spite of the fact that the parent oils contained negligible quantities of these fatty acids.

### Uptake of [$^{14}$C]oleate- and [$^3$H]cholesterol-labelled chylomicron remnants by the perfused liver

Both [$^{14}$C]oleate and [$^3$H]cholesterol incorporated into the different remnants were used to follow their clearance by the perfused liver. During liver perfusion, remnant [$^{14}$C]oleate and [$^3$H]cholesterol label in the perfusate decreased rapidly over the first hour and then more slowly over the remainder of the 4 h perfusion reflecting their uptake by the liver. The type of fat from which the remnant was derived influenced the extent of disappearance of [$^{14}$C]oleate-labelled remnant from the perfusate

**Table 1 Fatty acid composition of chylomicron remnants derived from olive, corn, palm and fish oil and butter fat**

Fatty acids were measured by separation of the fatty acid methyl esters by GLC as described in the text. Individual fatty acids were identified by comparison with standard fatty acids, and the results are expressed as the percentage composition by mass. Values represent means ± S.E.M. for three chylomicron remnant preparations.

| | Composition (%) | | | | |
|---|---|---|---|---|---|
| Fatty acid | Olive oil | Corn oil | Palm oil | Fish oil | Butter fat |
| $C_{14:0}$ | 0.89 ± 0.16 | 1.01 ± 0.09 | 1.01 ± 0.04 | 3.04 ± 0.51 | 4.84 ± 1.51 |
| $C_{16:0}$ | 18.53 ± 0.23 | 20.25 ± 0.50 | 29.12 ± 0.91 | 20.11 ± 0.37 | 27.98 ± 1.66 |
| $C_{16:1}$ | 3.13 ± 0.24 | 3.97 ± 0.05 | 3.02 ± 0.18 | 7.65 ± 0.65 | 3.59 ± 0.72 |
| $C_{18:0}$ | 5.12 ± 0.33 | 5.11 ± 0.24 | 4.99 ± 0.23 | 5.12 ± 0.53 | 8.30 ± 0.30 |
| $C_{18:1}$ | 46.22 ± 3.80 | 22.92 ± 0.79 | 33.86 ± 0.63 | 15.69 ± 0.63 | 21.11 ± 0.53 |
| $C_{18:2}$ | 18.16 ± 1.12 | 33.02 ± 0.85 | 18.14 ± 1.50 | 14.89 ± 1.49 | 16.51 ± 1.43 |
| $C_{18:3}$ | 0.71 ± 0.06 | 0.71 ± 0.04 | 0.41 ± 0.07 | 0.60 ± 0.14 | 0.79 ± 0.22 |
| $C_{20:5}$ | 0.79 ± 0.03 | 1.62 ± 0.31 | 0.74 ± 0.04 | 12.75 ± 1.31 | 1.44 ± 0.65 |
| $C_{22:6}$ | 2.62 ± 0.46 | 2.57 ± 0.80 | 2.50 ± 0.36 | 7.00 ± 0.36 | 2.64 ± 0.26 |
| Total | | | | | |
| Saturated | 24.53 ± 0.36 | 26.51 ± 0.43 | 35.12 ± 1.11 | 28.28 ± 0.42 | 41.16 ± 3.31 |
| Monounsaturated | 49.35 ± 3.70 | 26.90 ± 0.75 | 36.88 ± 0.46 | 23.34 ± 0.33 | 24.71 ± 0.86 |
| Polyunsaturated | 22.06 ± 1.04 | 37.68 ± 0.63 | 21.79 ± 1.81 | 35.86 ± 0.53 | 22.85 ± 2.95 |

