Modulation of hepatic apolipoprotein B, 3-hydroxy-3-methylglutaryl-CoA reductase and low-density lipoprotein receptor mRNA and plasma lipoprotein concentrations by defined dietary fats

Comparison of trimyristin, tripalmitin, tristearin and triolein

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Different dietary fatty acids exert specific effects on plasma lipids but the mechanism by which this occurs is unknown. Hamsters were fed on low-cholesterol diets containing triacylglycerols enriched in specific saturated fatty acids, and effects on plasma lipids and the expression of genes involved in hepatic lipoprotein metabolism were measured. Trimyristin and tripalmitin caused significant rises in low-density lipoprotein (LDL) cholesterol which were accompanied by significant reductions in hepatic LDL receptor mRNA levels. Tripalmitin also increased hepatic expression of the apolipoprotein B gene, implying an increased production of LDL via very-low-density lipoprotein (VLDL) and decreased removal of LDL in animals fed this fat. Hepatic

levels of 3-hydroxy-3-methylglutaryl-CoA reductase mRNA did not vary significantly between the groups. Compared with triolein, tristearin had little effect on hepatic gene expression or total plasma cholesterol. However, it caused a marked decrease in VLDL cholesterol and a rise in LDL cholesterol such that overall it appeared to be neutral. Lipid analysis suggested a rapid desaturation of much of the dietary stearate. The differential changes in plasma lipids and hepatic mRNA levels induced by specific dietary fats suggests a role for fatty acids or a metabolite thereof in the regulation of the expression of genes involved in lipoprotein metabolism.

INTRODUCTION

Atherosclerosis, presenting clinically as coronary heart disease, cerebrovascular disease or peripheral artery disease, is the most frequent cause of death in Western societies, and there is little doubt that hypercholesterolaemia is a major causative factor of premature atherosclerosis [1]. The amount and type of dietary fat can have a strong influence on plasma total and low-density lipoprotein (LDL) cholesterol, and diets enriched in certain saturated fatty acids raise LDL cholesterol concentrations relative to those observed with unsaturated fatty acid-enriched diets [2]. Short-chain saturated fatty acids ($C < 10$) have little effect on plasma lipids [3] but the longer-chain saturates, i.e. laurate, myristate and palmitate, tend to increase both plasma cholesterol and triacylglycerol [4,5]. Interestingly, stearate appears to have a neutral action [6]. Poor absorption of stearate or desaturation to oleate have been implicated to explain why this fatty acid, unlike the other saturates, is not hypercholesterolaemic [7,8]. However, Bonanome and Grundy and co-workers [9,10] showed that stearate is well absorbed from diets containing high amounts of the fatty acid and that there appeared to be a reduction in LDL cholesterol in direct proportion to the stearate content of the diet.

The mechanism by which dietary fat may alter plasma cholesterol, particularly LDL cholesterol, concentrations is unclear. The detailed studies of Woollett et al. [11,12] on feeding diets enriched in triacylglycerols containing fatty acids of defined carbon chain length indicated that saturated fatty acids of 12, 14 and 16 carbons decrease LDL receptor (LDLR) activity and increase LDL production rate, leading to marked increases in

plasma LDL cholesterol levels. Interestingly, the two effects appeared to be independent of one another. Since these changes occurred in the absence of any alteration in external sterol balance, it was suggested that they were due to some change in a supposed hepatic sterol regulatory pool. Shorter-chain saturates and stearic acid (C_{18}) were without effect on LDL cholesterol levels. In a recent study [13], the decrease in LDLR activity observed when hamsters were fed a diet rich in hydrogenated coconut oil, which is rich in saturated fatty acids (particularly laurate and myristate), was found to correlate with a decrease in hepatic LDLR mRNA levels. This suggests that alterations in hepatic gene expression may be responsible for the hypercholesterolaemic effect of dietary saturated fats. In the present studies the effects of diets enriched in specific fatty acids on lipoprotein profiles and on the hepatic expression of key genes involved in lipoprotein metabolism in the hamster were measured. Any possible interactive effects with dietary cholesterol were minimized by keeping the dietary cholesterol concentration at a very low level (0.005%). The results suggest that saturated fats of defined chain length have differential effects on plasma lipoprotein cholesterol and on the hepatic expression of genes involved in cholesterol homeostasis.

MATERIALS AND METHODS

The three laboratories involved in this study worked under Good Laboratory Practice following certification by the U.K. Department of Health.

Abbreviations used: LDL, low-density lipoprotein; LDLR, LDL receptor; VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein; HDL, high-density lipoprotein; apoB, apolipoprotein B; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA.

