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The type of dietary fat alters the hepatic uptake and biliary excretion of cholesterol from chylomicron remnants

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The consumption of fat-enriched diets may alter the uptake and metabolism of chylomicron remnant cholesterol by the liver. To test this hypothesis, [^{3}H]cholesterol-labelled chylomicron remnants derived from different dietary fats were studied in perfused livers both from rats fed on diets enriched in the corresponding fats and from rats fed on a low-fat diet. Livers from rats fed on each of the fat-enriched diets removed similar amounts (34–40 %) of the [^{3}H]cholesterol-labelled remnants added, whereas livers from rats fed on the low-fat diet removed significantly more labelled fish-oil and butter-fat remnants than olive-, maize- or palm-oil remnants. Significantly more remnant [^{3}H]cholesterol was secreted into the perfusate HDL by livers from rats fed on the olive-oil, fish-oil and butter-fat diets when compared with those from rats fed on the low-fat diet or the maize-oil diet. Furthermore, the excretion of remnant [^{3}H]cholesterol via the bile acid was increased by the olive-, maize-, palm- or fish-oil diets, and decreased by the butter-fat diet when compared with the low-fat diet, although the [^{3}H]bile acid excreted remained less on saturated fat diets. This investigation shows that the hepatic uptake and subsequent metabolism of cholesterol from chylomicron remnants is influenced by the type of fat in the diet as well as the fatty acid composition of the particles themselves, and may help to explain some of the hyper- and hypocholesterolaemic effects of saturated and unsaturated fatty acids.

Dietary fat: Chylomicron remnants: Cholesterol metabolism: Biliary steroids

Cholesterol and fat (predominantly triacylglycerol) from the diet enter the blood in triacylglycerol-rich chylomicrons. The action of lipoprotein lipase in the extrahepatic capillary beds hydrolyses most of the chylomicron triacylglycerol and generates smaller cholesterol-enriched chylomicron remnant particles which are removed from the circulation by the liver (Redgrave, 1970). Regular consumption of fat-enriched diets is likely to maintain the presence of chylomicron remnants in the blood for much of the day, and these lipoproteins are now considered to contribute to the development of both peripheral and coronary atherosclerosis (Zilversmit, 1979; Melchior *et al*. 1981; Fainaru *et al*. 1982). Therefore, all factors that affect the hepatic clearance and subsequent fate of cholesterol from chylomicron remnants are likely to be important determinants for the development of cardiovascular disease.

In our laboratory, studies in the rat *in vivo* have shown that [^{3}H]cholesterol carried in chylomicrons derived from maize oil (enriched in *n*-6 polyunsaturated fatty acids (PUFA)) or fish oil (enriched in *n*-3 PUFA) is cleared from the blood and excreted into the bile more rapidly than that from palm-oil (enriched in long-chain saturated fatty acids) or olive-oil chylomicrons (enriched in mono-unsaturated fatty acids) (Bravo *et al*. 1995), and results consistent with these observations were also found using the isolated perfused liver (Lambert *et al*. 1995). Also, chylomicron remnants derived from maize oil were found to stimulate bile acid synthesis by isolated hepatocytes, whereas those derived from palm oil were ineffective and stimulated the secretion of cholesterol-enriched VLDL (Bravo *et al*. 1996). Taken together, these investigations show that in animals fed on a low-fat diet, the type of fat from which chylomicron remnants are derived can influence the uptake and subsequent metabolism of their cholesterol by the liver.

In the longer term, however, adaptation of the liver to high-fat diets leads to changes in its lipid composition (Kritchevsky *et al*. 1988; Hostmark *et al*. 1989) and metabolism (Kritchevsky *et al*. 1983; Jackson *et al*. 1990), and these are likely to have further effects on the uptake and processing of lipids from chylomicron remnants, particularly cholesterol. Consistent with this hypothesis, an earlier investigation in this laboratory reported that feeding rats on diets enriched in different types of fat abolished the

Abbreviations: Apo, apolipoprotein; PUFA, polyunsaturated fatty acids.