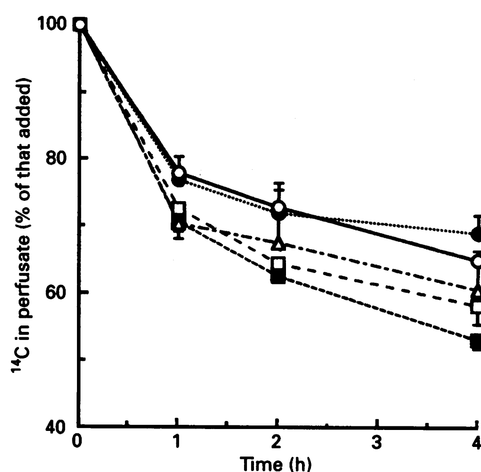


**Figure 1 Removal of [$^{14}$C]oleate-labelled chylomicron remnants from the perfusate of isolated livers**

Remnants were derived from olive oil (○), corn oil (●), palm oil (△), fish oil (□) and butter fat (■). Values are means ± S.E.M. for four to six perfusions. Significance limits: 2 h, olive oil remnants compared with fish oil or butter fat remnants, $P < 0.05$; 4 h, butter fat remnants compared with corn oil remnants, $P < 0.05$, butter fat remnants compared with olive oil remnants, $P < 0.01$.

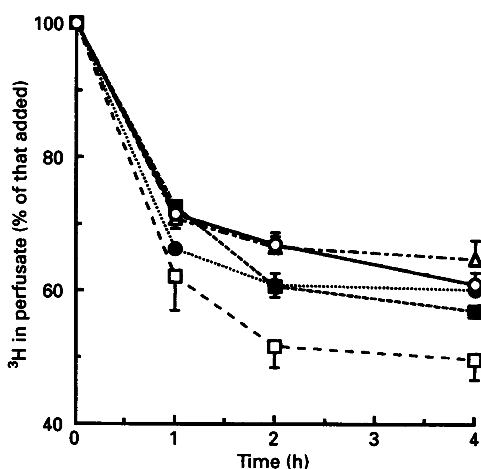


**Figure 2 Removal of [$^{3}$H]cholesterol-labelled chylomicron remnants from the perfusate of isolated livers**

Remnants were derived from olive oil (○), corn oil (●), palm oil (△), fish oil (□) and butter fat (■). Values are means ± S.E.M. for four to six perfusions. Significance limits: 2 h, fish oil remnants compared with olive oil or palm oil remnants, $P < 0.05$; 4 h, fish oil remnants compared with olive, corn or palm oil remnants, $P < 0.05$.

(Figure 1). At 1 h, 22–30 % of the added [$^{14}$C]oleate-labelled remnants derived from all the fats was removed from the perfusate. At 2 h, the amount of label from olive oil remnants remaining in the perfusate was significantly greater than that from fish oil or butter fat remnants ($P < 0.05$), but not significantly different from that from corn and palm oil remnants. After 4 h, butter fat remnants were removed to the extent of 47 %, with fish oil remnants at just under 42 % followed by palm oil (39 %), then olive oil (35 %) and, finally, corn oil remnants being removed the least at 31 %. The removal of [$^{14}$C]oleate-labelled butter fat remnants was significantly greater than that of

**Table 2 Recovery of remnant [$^{14}$C]oleate and [$^{3}$H]cholesterol in HDL after 4 h of liver perfusion**

HDLs were separated by ultracentrifugation of the perfusate as described in the text. Values represent the net percentage of [$^{3}$H]cholesterol and [$^{14}$C]oleate recovered in HDL after 4 h and are expressed as means ± S.E.M. for four to six liver perfusions. *$P < 0.05$, ** $P < 0.01$ compared with fish oil; ‡$P < 0.05$, ††$P < 0.01$ compared with butter fat.