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All chemicals were of the highest purity available commercially unless stated, and solvents were of AnalaR grade. Semi-synthetic hamster diet, corn starch, alphacell, salt mixture (Hegsted), vitamin-mix, triolein (technical grade; 65–70% triolein), linseed oil and cholesterol were from ICN Flow, High Wycombe, Bucks., U.K. Trimyristin (97% pure) and tripalmitin (95% pure) were from Fluka, Glossop, Derbys., U.K. Tristearin (> 90% pure) and L-cysteine were from Sigma Chemical Co., Poole, Dorset, U.K. Radioisotopes and Hybond N nylon membrane were obtained from Amersham International, Amersham, Bucks., U.K. Bluescript SK and KS phagemids and *in vitro* transcription kits were obtained from Stratagene Ltd., Cambridge, U.K. Restriction endonucleases, RNase-free DNase, Taq DNA polymerase and T4 polynucleotide kinase were obtained from Boehringer Mannheim U.K. Ltd., Lewes, E. Sussex, U.K. RPA II ribonuclease protection kits were obtained from AMS Biotechnology (U.K.) Ltd., Witney, Oxon., U.K. Oligo(dT)₁₈ homopolymer, Sephadex G50 and dNTPs were obtained from Pharmacia LKB Biotechnology Division, Milton Keynes, Bucks., U.K. Polyadenylic acid and salmon testes DNA were obtained from Sigma. T4 DNA ligase and Superscript reverse transcriptase were obtained from Gibco BRL Life Technologies Ltd., Paisley, Scotland, U.K.

Animals and diets

Twenty-four male DNSI Golden Syrian hamsters bred in-house were divided at random into four groups of six. The animals were housed individually in rooms lit from 06:00 to 18:00 h and allowed free access to food and water. They were fed on a commercial semi-synthetic hamster diet, purchased from ICN Flow, for 2 weeks prior to transfer to the test diets which contained 0.005% cholesterol and 46.8% (by energy) fat, of which 50% was the test fat and 50% was triolein. Thus four different diets enriched in triolein (TO), trimyristin (TM), tripalmitin (TP) and tristearin (TS) were used. On alternate days 30 g of test diet was placed in each food hopper and food intake and body mass were measured. Diets (1.5 kg of each) were prepared every 2 weeks and stored in portions of approx. 200 g at –20 °C. Faeces were collected on days 23–26. On day 28, after an overnight fast, the hamsters were anaesthetized with Sagatal (1 ml/kg body weight; 60 mg of sodium pentobarbitone/ml) and approx. 4 ml of blood was collected by cardiac puncture into plastic-EDTA tubes. Bile was removed from the gall bladder and the liver was perfused with ice-cold 0.9% NaCl (10 ml) prior to removal, freezing in portions (1 g) in liquid nitrogen and storage at –80 °C. Perirenal fat was removed, placed immediately in liquid nitrogen and stored at –20 °C.

Separation of plasma lipoproteins

Lipoprotein fractions were separated sequentially, on the basis of their density, by centrifugation of plasma at 100 000 g for 2.5 h at 12 °C in a Beckman Optima TLX benchtop centrifuge, at each of the final density ranges (g/l) of < 1.006, 1.006–1.02, 1.02–1.06 and > 1.06 for very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), LDL and high-density lipoprotein (HDL) respectively [14]. Densities were adjusted using solid KBr. Lipoprotein fractions were collected and stored at –20 °C until analysis. Cholesterol and triacylglycerol concentrations in plasma and lipoprotein fractions were assayed automatically on a COBAS MIRA autoanalyser using the Olympus system reagent 5000-cholesterol and triacylglycerol (GPO Trinder) kits respectively.

Fatty acid analysis

Fatty acids were converted into their methyl esters by trans-methylation [15] and analysed by GLC on a CP-Sil 88 capillary column (50 m × 0.25 mm; Chrompak) in a Perkin Elmer 8300 chromatograph. Diets (50 mg) and freeze-dried faeces (1 g) were extracted with chloroform/methanol (1:2, v/v; 2 ml and 10 ml respectively) and aliquots (200 µl) were taken for trans-methylation. Samples (250 mg) of adipose tissue were homogenized in 0.3 M sucrose containing 10 mM EDTA buffered to pH 7.4 with 50 mM potassium phosphate. Aliquots of adipose tissue homogenate and VLDL fractions were taken directly for transesterification. Aliquots of all transmethylation reactions were subjected to analysis. Fatty acid methyl ester standards were purchased from Sigma Chemical Co. and were routinely chromatographed each day.