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differential uptake and altered the metabolism of triacylglycerol from chylomicron remnants which had been observed with livers from rats fed on a low-fat diet (Lambert *et al.* 1998). In addition, other workers have shown that diets enriched in *n*-6 and *n*-3 PUFA increase the clearance of postprandial lipoproteins (chylomicrons and their remnants) from the plasma compared with those enriched in saturated fatty acids (Weintraub *et al.* 1988; Demacker *et al.* 1991). The consumption of diets enriched in monounsaturated fatty acids and PUFA has also been reported to increase bile acid synthesis (Botham & Boyd, 1983), and bile acid and cholesterol excretion in the rat (Byers & Friedman, 1958; Wood *et al.* 1963; McGovern & Quackenbush, 1973), although other studies have been unable to demonstrate any effect (Wilson & Siperstein, 1959; Reddy *et al.* 1977; Bochenek & Rodgers, 1978). Clearly, more evidence is required to fully understand the longer-term adaptive effects of different dietary fats on the uptake and metabolism of chylomicron remnant cholesterol by the liver.

In the present work the uptake and processing of [^{3}H]-cholesterol-labelled chylomicron remnants derived from olive oil, maize oil, palm oil, fish oil or butter fat were studied, using the perfused livers from rats fed on a diet enriched in the corresponding fat (e.g. metabolism of olive-oil remnants by perfused livers from rats fed on a diet enriched with olive oil). These results were also compared with those obtained using livers from rats fed on a low-fat diet. Before commencing any of the experiments, the diets were fed for 21 d and this was considered to be a sufficient time period for the adaptive changes in the liver to occur. This experimental design enabled the longer-term adaptive effects of dietary fats on hepatic remnant cholesterol metabolism to be compared with the effects of changes in the fatty acid composition of the remnants alone.

Materials and methods

Dietary studies

Male Wistar rats (Charles River) were housed under constant day length (12 h) and temperature (25°). Animals (starting mass 170–188 g) used for liver perfusions were fed on either a standard low-fat pellet diet or the same diet containing olive oil, maize oil, palm oil, fish oil or butter fat (40 % of the energy content) for 21 d before the experiments. The standard low-fat pellet diet contained (g/kg): fat 26, carbohydrate 523, protein 147; digestible energy was 12·10 kJ/kg. The source of fat for the low-fat diet was vegetable oil and this provided the following essential and non-essential fatty acids (g/100 g diet): oleic acid 0·76, linoleic acid 0·71, palmitic acid 0·32, myristic acid 0·14, arachidonic acid 0·13, palmitoleic acid 0·10, linolenic acid 0·06, stearic acid 0·04, myristoleic acid 0·02, lauric acid 0·02. The cholesterol content of the low-fat diet (0·21 mg/kg diet), and the range for each of the high-fat diets (0·3–1·1 mg/g diet) were relatively low. Therefore, in these studies, it was assumed that the differential metabolic effects of these diets were related to the fatty acid composition of the fats used, and not their cholesterol content. The amount of protein in both the low- and high-fat diets was sufficient to meet the recommended daily requirements for the rat (Chwalibog, 1994), and the body masses of the rats at the end of the 21 d period (300–350 g) were similar for each of the diets.

Materials

[1,2-^{3}H]Cholesterol was obtained from Amersham International, Amersham, Bucks, UK, and sodium pentobarbital, cholesterol oxidase, ampicillin and Menhaden fish oil were supplied by Sigma Chemical Company, Poole, Dorset, UK. Triacylglycerol and cholesterol assay kits were from Boehringer Mannheim, Lewes, E. Sussex, UK, and palm oil from Rhone Poulenc, Manchester, Lancs., UK. Olive oil, maize oil, and butter were obtained from domestic suppliers. All other chemicals were obtained from BDH, Dagenham, Essex, UK.

[^{3}H]Cholesterol-labelled chylomicron remnant preparation

Olive oil, maize oil, palm oil, fish oil or butter fat (0·5 ml) supplemented with α-tocopherol (4 mg/ml) was tube fed to a rat (weight 350 g; maintained on standard low-fat pellet diet) and after approximately 1 h, the rat was anaesthetized using sodium pentobarbital (60 mg/kg) and the thoracic duct cannulated. When the chyle was flowing satisfactorily, 0·5 ml of the same oil or fat fed initially, containing [1,2-^{3}H]cholesterol (18·5 MBq), was injected through the wall of the pyloric region of the stomach. Following this procedure, the abdominal wall was 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 for 16–18 h. The radiolabelled chyle, to which was added 1 mg ampicillin, was collected and layered (2 ml/tube) under NaCl (d 1·006 g/ml) in 6·5 ml polyallomer tubes and centrifuged at 20 000 rev./min for 21 min (6×10^5 ***g*** min) in a fixed angle rotor at 12°. [^{3}H]-Cholesterol-labelled large chylomicrons (diameter $>$ 100 nm) were then harvested from the top 10–15 mm of the tubes using a Beckman tube slicer.