| Source of remnant | [$^{3}$H]Cholesterol recovered in HDL (% of administered dose) [$^{14}$C]Oleate | [$^{3}$H]Cholesterol | (% of label remaining in perfusate) [$^{14}$C]Oleate | [$^{3}$H]Cholesterol |
|---|---|---|---|---|
| Olive oil | 0.87 ± 0.19 | 9.04 ± 1.95*† | 1.67 ± 0.25 | 14.80 ± 2.10**†† |
| Corn oil | 0.70 ± 0.15 | 10.04 ± 1.08* | 1.44 ± 0.66 | 17.29 ± 2.50**† |
| Palm oil | 1.15 ± 0.31 | 9.15 ± 3.08 | 2.49 ± 0.28 | 13.76 ± 4.10**† |
| Fish oil | 1.08 ± 0.30 | 15.72 ± 1.72 | 2.20 ± 0.69 | 31.42 ± 0.70†† |
| Butter fat | 1.10 ± 0.31 | 13.99 ± 0.35 | 2.18 ± 0.66 | 24.46 ± 1.60** |

corn oil ($P < 0.05$) and olive oil ($P < 0.01$) remnants, but not greater than remnants derived from palm or fish oil.

The influence of the different dietary oils and fats on the clearance of remnant [$^{3}$H]cholesterol is shown in Figure 2. After 1 h, approx. 34–38 % of the added [$^{3}$H]cholesterol label in corn and fish oil remnants was removed from the perfusate compared with less than 30 % with all other types of remnants, whereas at later time points the amount of fish oil remnant [$^{3}$H]cholesterol removed was significantly more than that of olive, palm and corn oil remnants, but not butter fat remnants. After 4 h, approx. 50 % of the labelled cholesterol from fish oil remnants had been removed, which was significantly more than that with olive-, corn- or palm-oil remnants ($P < 0.05$) at 35–39 %, but not significantly different from that with buffer fat remnants (43 %).

During these perfusions, a significant amount of the added remnant [$^{3}$H]cholesterol, but only a very small quantity of the remnant [$^{14}$C]oleate label, was recovered in high-density lipoprotein (HDL) of $d$ 1.050–1.210 (Table 2). After 4 h, significantly more of the [$^{3}$H]cholesterol added to the perfusate was recovered in HDL from fish oil remnants than in corn oil and olive oil remnants ($P < 0.05$), and the amount found with butter fat remnants was also significantly higher than that from olive oil remnants ($P < 0.05$). When the data were expressed in terms of the proportion of the [$^{3}$H]cholesterol label remaining in the perfusate found in HDL, the value for fish oil was significantly higher than those for all other remnants ($P < 0.01$), and that for butter fat was also significantly increased compared with the values obtained with olive ($P < 0.01$), corn ($P < 0.05$) and palm ($P < 0.05$) oil remnants. The amount of [$^{3}$H]cholesterol label (15–20 % of the amount added) incorporated into erythrocytes was similar for each of the remnants studied. Under the same conditions the [$^{14}$C]oleate label remained predominantly in the $d < 1.006$ lipoprotein fraction, and less than 1 % was found associated with erythrocytes.

After 4 h of liver perfusion, the percentage of [$^{3}$H]cholesterol and [$^{14}$C]oleate found in the liver (Table 3) enabled the remnants derived from the different oils and fat to be divided into two groups with respect to the net hepatic uptake of [$^{3}$H]cholesterol. A significantly lower amount of the added [$^{3}$H]cholesterol in remnants derived from corn, olive and palm oil was taken up by the liver at 4 h, than in remnants derived from fish oil and butter fat ($P < 0.01$). A similar trend was also observed with the added remnant [$^{14}$C]oleate label, but in this case the palm oil remnants gave an intermediate value between the two other groups. The

**Table 3 Uptake of [$^{14}$C]oleate- and [$^{3}$H]cholesterol-labelled chylomicron remnants by the perfused liver**

Livers were perfused with dual-labelled chylomicron remnants derived from olive, corn, palm or fish oil or butter fat as described in the text. Values (means ± S.E.M.) represent the percentage of added remnant labels recovered in liver lipid at 4 h, and are slightly lower than might be expected from the data shown in Figures 1 and 2 partly because of metabolism of the labels to $^{14}CO_2$ and other water-soluble intermediates, and to excretion via the bile. $^{**}P < 0.01$ compared with fish oil or butter fat remnants.