Determination of hepatic free cholesterol and cholesteryl ester

Liver samples were homogenized in 50 mM Tris/HCl, 150 mM NaCl, pH 7.4, and the lipids were extracted using the method of Bligh and Dyer [16]. Trace amounts of [$1\alpha,2\alpha(n)^3\text{H}$]cholesterol and [$1\text{-}^{14}\text{C}$]cholesteryl oleate were added and lipids were separated by TLC in light petroleum (b.p. 40–60 °C)/diethyl ether/acetic acid (90:30:1, by vol.). Lipids were then extracted from the silica gel using hexane/propan-2-ol (3:2, v/v), and cholesterol and cholesterol ester were assayed enzymically using the Wako cholesterol reagent in the presence of 10 mM sodium deoxycholate.

Determination of faecal total cholesterol and bile acids

Cholesterol

Ground freeze-dried faeces (100 mg approx.) were hydrolysed by heating with 1 M NaOH in 90% (v/v) methanol (2 ml) for 18 h at 110 °C. An aliquot (1 ml) of the hydrolysate was diluted with water (1 ml) and extracted twice with light petroleum (b.p. 40–60 °C). The combined extracts were dried down under nitrogen and assayed for cholesterol as above.

Bile acids

Bile acids were extracted from freeze-dried faeces in butan-2-ol/water (1:1, v/v) [17]. Extracted bile acids were assayed using the optimal conditions for 3α -hydroxysteroid dehydrogenase using deoxycholate as standard [18].

Extraction of total RNA from hamster liver

Approx. 100 mg of frozen liver tissue was homogenized in 2 ml of acidic guanidinium thiocyanate and the RNA was extracted according to the method of Chomczynski and Sacchi [19]. Total RNA was quantified by absorbance at 260 nm, assuming that 1 absorbance unit = 37 mg of RNA/ml.

Transcription vector construction

The Syrian hamster apolipoprotein B (apoB) partial cDNA clone pT13 R2 was obtained from Dr. N. Maeda, Department of Pathology, University of North Carolina, Chapel Hill, NC, U.S.A. The complete Syrian hamster 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase cDNA clone pDGS6 was a gift from Professor R. Simoni, Department of Biological Sciences, Stanford University, CA, U.S.A. ApoB cDNA (a 282 bp *EcoRI*–*SphI* fragment corresponding to nucleotides 8137–8419 of the human apoB cDNA sequence) and HMG-CoA reductase cDNA (a 362 bp *PstI* fragment corresponding to nucleotides

2024–2386 of the cDNA sequence) were subcloned into the Bluescript SK phagemid. Since the Syrian hamster LDL receptor cDNA was not available, reverse transcription PCR was used to clone a 192 bp cDNA fragment. Oligonucleotide primers (5'-GCTTCTGGTACACTGGGTTGTC-3' and 5'-TCGGTGAC-AATGTCGCACCAAG-3') were designed using regions of 100% identity between the Chinese hamster and rat sequences. PCR was carried out (30 s at 93 °C, 30 s at 55 °C and 1 min at 72 °C) for 30 cycles in a Trio Thermoblock (Biometra Ltd.). The PCR product was then blunt-end cloned into the *Sma*I site of pBluescript I KS and sequenced to confirm the identity and orientation of the clone.

Synthesis of radiolabelled cRNA probes and synthetic mRNA standards

³²P-labelled cRNA probes and synthetic mRNA standards were synthesized using a T3/T7 RNA transcription kit (Stratagene Ltd.) according to the manufacturer's instructions. Transcription vector DNA was removed by digestion with RNase-free DNase for 15 min at 37 °C. Unincorporated nucleotides were removed using Quick Spin Sephadex G50 columns. cRNA probes were Čerenkov-counted to determine incorporation of radionucleotide and synthetic mRNA was quantified by absorbance at 260 nm.

Solution hybridization analysis of mRNA

All mRNA levels were quantified by solution hybridization assay using an RPA II RNase protection kit (AMS Biotechnology Ltd.). Total RNA (5 µg for apoB and 25 µg for LDLR and HMG-CoA reductase) and synthetic mRNA (50–500 amol for apoB and 10–50 amol for LDLR and HMG-CoA reductase) were hybridized overnight at 45 °C with 1×10^5 c.p.m. of cRNA probe in a final volume of 20 µl. Samples were then treated with RNase and precipitated in accordance with the manufacturer's instructions. The RNase-resistant products were then separated by electrophoresis on a 6% acrylamide gel (50 cm × 30 cm) containing 7 M urea, 2 mM EDTA and 90 mM Tris/borate, pH 8.0. Gels were dried down under vacuum and subjected to autoradiography. The bands representing the protected cRNA probe were excised and assayed for radioactivity by liquid scintillation spectrometry.