[^{3}H]Cholesterol-labelled chylomicron remnants were prepared from these labelled large chylomicrons by intravenous administration into post-absorptive rats (350–370 g body weight, maintained on a low-fat diet) which had been anaesthetized and functionally hepatectomized by ligation of all blood vessels supplying the liver, as previously described (Lambert *et al.* 1996). After 45 min, the rats was exsanguinated and serum prepared by low-speed centrifugation. The serum was layered under NaCl solution (d 1·006 g/ml) in 6·5 ml polyallomer tubes and ultracentrifuged at 6×10^7 ***g*** min at 12°, and the top fraction containing labelled chylomicron remnants was further purified by ultracentrifugation for $3{\cdot}2\times10^7$ ***g*** min 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 was minimized by using post-absorptive rats and the two ultracentrifugation steps indicated earlier. As reported in previous studies, chylomicron remnants prepared by this method were shown to contain apolipoprotein (Apo) B48 (220 kDa) and ApoE (33 kDa) but not ApoB-100 or ApoC (Guldur & Mayes, 1990; Guldur *et al.* 1997). No significant differences in cholesterol and triacylglycerol content were observed

between the different types of remnants. 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 distribution of [^{3}H]cholesterol between the esterified and non-esterified forms was approximately 70 : 30 and was similar for each of the different types of remnants. A detailed analysis of the fatty acid composition of each of the types of chylomicron remnants has been reported previously (Lambert *et al*. 1996).

Liver perfusions

Rat livers were isolated for perfusion as described by Lambert *et al*. (1995, 1998). Briefly, the common bile duct and the portal vein (perfusion inflow) were cannulated. After cutting the adbominal vena cava, blood was flushed out of the liver by perfusing with Krebs and Henseleit bicarbonate buffer containing (mmol/l): 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 inferior vena cava (perfusion outflow) was cannulated in the thorax, the abdominal vena cava and hepatic artery were ligated, and the liver was connected to a recirculating blood perfusate (115 ml) in a thermostatically controlled cabinet at 37°. At zero time, [^{3}H]cholesterol-labelled chylomicron remnants, prepared from one of the test fats, were added to the recirculating perfusate of a liver from an animal fed on either a low-fat diet or a diet enriched with the corresponding fat and perfusion continued 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. Bile was collected continuously during the 4 h perfusion, after which, the perfusion was terminated and the liver flushed free of blood with a Krebs and Henseleit bicarbonate buffer containing bovine serum albumin (10 g/l).

Lipid extractions

Lipid extracts were prepared from the perfusate serum and the liver using 20 volumes of chloroform–methanol (2 : 1, v/v), and partitioned with 0·4 volumes 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. The silica-gel bands containing esterified and non-esterified cholesterol were located with I_2, and transferred into scintillation vials for counting. Erythrocytes from the perfusate were washed with NaCl (9 g/l, three times), haemolysed with distilled water, and their lipids were extracted using 20 volumes propan-2-ol–chloroform (11 : 7, v/v) (Rose & Oklander, 1965). 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. Lipids were extracted from bile using 20 volumes of chloroform–methanol (2 : 1, v/v), and partitioned with 0·4 volumes of distilled water. Portions of the aqueous methanol phase containing labelled bile acids were counted for radioactivity using the scintillant Instagel (Packard Instruments). The radioactivity in the chloroform phase containing labelled non-esterified cholesterol was counted using the toluene-based scintillant described earlier.

Cholesterol and triacylglycerol mass determination

Cholesterol mass was determined in lipid extracts after resuspending portions of the dried extract in propan-2-ol. Total, non-esterified cholesterol and triacylglycerol content in these and aqueous lipoprotein samples were assayed using commercially available kits from Boehringer Mannheim.

Statistical analysis

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. For comparisons between two groups (low-fat *v*. high-fat diets) Student's *t* test (unpaired) was used to determine statistical significance. All statistics were performed using Statview software, version 1.03, 1988 (Abacus Concepts Inc., Berkeley, CA, USA) and a P value $< 0{\cdot}05$ was considered to be statistically significant.