| Source of remnants | Remnant label recovered in liver lipids (% of that added) | |
|---|---|---|
| | [$^{14}$C]Oleate | [$^{3}$H]Cholesterol |
| Olive oil | 25.09 ± 1.39** | 29.58 ± 0.91** |
| Corn oil | 26.00 ± 1.72** | 30.02 ± 1.13** |
| Palm oil | 30.08 ± 4.53 | 30.59 ± 1.00** |
| Fish oil | 35.90 ± 2.41 | 41.20 ± 1.28 |
| Butter fat | 37.13 ± 1.98 | 41.11 ± 1.65 |

**Table 4 Oxidation of chylomicron remnant [$^{14}$C]oleate-labelled lipids to $^{14}CO_2$ by the perfused liver**

Livers were perfused with [$^{14}$C]oleate-labelled remnants derived from olive oil, corn oil, palm oil, fish oil or butter fat as described in the text. The values represent the total $^{14}CO_2$ in respiratory gases produced by the liver over 4 h, and are expressed as means ± S.E.M. for four to six perfusions. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with corn oil or palm oil remnants.

| Source of remnants | Oxidation to $^{14}CO_2$ | |
|---|---|---|
| | (% of $^{14}$C label added) | (% of $^{14}$C label in liver after 4 h) |
| Olive oil | 0.97 ± 0.12** | 5.38 ± 1.71* |
| Corn oil | 0.32 ± 0.02 | 1.24 ± 0.06 |
| Palm oil | 0.29 ± 0.07 | 0.79 ± 0.26 |
| Fish oil | 2.50 ± 0.72** | 6.64 ± 1.29*** |
| Butter fat | 1.52 ± 0.19** | 4.17 ± 0.67*** |

percentage of the added remnant labelled fatty acid found in the liver was, in general, 4–5 % less than the corresponding amount of labelled cholesterol for each type of remnant.

## Oxidation of remnant [$^{14}$C]oleate-labelled lipids

The percentage of added remnant [$^{14}$C]oleate label (mainly in triacylglycerol) oxidized to $^{14}CO_2$ for butter fat, fish oil and olive oil remnants was three to eight times greater than with corn and palm oil remnants (Table 4). The greater hepatic uptake of [$^{14}$C]oleate in butter fat and fish oil remnants could have partially accounted for its increased oxidation. However, when the percentage of $^{14}CO_2$ produced was normalized for the differences in remnant [$^{14}$C]oleate uptake, oxidation of olive oil, fish oil and butter fat remnants was still four to seven times more than that observed with remnants derived from corn and palm oil.

## Conversion of remnant [$^{14}$C]oleate-labelled lipids into $^{14}$C-labelled phospholipids

In the liver there was a net conversion of remnant [$^{14}$C]oleate-labelled lipids (mainly in triacylglycerol) into [$^{14}$C]oleate-labelled phospholipid with all the remnants studied (Table 5). The amount of labelled phospholipid synthesized was greater with fish oil and butter fat remnants than with corn, olive and palm oil remnants. Normalization of the amount of phospholipid synthesized with respect to differences in the hepatic uptake of [$^{14}$C]oleate in remnants indicated that between 15 and 25 % of the $^{14}$C label taken up into the liver was converted into phospholipid, for all the remnants studied. However, significantly more labelled phospholipid was synthesized after the uptake of fish oil remnants than after the uptake of olive ($P < 0.01$), palm ($P < 0.01$) and corn ($P < 0.05$) oil remnants. Because of greater variation in the results, the synthesis of labelled phospholipid from butter fat remnants was not significantly different from the fish oil remnants.