Quantification of mRNA levels

The results obtained from hybridization to increasing amounts of synthetic mRNA were used to construct standard curves, from which the amount of each mRNA in terms of amol/µg of total RNA was calculated. Standard curves gave routinely an *r* value of > 0.95. Results were corrected for any variation in the mRNA content of total RNA samples by quantification of poly(A)⁺ RNA using oligo(dT)₁₈ hybridization [20]. All mRNA values are expressed in amol of mRNA/µg of total RNA, normalized to 0.02 µg of poly(A)/µg of total RNA.

Statistical analysis

Data were analysed by one-way analysis of variance (ANOVA). If significance at *P* < 0.05 was obtained, further comparison was performed between groups using the Tukey–Kramer multiple comparison test.

RESULTS

The fatty acid profiles (mol%) of the individual diets showed that diets TO, TM, TP and TS were enriched in oleate (69%), myristate (74%), palmitate (68%) and stearate (58%) respect-

Table 1 Fatty acid composition of dietary lipids

Dietary fatty acids were converted into their methyl esters and analysed by GLC as described in the Materials and methods section. Samples from each dietary batch were taken for analysis at the beginning of the feeding trial and thereafter at 2 week intervals until the end of the trial. No variation in fatty acid composition was observed between the samples of diets taken at the different time points. Results shown are the means ± S.D. of three separate determinations.

Diet	Distribution of fatty acids (mol%)					
	C _{14:0}	C _{16:0}	C _{18:0}	C _{18:1}	C _{18:2}	C _{18:3}
TO	6.78 ± 0.64	9.94 ± 0.45	2.14 ± 0.10	68.97 ± 2.31	10.82 ± 0.27	1.35 ± 0.10
TM	74.39 ± 1.70	3.50 ± 0.04	0.81 ± 0.17	17.62 ± 0.81	3.03 ± 0.25	0.65 ± 0.14
TP	2.19 ± 0.13	67.88 ± 0.09	1.10 ± 0.08	23.93 ± 0.08	4.12 ± 0.08	0.85 ± 0.04
TS	2.50 ± 0.21	4.75 ± 0.24	57.52 ± 2.92	29.02 ± 2.20	5.00 ± 0.02	1.21 ± 0.32

ively (Table 1). Apart from three animals which lost more than 10% body weight during the study and were removed, all animals consumed similar amounts of food during the trial period and gained weight at similar rates. Analysis of the fat content of freeze-dried faeces indicated that the amount of lipid excreted by each group of animals was similar, of the order of 15 mg of total fat per g dry weight of faeces, and the fatty acid composition of faecal lipids reflected that of the individual diets. In no case was the faecal lipid in excess of 6% of the total dietary lipid intake.

The major fatty acid deposited in adipose tissue reflected the major fatty acid of the diet except in the case of the TS group (Table 2a). Thus, in the TO group, oleic acid accounted for 45 mol% of the total, with linoleate at 16% and palmitate at 25%. The level of linoleate was the same in all groups. In the TM group myristate accumulated in adipose tissue at the expense of oleate (23 mol%) and constituted 38% of the total triacylglycerol fatty acids. Palmitate was at the same level as in the oleate group (22%). In the TP group, palmitate was the major saturated fatty acid (44%) of adipose tissue triacylglycerols, with only small contributions from myristate (3%) and stearate (2%). Feeding the TS diet proved to be the exception to the pattern seen with the other dietary fats in that, although the adipose tissue triacylglycerols contained three times as much stearate as those in the other groups, stearate still represented only 7% of the total fatty acids. The fatty acid composition of the adipose tissue triacylglycerols of these animals was very similar to that of TO-fed animals. Thus oleate (43%) and linoleate (14%) were the major unsaturated fatty acids, while palmitate (24%) represented the major saturated fatty acid with minor contributions from myristate (4%) and stearate (7%). The fatty acid profile of VLDL lipids, which presumably reflects the composition of hepatic triacylglycerols, contrasts with that of the adipose tissue triacylglycerols (Table 2b). Only very small amounts of myristate were present in VLDLs of TM-fed animals, while the VLDL fractions of the other groups were enriched in the major fatty acid of their respective diets. This includes the TS group, where there was a doubling of stearate content compared with the other groups.