Results

[^{3}H]Cholesterol-labelled chylomicron remnant removal by perfused livers from rats fed on the low- or high-fat diets

Studies of the time courses for the removal of [^{3}H]radioactivity from the perfusate during the 4 h experimental period indicated that livers from rats fed on the low-fat diet removed labelled fish-oil and butter-fat remnants more rapidly than olive-, maize- or palm-oil remnants (Fig. 1(a–e)). In comparison, livers from rats fed on the fish-oil diet removed fish-oil remnants at a significantly slower rate (Fig. 1(d), $P < 0{\cdot}05$), although none of the observed differences in remnant removal associated with the other high-fat diets achieved statistical significance (Fig. 1(a,c,e)). Comparison of the different high-fat diet groups indicated that the rates of removal of the various types of remnants from the perfusate were no longer significantly different (Fig. 1(a–e)). These results were confirmed by measurements of the recovery of [^{3}H] in the livers on termination of perfusion after 4 h (Table 1). For the livers from rats fed on a low-fat diet, more ^{3}H was recovered from labelled fish-oil and butter-fat remnants than from olive-, maize- and palm-oil remnants ($P < 0{\cdot}01$). However, in the same period, the percentages of ^{3}H recovered in the livers from rats fed on each of the high-fat diets were not significantly different. These results show that fat-feeding eliminates the differential hepatic removal and uptake of cholesterol from remnants of different fatty acid composition found with livers from rats fed on a low-fat diet.

After 4 h, most of the perfusate [^{3}H]cholesterol that was not associated with chylomicron remnants or newly secreted VLDL (d $< 1{\cdot}006$ g//ml) was recovered in the fraction d 1·050–1·210 g/ml (HDL) (Table 2). The percentage of added radioactivity recovered in HDL was significantly

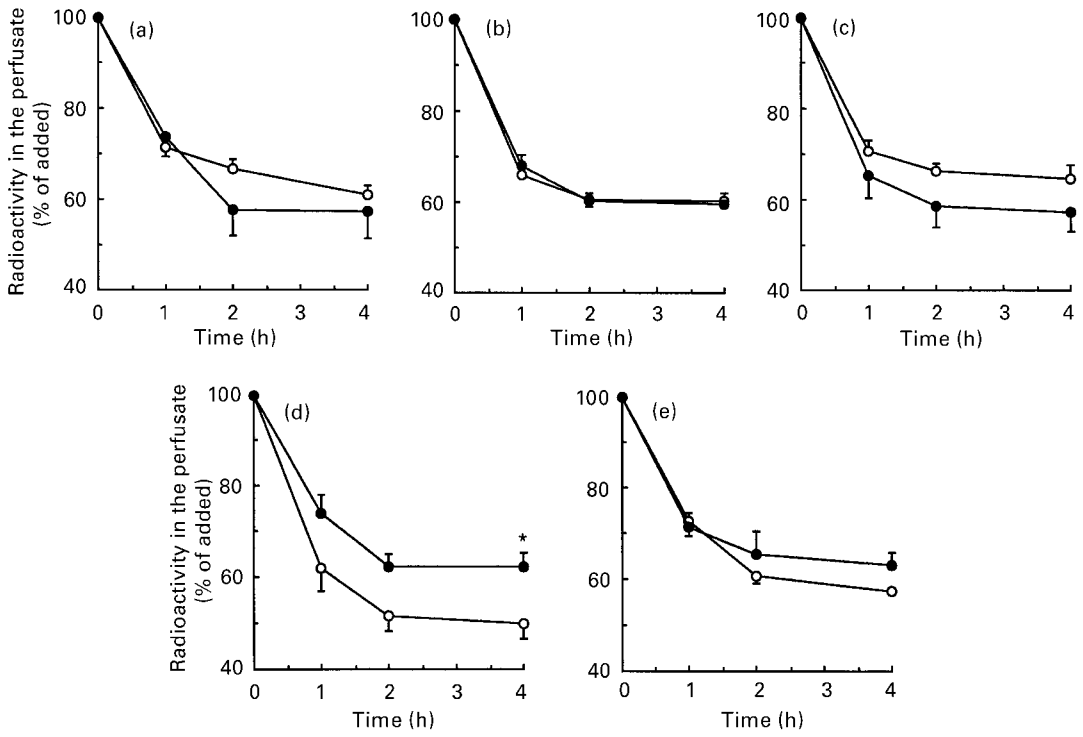

Fig. 1. Removal of [^{3}H]cholesterol-labelled remnants from the perfusate of isolated livers from rats fed on a low- or high-fat diet. Livers from rats fed on the low- (○) or high-fat (●) diets were perfused with [^{3}H]cholesterol-labelled remnants derived from (a), olive oil; (b), maize oil; (c), palm oil; (d), fish oil; (e), butter fat. For details of procedures, see pp. 432–433. Values are means with their standard errors represented by vertical bars for the following number of perfusions; low-fat diet: all types of remnants *n* 4; high-fat diets: olive-oil diet *n* 5, maize-oil diet *n*-6, palm-oil diet *n* 6, fish-oil diet *n* 4, butter-fat diet *n* 6. * Mean values for high- and low-fat diets were significantly different $P < 0{\cdot}05$.