**Table 5 Conversion of chylomicron remnant [$^{14}$C]oleate-labelled lipids into phospholipid by the perfused liver**

Livers were perfused with [$^{14}$C]oleate-labelled remnants derived from olive oil, corn oil, palm oil, fish oil or butter fat as described in the text. The values represent the net amount of remnant [$^{14}$C]oleate-labelled lipid converted into phospholipid by the liver over 4 h, and are expressed as means ± S.E.M. for four to six perfusions. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with fish oil remnants.

| Source of remnants | Net conversion of $^{14}$C-labelled lipids into phospholipid | |
|---|---|---|
| | (% of $^{14}$C label added) | (% of hepatic $^{14}$C label at 4 h) |
| Olive oil | 4.33 ± 1.00** | 14.89 ± 2.18** |
| Corn oil | 5.47 ± 0.52** | 20.94 ± 1.38* |
| Palm oil | 6.16 ± 0.77* | 20.78 ± 0.92** |
| Fish oil | 9.25 ± 0.42 | 25.89 ± 1.07 |
| Butter fat | 7.47 ± 1.47 | 19.88 ± 3.40 |

## Hydrolysis of remnant esterified [$^{3}$H]cholesterol to non-esterified [$^{3}$H]cholesterol

A significant proportion of remnant esterified [$^{3}$H]cholesterol taken up into the liver was hydrolysed to non-esterified [$^{3}$H]cholesterol in all experiments. Expressed as a percentage of the added esterified [$^{3}$H]cholesterol, the livers hydrolysed 1.5 times more of the esterified cholesterol in fish oil and butter fat remnants than in corn, olive and palm oil remnants (Table 6). However, when these data were normalized for differences in the total amount of esterified [$^{3}$H]cholesterol removed by the liver, the above trends were evident but did not reach statistical significance, with approx. 50 % of the labelled esterified chol-

**Table 6 Conversion of chylomicron remnant esterified [$^{3}$H]cholesterol into non-esterified [$^{3}$H]cholesterol by the perfused liver**

Livers were perfused with [$^{3}$H]cholesterol-labelled remnants derived from olive oil, corn oil, palm oil, fish oil or butter fat as described in the text. The values represent the net amount of remnant esterified [$^{3}$H]cholesterol hydrolysed in the liver over 4 h, and are expressed as means ± S.E.M. for four to six perfusions. $^{**}P < 0.01$ compared with fish oil or butter fat remnants.

| Source of remnants | Net hydrolysis of esterified [$^{3}$H]cholesterol | |
|---|---|---|
| | (% of esterified [$^{3}$H]cholesterol added) | (% of esterified [$^{3}$H]cholesterol taken up by liver in 4 h) |
| Olive oil | 13.99 ± 1.10** | 47.69 ± 3.90 |
| Corn oil | 14.92 ± 0.72** | 49.99 ± 1.03 |
| Palm oil | 15.05 ± 1.24** | 49.02 ± 2.63 |
| Fish oil | 22.85 ± 1.49 | 55.48 ± 2.10 |
| Butter fat | 23.42 ± 1.57 | 57.20 ± 4.77 |

**Table 7 Excretion of labelled bile acids and non-esterified cholesterol resulting from the hepatic uptake of [$^3$H]cholesterol-labelled remnants**

Livers were perfused with labelled remnants derived from olive oil, corn oil, palm oil, fish oil or butter fat as described in the text. The values represent the total $^3$H label recovered in the bile as bile acid and non-esterified cholesterol over 4 h and are expressed as means ± S.E.M. for four to six perfusions. *$P < 0.05$, **$P < 0.01$ compared with palm oil remnants.