The effects of the different fats on plasma lipids are shown in Table 3. Both the TM and TP diets caused significant increases in total plasma cholesterol relative to the TO diet, with the TP diet being significantly more hypercholesterolaemic than the TM diet. Analysis of the individual lipoprotein fractions showed that these differences were manifest in the LDL cholesterol for each group. HDL cholesterol in the TP group was significantly greater than in the TM group, and both were significantly greater than

Table 2 Fatty acid composition of (a) adipose tissue and (b) VLDL

Samples of adipose tissue (250 mg) or VLDL fractions (100 μ l) were subjected to transmethylation as described in the Materials and methods section. Aliquots were analysed by GLC. The results are given as means $\pm$ S.D. for five individual samples.

(a)							
Distribution of fatty acid (mol%)							
Diet	C _{14:0}	C _{16:0}	C _{16:1}	C _{18:0}	C _{18:1}	C _{18:2}	C _{22:6}
TO	3.66 $\pm$ 0.22	23.64 $\pm$ 3.20	5.46 $\pm$ 0.97	2.11 $\pm$ 0.30	42.53 $\pm$ 4.14	13.60 $\pm$ 1.66	0.29 $\pm$ 0.05
TM	37.72 $\pm$ 6.99	21.59 $\pm$ 2.44	4.50 $\pm$ 1.20	1.64 $\pm$ 0.20	23.49 $\pm$ 0.98	10.61 $\pm$ 2.21	0.45 $\pm$ 0.08
TP	2.73 $\pm$ 1.38	44.32 $\pm$ 1.17	5.99 $\pm$ 1.03	2.45 $\pm$ 0.18	32.02 $\pm$ 1.82	11.30 $\pm$ 1.43	0.56 $\pm$ 0.09
TS	4.75 $\pm$ 1.13	27.09 $\pm$ 4.23	5.65 $\pm$ 2.16	6.68 $\pm$ 1.43	39.65 $\pm$ 3.26	15.31 $\pm$ 2.96	0.87 $\pm$ 0.13

(b)							
Distribution of fatty acid (mol%)							
Diet	C _{14:0}	C _{16:0}	C _{18:0}	C _{18:1}	C _{18:2}	C _{20:4}	C _{18:3}
TO	0.18 $\pm$ 0.40	24.92 $\pm$ 2.74	7.82 $\pm$ 1.57	44.78 $\pm$ 3.20	15.56 $\pm$ 0.86	2.24 $\pm$ 1.28	1.88 $\pm$ 1.25
TM	5.78 $\pm$ 1.38	22.82 $\pm$ 1.05	6.33 $\pm$ 1.25	35.92 $\pm$ 2.13	20.18 $\pm$ 2.27	2.77 $\pm$ 0.49	2.27 $\pm$ 0.29
TP	1.42 $\pm$ 1.28	34.42 $\pm$ 0.40	5.68 $\pm$ 1.18	33.94 $\pm$ 1.74	16.88 $\pm$ 1.70	2.08 $\pm$ 0.23	2.32 $\pm$ 0.91
TS	1.22 $\pm$ 1.67	25.48 $\pm$ 1.83	14.48 $\pm$ 1.61	35.46 $\pm$ 3.00	17.70 $\pm$ 1.53	2.80 $\pm$ 1.62	2.20 $\pm$ 1.33

Table 3 Cholesterol and triacylglycerol concentrations of plasma and lipoprotein fractions

Individual lipoprotein fractions were separated sequentially on the basis of their density as described in the Materials and methods section. Cholesterol and triacylglycerol concentrations were assayed automatically on the COBAS MIRA autoanalyser. Results are expressed as means $\pm$ S.D. ($n = 5$ for TO, TP and TS; $n = 6$ for TM). Significance of differences is indicated by: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

	TO group	TM group	TP group	TS group
Cholesterol (mM)				
Total plasma	3.40 $\pm$ 0.190	4.15 $\pm$ 0.31 (TO*, TS*)	5.05 $\pm$ 0.39 (TO***, TS**)	3.43 $\pm$ 0.59
VLDL	0.931 $\pm$ 0.354	1.090 $\pm$ 0.140	0.950 $\pm$ 0.310	0.369 $\pm$ 0.162 (TO**, TM***, TP**)
IDL	0.113 $\pm$ 0.354	0.077 $\pm$ 0.016 (TS*)	0.231 $\pm$ 0.048 (TO**, TM***)	0.163 $\pm$ 0.063
LDL	0.582 $\pm$ 0.115	0.796 $\pm$ 0.145 (TO*)	1.23 $\pm$ 0.060 (TO***, TM***, TS***)	0.824 $\pm$ 0.111 (TO**)
HDL	2.10 $\pm$ 0.41	2.39 $\pm$ 0.20 (TO**, TS**)	2.65 $\pm$ 0.08 (TO***, TM*, TS***)	2.02 $\pm$ 0.17
Recovery (%)	92 $\pm$ 22	89 $\pm$ 5	88 $\pm$ 4	91 $\pm$ 12
Triacylglycerol (mM)				
VLDL	1.02 $\pm$ 0.50	1.68 $\pm$ 0.34	1.26 $\pm$ 0.46	0.51 $\pm$ 0.15 (TM***, TP*)