greater when livers from rats fed on the olive-oil, fish-oil and butter-fat diets were compared with those from rats fed on the low-fat diet ($P < 0{\cdot}01$), or those from rats fed on the maize-oil diet ($P < 0{\cdot}05$).

[^{3}H]Cholesterol-labelled chylomicron remnant metabolism by perfused livers from rats fed on the low- or high-fat diets

The net percentage hydrolysis of added remnant esterified [^{3}H]cholesterol (includes effects of cholesterol re-esterification) by livers from rats fed on the high-fat or low-fat diets is shown in Table 3. The values were calculated by comparing the proportions of labelled non-esterified : esterified cholesterol in the dose with those at the end of the experiment in the combined liver, perfusate (serum and erythrocytes) and bile. The proportion of radioactivity used to synthesize labelled bile acids (a maximum of 1·6 % of added) was excluded from the calculations, because it was not possible to determine the separate contributions of esterified or non-esterified cholesterol to bile acid synthesis. Due to their differential uptake, livers from rats fed on the low-fat diet hydrolysed significantly more of the added esterified [^{3}H]cholesterol from fish-oil or butter-fat remnants when compared with olive- ($P < 0{\cdot}01$), maize- ($P < 0{\cdot}01$) or

Table 1. Uptake of [^{3}H]cholesterol-labelled chylomicron remnants derived from different fats, by perfused livers of rats fed on a low-fat diet or a high-fat diet containing the same fat as in the remnants*

(Mean values with their standard errors after 4 h perfusion)

Source of remnants	Added [^{3}H]cholesterol taken up by the liver after 4 h (%)						Change (%)
	Low-fat diet			High-fat diet			
	Mean	SE	*n*	Mean	SE	*n*	
Olive oil	29·58[a]	0·91	4	34·05	6·11	5	+15·11
Maize oil	30·02[a]	1·13	6	40·20	4·62	6	+33·91
Palm oil	30·59[a]	1·00	4	38·58	5·19	6	+26·15
Fish oil	41·20[b]	1·28	4	36·78	1·06	4	−12·02
Butter fat	41·11[b]	1·65	4	36·01	4·48	6	−14·16

[a,b] Mean values within a column not sharing a common superscript letter were significantly different: $P < 0{\cdot}01$.
* For details of procedures, see pp. 432–433.

Table 2. Recovery of [^{3}H]cholesterol from [^{3}H]cholesterol-labelled chylomicron remnants derived from different fats, in HDL in the perfusate from livers of rats fed on a low-fat diet or a high-fat diet containing the same fat as in the remnants†

(Mean values with their standard errors after 4 h of perfusion)

Source of remnants	Added [^{3}H]cholesterol recovered in HDL (%)					
	Low-fat diet			High-fat diet		
	Mean	SE	*n*	Mean	SE	*n*
Olive oil	9·04^{a}	1·95	4	25·11^{a}**	3·10	4
Maize oil	10·04^{ac}	1·08	6	12·23^{b}	3·32	6
Palm oil	9·15^{abc}	3·08	4	16·53^{ab}	3·41	6
Fish oil	15·72^{b}	1·72	4	24·16^{a}**	1·07	4
Butter fat	13·99^{bc}	0·35	4	25·57^{a}**	2·86	5

^{a,b,c} Mean values within a column not sharing a common superscript letter were significantly different: $P < 0·05$ or better.
Mean values were significantly different from those for the corresponding low-fat diet: ** $P < 0·01$.
† For details of procedures, see pp. 432–433.

palm-oil remnants ($P < 0·01$), although the overall proportion of non-esterified cholesterol (48–57 % total cholesterol) in each of the livers was not significantly different. Livers from rats fed on the fish-oil ($P < 0·05$) and maize-oil diets ($P < 0·01$) hydrolysed significantly more of the added esterified [^{3}H]cholesterol from each of the remnants when compared with those from rats fed on a low-fat diet, although for the other diets the observed increase in hydrolysis did not achieve statistical significance. However, each of the groups of livers from rats fed on the high-fat diets contained a significantly greater overall proportion of non-esterified cholesterol (64–80 % total cholesterol) compared with the corresponding low-fat groups.