| Source of remnants | Percentage of added $^3$H label in: | | Percentage of $^3$H label in liver at 4 h in: | |
|---|---|---|---|---|
| | Bile acid | Bile cholesterol | Bile acid | Bile cholesterol |
| Olive oil | 0.74 ± 0.10** | 0.10 ± 0.01* | 2.50 ± 0.26** | 0.32 ± 0.03* |
| Corn oil | 0.55 ± 0.10* | 0.12 ± 0.04 | 1.89 ± 0.39* | 0.38 ± 0.10 |
| Palm oil | 0.22 ± 0.03 | 0.06 ± 0.01 | 0.73 ± 0.13 | 0.19 ± 0.03 |
| Fish oil | 0.84 ± 0.13** | 0.14 ± 0.02** | 2.08 ± 0.38* | 0.34 ± 0.04* |
| Butter fat | 0.81 ± 0.04** | 0.15 ± 0.01** | 1.97 ± 0.13** | 0.37 ± 0.03** |

**Table 8 Mass of taurine-conjugated bile acids excreted after the hepatic uptake of chylomicron remnants**

Livers were perfused with the labelled remnants derived from olive oil, corn oil, palm oil, fish oil or butter fat as described in the text. The values represent the total mass of each taurine-conjugated bile acid excreted by the liver over 4 h, and are expressed as means ± S.E.M. for four to six perfusions. *$P < 0.05$, **$P < 0.01$ compared with palm oil remnants.

| Source of remnants | Mass of bile acid excreted over 4 h ($\mu$mol) | | | |
|---|---|---|---|---|
| | Total | Tauro-$\beta$-muricholic acid | Taurocholic acid | Taurochenodeoxycholic acid |
| Olive oil | 8.14 ± 0.85** | 6.01 ± 0.70* | 1.64 ± 0.13 | 0.49 ± 0.08 |
| Corn oil | 4.58 ± 0.45 | 3.38 ± 0.51 | 0.91 ± 0.05 | 0.29 ± 0.06 |
| Palm oil | 4.50 ± 0.61 | 2.94 ± 0.38 | 1.27 ± 0.20 | 0.29 ± 0.05 |
| Fish oil | 8.21 ± 1.09** | 5.83 ± 0.80* | 1.74 ± 0.20 | 0.64 ± 0.17 |
| Butter fat | 6.92 ± 0.25** | 4.73 ± 0.26* | 1.80 ± 0.15 | 0.39 ± 0.03 |

esterol taken up in each case being hydrolysed to non-esterified [$^3$H]cholesterol.

### Biliary excretion of [$^3$H]bile acids and [$^3$H]cholesterol

After the uptake of chylomicron remnant cholesterol by the liver, the synthesis of bile acids and their excretion via the bile together with non-esterified cholesterol represents a major route of catabolism of dietary cholesterol. The total amount of $^3$H label recovered in the bile over 4 h for each of the remnants is shown in Table 7. Metabolism of corn, olive and fish oil and butter fat remnants resulted in two to four times more $^3$H label being recovered in the bile than for palm oil remnants. When the amount of $^3$H label excreted was expressed in terms of total hepatic [$^3$H]cholesterol uptake, between 2.3 and 2.8 % of the total at 4 h was excreted in the bile for corn, olive or fish oil or butter fat remnants compared with less than 1 % for palm remnants ($P < 0.01$). The $^3$H label in bile was recovered mainly in bile acids (80–85 %) with the remainder in non-esterified cholesterol, and the amounts of label in both were significantly lower in experiments with palm oil remnants (Table 7). Analysis of the [$^3$H]bile acids indicated that for all the remnants 80–90 % of the $^3$H label was in the taurine-conjugated trihydroxybile acids, $\beta$-muricholic and cholic acid, and the remainder was mainly in the dihydroxybile acid, taurochenodeoxycholic acid, with a small amount in the glycoconjugated bile acids. In terms of mass, the trihydroxybile acids were also the main bile acids excreted during the perfusion. Livers perfused with palm oil remnants secreted significantly less ($P < 0.01$) bile acid mass than remnants derived from all other fats except corn oil (Table 8), because of a 50 % lower excretion of tauro-$\beta$-muricholic acid. For all the remnants, the relative specific radioactivity of taurochenodeoxycholic acid was three times higher than the value for tauro-$\beta$-muricholic and taurocholic acids (results not shown).