Table 4 Hepatic and faecal sterol and bile acids

Hepatic lipids were extracted by the method of Bligh and Dyer [16] and separated by TLC on silica gel. Cholesterol and cholesterol ester were extracted from the gel and assayed enzymically. Ground freeze-dried faeces (100 mg) were hydrolysed with 1 M NaOH at 110 °C for 18 h. Cholesterol was extracted from the hydrolysate with light petroleum (b.p. 40–60 °C) and assayed enzymically. Bile acids were extracted from freeze-dried faeces into butan-2-ol/water (1:1, v/v) and assayed enzymically as described [18]. Results are given as means $\pm$ S.D. ($n = 5$ for TO, TP and TS; $n = 6$ for TM). Hepatic free cholesterol and cholesterol ester are expressed as mg of cholesterol/g of liver tissue; faecal cholesterol is expressed as mg of cholesterol excreted/3 days; faecal bile acids are expressed as μ mol of bile acid excreted/3 days. Significance of differences is indicated by: ** $P < 0.01$; *** $P < 0.001$.

Parameter	TO group	TM group	TP group	TS group
Hepatic free cholesterol (mg/g)	3.351 $\pm$ 0.393	3.192 $\pm$ 0.232	3.093 $\pm$ 0.316	3.017 $\pm$ 0.369
Hepatic cholesterol ester (mg/g)	5.907 $\pm$ 3.066	6.017 $\pm$ 2.278	4.276 $\pm$ 1.482	2.184 $\pm$ 1.615
Faecal cholesterol (mg/3 days)	5.723 $\pm$ 1.659	3.022 $\pm$ 0.227	5.082 $\pm$ 1.033	10.012 $\pm$ 3.037 (TO**, TM***, TP**)
Faecal bile acid (μ mol/3 days)	17.37 $\pm$ 6.21	28.96 $\pm$ 6.57	22.60 $\pm$ 14.26	70.62 $\pm$ 28.66 (TO***, TM**, TP**)

in the TS and TO groups. The apparent increases in the VLDL triacylglycerol concentrations in the TM and TP groups were not statistically significant. The effect of tristearin contrasted markedly with that of the two shorter-chain saturates. While total plasma cholesterol was similar to that of the TO group, VLDL

cholesterol was reduced by 60%. A dramatic fall was also seen in the VLDL triacylglycerol concentration, such that the cholesterol/triacylglycerol ratio in the TO and TS groups remained approximately constant (Table 3). The concentrations of both cholesterol and bile acids in the faeces were significantly

Table 5 Effect of dietary fatty acids on hepatic gene expression

Total RNA was extracted from hamster liver and assayed for mRNA levels using a solution hybridization/RNase protection assay as described in the Materials and methods section. Results are expressed as means $\pm$ S.D. ($n = 5$ for TO, TP and TS; $n = 6$ for TM). mRNA levels are expressed as amol of mRNA/ μ g of total hepatic RNA, normalized to 0.02 μ g of poly(A)/ μ g of total hepatic RNA as described [20]. Significance of differences is indicated by: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Diet	mRNA (amol/ μ g)		
	ApoB	HMG-CoA reductase	LDLR
TO	63.40 $\pm$ 8.02	1.364 $\pm$ 0.285	3.876 $\pm$ 1.335
TM	72.83 $\pm$ 13.03	1.252 $\pm$ 0.263	2.058 $\pm$ 0.572 (TO*)
TP	105.75 $\pm$ 9.71 (TO***, TM**, TS**)	0.928 $\pm$ 0.215	1.773 $\pm$ 0.415 (TO*)
TS	67.25 $\pm$ 13.84	1.293 $\pm$ 0.344	3.113 $\pm$ 0.857

higher in the TS group than in each of the other three groups (Table 4). There were no significant differences in the concentrations of either non-esterified or esterified cholesterol in the liver between any of the groups. The other difference of note between the TO and TS groups was a significant rise in LDL cholesterol in the TS group, although this was not as great as that seen in the TP group.