The olive-, maize- or palm-oil diets significantly increased (2·0–3·3-fold) and butter-fat diets significantly decreased (50 %) the excretion of added [^{3}H]radioactivity into bile acids when compared with the values found with livers from rats given the low-fat diets, but feeding fish oil had little effect (Table 4). However, when these data were expressed as a percentage of the total liver [^{3}H]radioactivity only the palm-oil diet significantly increased the excretion of [^{3}H]radioactivity into bile acids when compared with the low-fat diet ($P < 0·01$). The secretion of the added radioactivity by this route was also higher for livers from rats fed on olive- or maize-oil diets (2·1–3·9-fold) when compared with those from rats fed on palm oil, fish oil or butter fat, and was higher for livers from rats fed on fish oil (2·3-fold; $P < 0·005$) compared with those fed on butter fat, and these trends were similar when these data were expressed as a percentage of the total liver [^{3}H]radioactivity.

Livers from rats fed on the olive-oil diet excreted significantly more of the added [^{3}H]radioactivity in biliary non-esterified cholesterol (1·9–2·5-fold) when compared with livers from rats fed on the low-fat ($P < 0·05$), maize-oil ($P < 0·05$) or butter-fat ($P < 0·05$) diets (Table 5). These trends were similar when these data were expressed as a percentage of the total liver [^{3}H]radioactivity, although the olive-oil diet only achieved statistical significance from the maize-oil diet ($P < 0·05$).

Discussion

[^{3}H]Cholesterol-labelled chylomicron remnants derived from a range of different dietary fats were perfused through livers from rats fed on the corresponding high-fat diet. These results were compared with those obtained with livers from rats fed on a low-fat diet. This experimental design enabled us to determine how the hepatic metabolism of cholesterol from chylomicron remnants is influenced by the longer-term consumption of different fats in the diet, compared with the effects due to changes in the fatty acid composition of the chylomicron remnant particles alone.

As the chylomicron remnants did not differ in any significant respect apart from their fatty acid composition, the increased removal of [^{3}H]cholesterol-labelled fish-oil and butter-fat remnants by livers from rats fed on a low-fat diet when compared with the other types of remnants demonstrates that the fatty acid composition of the remnant particles influences their hepatic uptake. Previous studies in this laboratory have also shown that [^{14}C]oleate-labelled fish-oil and butter-fat remnants (labelled predominantly in the triacylglycerol) were taken up by the perfused rat liver in a similar pattern, i.e. more rapidly than those derived from olive, maize or palm oils (Lambert *et al*. 1998). Thus, it would appear that the change in fatty acid composition

Table 3. Hydrolysis of esterified [^{3}H]cholesterol in chylomicron remnants derived from different fats, by the perfused livers of rats fed on a low-fat diet or a high-fat diet containing the same fat as in the remnants†

(Mean values with their standard errors after 4 h of perfusion)

Source of remnants	Added esterified [^{3}H]cholesterol hydrolysed (net %)						Liver non-esterified [^{3}H]cholesterol (% total liver [^{3}H]cholesterol)					
	Low-fat diet			High-fat diet			Low-fat diet			High-fat diet		
	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*
Olive oil	13·99^{a}	1·10	4	18·63	6·75	3	47·69	3·90	4	63·73*	3·07	3
Maize oil	14·92^{a}	0·72	6	29·43**	4·47	6	49·99	1·03	6	71·76***	4·66	6
Palm oil	15·05^{a}	1·24	4	26·49	6·29	4	49·02	2·63	4	73·66***	3·11	4
Fish oil	22·85^{b}	1·49	4	29·54*	1·44	4	55·48	2·10	4	80·17***	1·72	4
Butter fat	23·42^{b}	1·57	4	27·00	3·71	6	57·20	4·77	4	74·37**	2·64	6

^{a,b} Mean values within a column not sharing a common superscript letter were significantly different: $P < 0·01$.
Mean values were significantly different from those for the corresponding low-fat diet: * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† For details of procedures, see pp. 432–433.