The mass of non-esterified cholesterol recovered in the bile over 4 h ranged from 0.13 to 0.49 $\mu$mol in all experiments and no significant differences were observed between the different remnants studied.

## DISCUSSION

The predominant fatty acids identified in each of the parent oils and fats remained abundant in each of the resultant remnants, although the extremes in fatty acid composition were attenuated by the physiological processes that led to the formation of chylomicron remnants. The increased content of both saturated and unsaturated fatty acids not identified in the parent oils and fats indicates a contribution from endogenous fatty acids during chylomicron and remnant formation (Table 1).

The metabolism of [$^3$H]cholesterol- and [$^{14}$C]oleate-labelled chylomicron remnants derived from dietary fats of different fatty acid composition were compared using perfused livers from rats fed on standard low-fat laboratory rations. The studies are therefore confined to the acute effects of a high-fat meal in animals not adapted to a high-fat diet. The mass of cholesterol added to the perfusate increased the total cholesterol concentration by less than 10 %, and the distribution of [$^{14}$C]oleate and [$^3$H]cholesterol labels between the major lipid classes and the specific radioactivities for triacylglycerol and cholesterol were similar for each of the remnants. Therefore differences in metabolism of the labels between the different types of remnant can only be attributed to the differences in their fatty acid composition. However, differences due to variations in the

position of the [14C]oleate label on the glycerol moiety of remnant triacylglycerol cannot be excluded.

The parallel uptake of remnant [14C]oleate and [3H]cholesterol by the liver is indicative of the remnant triacylglycerol and cholesterol undergoing endocytosis simultaneously. However, the small increase (up to 5%) in average [3H]cholesterol uptake compared with [14C]oleate uptake may be ascribed to cholesterol exchange with liver membranes. There was little evidence of significant exchange involving the $^{14}C$ label, e.g. with erythrocytes, indicating that this label is the better indicator of remnant uptake by the liver. The amount of [3H]cholesterol derived from remnants recovered in perfusate HDL at 4 h was related to the differences in the extent of their uptake and metabolism by the liver. This suggests that, in addition to direct transfer of labelled non-esterified cholesterol into HDL by exchange, remnant [3H]cholesterol is significantly incorporated into newly synthesized HDL by the liver, as radioactivity arising from exchange would not be expected to be influenced significantly by the fatty acid composition of the remnants. Thus the enhanced metabolism of fish oil and butter fat resulted in a greater incorporation of their cholesterol into HDL when compared with the other remnants.

The increased recovery (25% greater) of labelled fish oil and butter fat remnants in the liver at 4 h compared with olive, corn and palm oil remnants demonstrates directly that the fatty acid composition of chylomicron remnants influences their uptake by the liver. Our finding of increased uptake of [14C]oleate from butter fat compared with corn oil remnants by the perfused liver is consistent with previous observations in experimental animals [14,15]. Also, in humans, olive oil remnants have been reported to remain in the circulation for a longer period than cream remnants [36]. However, the large differences between uptake of corn and olive oil remnants on the one hand and fish oil and butter fat remnants on the other, and the intermediate position of palm oil remnants (Table 3), suggests that the effects of individual fatty acids and their combinations and positions in the remnant triacylglycerol are more important in determining the rate of hepatic uptake than the degree of saturation or unsaturation. Both fish oil and butter fat remnants were observed to contain a significantly higher proportion of myristic acid ($C_{14:0}$) than the other remnants (Table 1). It is possible therefore that this fatty acid may determine their increased hepatic uptake, although in the case of fish oil remnants the relative abundance of long-chain $n-3$ polyunsaturated fatty acids may also be involved.