In an attempt to define the mechanisms involved in the lipoprotein changes described, the effect of the four diets on the hepatic expression of the apoB, HMG-CoA reductase and LDLR genes was also assessed. Since the different diets were not expected to cause major changes in gene expression, the absolute levels of hepatic mRNA for these three genes were measured using a highly sensitive solution hybridization/RNase protection assay capable of detecting accurately small changes in mRNA concentration. Differences in absolute levels of mRNA in the total RNA samples used in this assay were corrected for by quantification of the poly(A) content of each sample using oligo(dT)₁₈ hybridization as described [20]. The mean dietary-induced changes in mRNA levels (Table 5) may account, in part, for the changes observed in serum lipoproteins. The TP and TM groups both had significantly lower levels of LDLR mRNA than the TO group. The TP and TM groups also had lower levels of HMG-CoA reductase mRNA relative to the TO and TS groups, but not to a statistically significant degree. Interestingly, the TP group also had significantly elevated apoB mRNA levels relative to all the other groups tested. The TS group was found not to have significantly different hepatic expression of these genes relative to the TO group, reflecting the different response of serum lipids to the TS diet compared with the other saturated fat diets.

DISCUSSION

In recent years the male Golden Syrian hamster has been used extensively as a model of human lipoprotein metabolism, particularly in the study of LDL kinetics *in vivo* [12]. This species shows several advantages over other small mammals, including the following: (i) a significant proportion of cholesterol is carried in the LDL fraction [21]; (ii) the hepatic cholesterol synthesis rate is similar to that of humans [22]; (iii) plasma lipoprotein concentrations change in response to dietary [11,12] and pharmacological [23] challenge. Although a number of studies have investigated the effects of dietary fats on lipoprotein metabolism in the hamster, it has often been difficult to attribute effects to specific fatty acids because of the use of naturally occurring fats and oils or the presence of relatively high concentrations of dietary cholesterol [12]. It is likely that under these circumstances significant interactive effects occur between dietary fatty acids and cholesterol [24]. In the present study semi-synthetic diets

containing purified triacylglycerols of defined composition and a cholesterol concentration of 0.005 % (w/w), which equates to the average daily intake in humans, were used to allow the effects of specific dietary fatty acids to be observed in the absence of an excessive accumulation of hepatic cholesteryl ester.

The similar weight gains by animals in all four dietary groups and the lack of any significant increase in total faecal fat implies that each of the fats, including stearate, was well absorbed. This is likely to be due to the inclusion of 50 % of the dietary fat as triolein, which improved the presentation of the test fat, and supports the earlier conclusions [10] that stearate is well absorbed from commercial fats in humans. The absence of myristate in the hepatic phospholipids of animals fed on the TM diet (results not shown) and the low level of myristate in VLDLs of the TM group (Table 2b) suggest that myristate undergoes rapid oxidation or elongation to palmitate in the liver. Wang and Koo [25] also observed differential hepatic metabolism of saturated fatty acids in the rat, where infusion of chylomicrons containing radio-labelled fatty acids was followed by rapid clearance of the fatty acids, with stearate being preferentially used for phospholipid synthesis and myristate being preferentially oxidized in the liver or utilized by peripheral tissues. Similar metabolic differences were seen in the present study where dietary myristate, palmitate and oleate, but not to any great extent stearate, were deposited in adipose tissue triacylglycerols. Furthermore, in another study [26], dietary polyunsaturated and monounsaturated fatty acids were well incorporated into adipose tissue of rabbits while deposition of stearate, presented as cocoa butter, was limited. Limited deposition of stearic acid in adipose tissue was also seen in hamsters fed on stearate-enriched diets [27]. The high levels of oleate in adipose tissue triacylglycerols in the TS group in the present study (Table 2a) implies either selection against stearate by this tissue or that stearate undergoes rapid desaturation by a Δ -9 desaturase in the gut [8] or even in adipose tissue itself, and may represent a protective mechanism against the deposition of hard fat. That a significant proportion of dietary stearate is absorbed and reaches the liver intact is also implied by the raised stearate content of VLDL isolated from the TS group of animals (Table 2b).

Of the four diets tested, the TP diet produced the highest plasma total cholesterol concentrations, with significant increases compared with animals on both the TO and TS diets. The TM diet also caused an increase in plasma total cholesterol, whereas the TS diet was without effect relative to the TO group. Major differences were also apparent in the cholesterol concentrations of the lipoprotein fractions. In particular, VLDL cholesterol in the TS group was reduced by 60 % relative to the TO group. It is not known whether this reflects the action of stearate to decrease hepatic VLDL synthesis and secretion and/or to in-