Table 4. Recovery of radioactivity from [^{3}H]cholesterol-labelled chylomicron remnants derived from different fats in bile acids from perfused livers of rats fed on a low-fat diet or a high-fat diet containing the same fat as in the remnants†

(Mean values with their standard errors after 4 h of perfusion)

Source of remnants	Added [^{3}H]cholesterol in bile acids (%)						Total liver [^{3}H]cholesterol as bile acids (%)					
	Low-fat diet			High-fat diet			Low-fat diet			High-fat diet		
	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*
Olive oil	0·74^{a}	0·10	4	1·51^{a}*	0·20	5	2·50^{a}	0·26	4	5·70^{ac}	1·77	5
Maize oil	0·55^{a}	0·10	6	1·56^{a}**	0·23	4	1·89^{a}	0·39	6	3·69^{a}	0·74	4
Palm oil	0·22^{b}	0·03	4	0·73^{bcd}*	0·19	5	0·73^{b}	0·13	4	1·86^{bc}**	0·28	5
Fish oil	0·84^{a}	0·13	4	0·92^{c}	0·11	4	2·08^{a}	0·38	4	2·52^{ac}	0·31	4
Butter fat	0·81^{a}	0·04	4	0·40^{bd}**	0·08	6	1·97^{a}	0·13	4	1·29^{b}	0·39	6

^{a,b,c,d} Mean values within a column not sharing a common superscript letter were significantly different: $P < 0·05$ or better.
Mean values were significantly different from those for the corresponding low-fat diet: * $P < 0·05$, ** $P < 0·01$.
† For details of procedures, see pp. 432–433.

affects the uptake of the whole remnant particle rather than a single component. Following the consumption of a high-fat diet, the differential hepatic removal of the individual [^{3}H]cholesterol-labelled remnants was abolished, due to the reduced uptake of labelled fish-oil and butter-fat remnants and the increased uptake of olive-, maize- and palm-oil remnants.

The longer-term consumption of dietary fats is likely to influence the fatty acid composition of the liver membranes (Kritchevsky *et al*. 1988; Hostmark *et al*. 1989) and this may have additional modulatory effects on the receptors associated with chylomicron remnant uptake. Since the LDL receptor is considered to mediate at least some of the remnant uptake by the liver (Choi *et al*. 1991), the reduced uptake of chylomicron remnants by livers from rats fed on the butter fat (enriched in saturated fatty acids) or fish oil (enriched in *n*-3 PUFA) could be explained by the down-regulatory effects of these fatty acids on LDL receptor activity (Fox *et al*. 1987; Wong & Nestel, 1987; Lindsey *et al*. 1992). Also, there is some evidence to suggest that the olive-oil (rich in monounsaturated fatty acids) and maize-oil (rich in *n*-6 PUFA) diets could be increasing remnant uptake by up-regulating the activity of this receptor (Ventura *et al*. 1989; Grundy & Denke, 1990). The explanation for the effect of the palm-oil diet in increasing chylomicron remnant uptake is not clear at present. Given that palm oil is similar to butter fat in respect to it being relatively enriched in saturated fatty acids, diets enriched in this oil might have been expected to decrease remnant uptake. However, a closer comparison of the fatty acid composition of these two fats reveals that palm oil contains a much greater proportion of monounsaturated fatty acids (particularly oleic acid) than butter fat, such that the ratio saturated : monounsaturated fatty acids is only 1·2 : 1·0 whereas in butter fat it is 3·72 : 1·0 (Lambert *et al*. 1996). Thus, the greater proportion of monounsaturated fatty acids in palm oil may act to increase chylomicron remnant uptake when compared with those fed on the butter-fat or the low-fat diet.

The recirculation of remnant-derived [^{3}H]cholesterol into perfusate HDL by livers from rats fed on a low-fat diet appeared to be determined by the degree of hepatic uptake, which is closely determined by the fatty acid composition of the remnant particle itself. However, when livers from rats fed on the high-fat diets were used, significantly less remnant cholesterol was recirculated into HDL when the donor animals were fed on the maize-oil (enriched in *n*-6 PUFA) as compared with the olive-oil, fish-oil or butter-fat diets. This observation is consistent with the tendency of *n*-6 PUFA to lower plasma HDL-cholesterol (De Bruin *et al*. 1993), and the retention of remnant-derived cholesterol in the liver would allow it to be used for bile acid synthesis, thus contributing further to the known hypocholesterolaemic effects of maize oil.