The reasons for the enhanced oxidation of [14C]oleate in olive oil remnants (Table 4) are not clear at present. However, moderate incorporation of $n-3$ polyunsaturated fatty acids into the diet of Syrian hamsters has been found to be associated with large increases in carnitine palmitoyltransferase I (CPT-I) activity [37], and short- and medium-chain saturated fatty acids were able to increase CPT-I activity by decreasing its sensitivity to malonyl-CoA inhibition [38]. As the amount of myristic acid (a medium-chain fatty acid) present in both fish oil and butter fat remnants was significantly higher than in the other remnants, the enhanced oxidation of [14C]oleate from both fish oil and butter fat remnants could be due to a decrease in the sensitivity of CPT-I to malonyl-CoA, and the presence of $n-3$ polyunsaturated fatty acids in fish oil remnants may increase CPT-I activity directly. In addition to the oxidation, the increased incorporation of fish oil remnant [14C]oleate into phospholipid may indicate increased initial hydrolysis of triacylglycerol from this type of remnant.

The inverse relationship between fatty acid oxidation and esterification [39] implies that increased oxidation of remnant tricylglycerol fatty acid represents a decrease in its availability for triacylglycerol synthesis and VLDL production. Therefore the production of VLDL after the uptake of olive oil, fish oil and butter fat remnants would be expected to be less than that with corn or palm oil remnants, leading to a lowering of plasma triacylglycerol. Hypotriacylglycerolaemia is a consistent finding with fish oil diets, and a reduced low-density lipoprotein (LDL) formation as a consequence of decreased VLDL formation could also explain the hypocholesterolaemic effects of olive oil diets [4]. As dietary butter fat is known to be hypercholesterolaemic and corn oil hypocholesterolaemic, our results suggest that the effects of these dietary fats are related to other factors such as down-regulation of LDL receptor synthesis by saturated fatty acids and up-regulation by unsaturated fatty acids [40].

With the notable exception of palm oil remnants, the proportion of $^{3}H$ label secreted into bile generally reflected the magnitude of remnant [3H]cholesterol uptake and esterified [3H]cholesterol hydrolysis, and was not apparently influenced by the type of oil or fat from which the remnants were derived. Our results clearly demonstrate that cholesterol carried in chylomicron remnants derived from palm oil is secreted into bile at a markedly lower rate than that from remnants derived from olive, corn or fish oil or butter fat, and thus show for the first time that this dietary fat has a direct influence on the removal of cholesterol from the body by the liver. The effect appears to be due to decreased bile acid synthesis, and/or reduced bile acid secretion as the proportion of esterified [3H]cholesterol hydrolysed by the liver was comparable for all the remnants. This may represent an important mechanism by which dietary palm oil increases blood cholesterol concentrations, as the retention of cholesterol in the liver would lead to down-regulation of the LDL receptor. The proportion of total saturated fatty acids in the palm oil remnants is unlikely to be the causative factor, as butter fat remnants contain a greater proportion of saturated fatty acids than palm oil remnants. However, the relative amounts of individual saturated fatty acids may be important; for example, there is nearly five times as much myristic acid in butter fat remnants than in palm oil remnants. This observation further emphasizes that individual fatty acids may be more important than the overall degree of saturation or unsaturation of a dietary oil or fat.

In summary, the present investigations demonstrate that both the hepatic uptake and the subsequent metabolism of cholesterol and triacylglycerol carried in chylomicron remnants is influenced by the type of fat from which they are derived. Fish oil and butter fat remnants are taken up more rapidly by the liver than olive, corn and palm oil remnants, and there are also differences in the utilization of the fatty acids for oxidation and phospholipid synthesis. In addition, label from [3H]cholesterol in remnants derived from palm oil is secreted into bile more slowly than that carried in remnants from the other oils and fat, suggesting that reduced biliary excretion of cholesterol may be one mechanism to explain the hypercholesterolaemic effects of palm oil.

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