crease lipoprotein lipase activity giving rise to the increased LDL cholesterol seen in the TS group. Both TM and TP diets also increased LDL and HDL cholesterol relative to the TO group. A relatively hypocholesterolaemic effect of stearate compared with the shorter-chain saturates in hamsters has been reported recently [27], although this difference was not apparent when cholesterol (0.2%) was included in the diets. Furthermore, addition of trilaurin, trimyristin or tripalmitin, but not tristearin, to a control diet containing 10% olive oil and 0.12% cholesterol raised LDL cholesterol significantly through a reduction in LDLR activity [11]. Concentrations in other lipoprotein fractions were not reported. The hypocholesterolaemic effect of the TS diet compared with the TP and TM diets seen in the present study may be associated with an increase in sterol excretion, since significantly more cholesterol and bile acids were detected in the faeces of the TS group. Whether this is as a result of increased biliary excretion or decreased reabsorption remains to be established.

The determination of hepatic apoB, LDLR and HMG-CoA reductase mRNA levels using a highly sensitive RNase protection assay allows speculation on potential mechanisms whereby these different dietary fats exert their effects. Animals in the TP and TM groups showed decreased levels of LDLR mRNA, indicating that decreased synthesis of LDLR might be responsible for the increased serum LDL cholesterol in these groups. This is in agreement with recent results in the hamster [13] which showed that feeding a coconut-oil-enriched diet containing a significant proportion (73%) of saturated fat led to a decrease in hepatic LDLR mRNA, protein and activity. In the present study the TP group also showed an unexpected increase in hepatic apoB mRNA, suggesting that increased production of apoB, as VLDL and consequently LDL, as well as decreased clearance of LDL, may contribute to the elevated LDL cholesterol levels in this group. By contrast, mRNA levels for these two genes were similar in the TS group and the TO group. The mechanism by which tripalmitin and trimyristin feeding affect hepatic LDLR gene expression is not clear. It was suggested [12] that saturated fats are poor substrates for cholesteryl ester formation and that feeding diets rich in saturated fats leads to a dose-dependent increase in the putative regulatory pool of non-esterified sterol, thus leading to disproportionate suppression of LDLR gene expression. However, evidence for this is based primarily on studies in which animals have accumulated relatively large amounts of cholesterol in the liver. In the present study there was no significant difference in hepatic cholesterol content in any of the groups (Table 4), although the possible redistribution of cholesterol between putative intracellular pools cannot be ruled out. Furthermore, the levels of HMG-CoA reductase mRNA in the TP and TM groups were not significantly different from those observed in the TS and TO groups. Rudling [28] showed that the hepatic expression of the LDLR and HMG-CoA reductase genes in the mouse is co-ordinately regulated *in vivo* by high levels of dietary cholesterol, while studies using HepG2 cells [29], human T cells [30] and fibroblasts [31] have demonstrated non-sterol-mediated regulation of the LDLR gene. In addition, non-esterified fatty acids regulate differentially the expression of hepatic genes involved in peroxisomal β -oxidation [32], glycolysis and lipogenesis [33], and also genes involved in the differentiation of pre-adipocytes into adipocytes [34]. Thus fatty acids may regulate LDLR gene expression in a manner unconnected with classical sterol-mediated regulation.

The mechanism leading to the increase in apoB gene expression found in the TP group (Table 5) is also unknown. Regulation of apoB production is thought to be controlled mainly at the post-translational level [35,36]. However, hepatic apoB mRNA levels may be modulated in both the rabbit and the rat by dietary

cholesterol [37,38], and hepatic apoB mRNA levels are elevated in cebus monkeys fed a coconut oil diet compared with those fed a corn oil diet [39]. Thus the result obtained in the present study is not unprecedented, and further analysis is required to elucidate the mechanism of action of dietary tripalmitin on apoB mRNA levels. Although there is no evidence *in vivo* that increasing apoB mRNA and/or protein affects the VLDL production rate, a recent study indicated that increases in apoB mRNA concentration and apoB protein synthesis lead to an increase in the secretion of apoB-containing lipoproteins by HepG2 cells [40]. The current results suggest that increased synthesis of apoB-containing lipoprotein as well as decreased clearance may be involved in the rise in plasma LDL cholesterol concentrations observed in this study of animals fed on diets rich in saturated fat. However, this hypothesis should be treated with caution. The possibility remains that dietary fat also influences conversion of VLDL into IDL and LDL by actions on lipoprotein lipase.

Although much work needs to be done to investigate the mechanism of these effects, the current experiments with defined fats have clearly demonstrated the hypercholesterolaemic effects of TM and TP diets in the hamster. In contrast to this, the major effect of a TS diet was a reduction in serum VLDL concentrations. It is also evident that modulation of hepatic gene expression plays a major part in these effects.

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