Livers from rats fed on each of the high-fat diets hydrolysed more of the added remnant esterified cholesterol and

Table 5. Recovery of radioactivity from [^{3}H]cholesterol-labelled chylomicron remnants derived from different fats in biliary cholesterol from perfused livers of rats fed on a low-fat diet or a high-fat diet containing the same fat as in the remnants†

(Mean values with their standard errors after 4 h of perfusion)

Source of remnants	Added [^{3}H]cholesterol in biliary cholesterol (%)						Total liver [^{3}H]cholesterol in biliary cholesterol (%)					
	Low-fat diet			High-fat diet			Low-fat diet			High-fat diet		
	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*
Olive oil	0·10^{a}	0·01	4	0·25^{a}*	0·05	5	0·32^{a}	0·03	4	0·90^{a}	0·26	5
Maize oil	0·12^{ab}	0·04	6	0·11^{bc}	0·02	6	0·38^{ab}	0·10	6	0·27^{b}	0·04	6
Palm oil	0·06^{b}	0·01	4	0·15^{abc}	0·05	5	0·19^{b}	0·03	4	0·35^{ab}	0·10	5
Fish oil	0·14^{a}	0·02	4	0·15^{abc}	0·04	4	0·34^{a}	0·04	4	0·40^{ab}	0·11	4
Butter fat	0·15^{a}	0·01	4	0·13^{c}	0·03	4	0·37^{a}	0·03	4	0·43^{ab}	0·12	4

^{a,b,c} Mean values within a column not sharing a common superscript letter were significantly different: $P < 0·05$ or better.
Mean values were significantly different from those for the corresponding low-fat diet: * $P < 0·05$.
† For details of procedures, see pp. 432–433.

therefore maintained a greater proportion of their hepatic cholesterol in the unesterified form than those fed on the low-fat diets. Since remnant cholesterol is efficiently utilized for bile acid synthesis (Bravo *et al*. 1992), the increased hydrolysis of remnant esterified cholesterol by livers from rats fed on a high-fat diet provides a general mechanism to increase the utilization of remnant cholesterol for bile acid synthesis. Consistent with this hypothesis, the diets enriched in olive, maize or palm oil increased the hepatic excretion of radioactivity as bile acids compared with the low-fat diet, which is in keeping with previous work showing that olive and maize oil feeding increases bile acid synthesis in rat liver hepatocytes (Botham & Boyd, 1983). In addition, only the olive-oil diet significantly increased the excretion of radioactivity into biliary non-esterified cholesterol when compared with all the other high-fat and the low-fat diets (Table 5). Overall, the radioactivity excreted into the bile (predominantly as bile acids) by livers from rats fed on the more saturated palm-oil and butter-fat diets was markedly less than that excreted by livers from olive- and maize-oil-fed rats. Since the removal of remnant-derived cholesterol from the body prevents its recirculation into the blood, these results provide further explanations for the hypercholesterolaemic effects of saturated fats and the hypocholesterolaemic effects of monounsaturated fats and *n*-6 PUFA.

To summarize, although livers from rats fed on a low-fat diet removed more cholesterol from remnants enriched with *n*-3 PUFA (fish oil) or long- and medium-chain saturated fatty acids (butter fat) when compared with those enriched in monounsaturated fatty acids (olive oil), *n*-6 PUFA (maize oil) or long-chain saturated fatty acids (palm oil), the addition of the corresponding fats to the diet abolished these differences, such that all types of remnants were removed at similar rates. When a diet rich in *n*-6 PUFA (maize oil) was fed, the transfer of cholesterol from chylomicron remnants to HDL was retarded compared with that found with the low-fat and all the other high-fat diets, but conversion to bile acids was enhanced. However, with the exception of the saturated fat (butter fat), all the high-fat diets caused an increase in metabolism of remnant cholesterol to bile acids, and this was particularly marked for monounsaturated and *n*-6 PUFA diets. Nevertheless, the excretion of remnant cholesterol into bile acids was slowest for remnants enriched in the saturated fatty acids (butter fat or palm oil) when compared with all the other types of fat. These data support our earlier reports on the effects of remnant fatty acid composition on remnant triacylglycerol metabolism and clearly indicate that adaptive changes in the rat liver caused by feeding different types of fat also have effects on the uptake and metabolism of chylomicron remnant cholesterol which are different from those that are due entirely to variations in the fatty acid composition of the remnant particles.

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