The effect of different dietary fatty acids on lipoprotein metabolism: concentration-dependent effects of diets enriched in oleic, myristic, palmitic and stearic acids

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While it is well established that the fatty acid composition of dietary fat is important in determining plasma lipoprotein cholesterol concentrations, the effects of changing the absolute quantities of the individual fatty acids are less clear. In the present study Golden Syrian hamsters were fed on isoenergetic, low cholesterol (0·05 g/kg) diets containing 100, 150 or 200 g added fat/kg. This consisted of triolein (TO) alone, or equal proportions of TO and either trimyristin (TM), tripalmitin (TP) or tristearin (TS). Each trial also included a control group fed on a diet containing 50 g TO/kg. As the mass of TO in the diet increased, plasma VLDL-cholesterol concentrations rose. The TM-rich diets produced a concentration-dependent increase in total plasma cholesterol which was a result of significant increases in both VLDL and HDL levels. The TP-rich diets increased plasma LDL- and HDL-cholesterol levels in a concentration-dependent manner. TS-containing diets did not increase the cholesterol content of any of the major lipoprotein fractions. Hepatic LDL-receptor mRNA concentrations were significantly decreased in animals fed on TP, while apolipoprotein B mRNA concentrations were significantly increased. Thus, on a low-cholesterol diet, increasing the absolute amount of dietary palmitic acid increases LDL-cholesterol more than either myristic or stearic acid. These effects on lipoprotein metabolism may be exerted through specific modulation of the expression of the LDL receptor and apolipoprotein B genes.

Fatty acids: Lipoproteins: LDL receptor

It is generally accepted that plasma cholesterol concentration is influenced by the fatty acid composition of dietary fat. Thus, relative to monounsaturated and polyunsaturated fatty acids, dietary long-chain saturated fatty acids increase plasma cholesterol levels (Grundy & Denke, 1990). However, not all saturated fatty acids have equivalent effects. Lauric (12 : 0), myristic (14 : 0) and palmitic (16 : 0) acids raise plasma cholesterol levels, though considerable controversy surrounds their relative potency (Denke & Grundy, 1992; Derr *et al.* 1993; Sundram *et al.* 1994, Tholstrup *et al.* 1994*b*; Zock *et al.* 1994). By contrast, stearic acid does not appear to have such an effect and has been described as 'neutral' (Bonanome & Grundy, 1988; Denke & Grundy, 1991; Derr *et al.* 1993; Tholstrup *et al.* 1994*a*). We have recently shown that high fat (200 g/kg), palmitic acid-rich diets increase plasma LDL-cholesterol concentrations in hamsters to a greater extent than those rich in myristic, stearic or oleic acids and that this is associated with decreased hepatic LDL receptor and increased apolipoprotein (Apo) B mRNA concentrations (Bennett *et al.* 1995*a*). The effect of changing the absolute amount of an individual fatty acid in the diet is less clear. Meta-analysis suggests that, depending on the fatty acid composition of the diet, increasing the amount of fat without altering the composition could have adverse effects on the lipoprotein profile (Mensink & Katan, 1992). In the present study we have

Abbreviations: Apo, apolipoprotein; CETP, cholesteryl ester transfer protein; dT_{18}, oligo deoxythymidine homopolymer (18 bases); HMG-CoA, hydroxymethylglutaryl-CoA; IDL, intermediate density lipoproteins; MTP, microsomal triacylglycerol transfer protein; TM, trimyristin; TO, triolein; TP, tripalmitin; TS, tristearin.

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extended our previous findings to look at the effects of increasing the concentration of individual fatty acids in the diet without major changes in the overall fatty acid composition.

Materials and methods

Chemicals

All reagents were of the highest purity commercially available and solvents were of analytical grade unless otherwise stated. Radioisotopes and Hybond N nylon membrane were obtained from Amersham International plc (Amersham, Bucks., UK). Bluescript SK and KS phagemids (hybrid type plasmids) and *in vitro* transcription kits were obtained from Stratagene Ltd (Cambridge, Cambs., UK). Restriction endonucleases, RNAse-free DNAse (*EC* 3.1.21.1), Taq DNA polymerase (*EC* 2.7.7.7) and bacteriophage T4 polynucleotide kinase were obtained from Boehringer Mannheim (Lewes, Sussex, UK). RPA II ribonuclease protection assay kits were obtained from AMS Biotechnology (Witney, Oxon., UK). Oligo deoxythymidine homopolymer (18 bases) (dT_{18}) and deoxynucleoside triphosphates were obtained from Pharmacia LKB (Milton Keynes, Bucks., UK). Polyadenylic acid and salmon testes DNA were obtained from Sigma Chemical Company (Poole, Dorset, UK). Bacteriophage T4 DNA ligase (*EC* 6.5.1.1) and reverse transcriptase (*EC* 2.7.7.49) were obtained from Gibco BRL (Paisley, Strathclyde, UK).

Maize starch, cellulose (Alphacel), salt mixture (Hegsted), vitamin mix, and cholesterol were obtained from ICN Flow (High Wycombe, Bucks., UK). Trimyristin (TM, 97% pure), tripalmitin (TP, 95% pure) and triolein (TO, technical grade 65–70% pure) were obtained from Fluka (Glossop, Derby., UK). Tristearin (TS, 95% pure) and L-cystine were obtained from Sigma Chemical Company.

Animals and diets

Four individual trials were conducted to test the effect of dietary oleic, myristic, palmitic and stearic acids respectively. For each trial thirty male Golden Syrian hamsters, 120–165 g (3–4 months old) were obtained from a colony housed at the Biomedical Services Unit, Queen's Medical Centre. This was originally derived from a colony established by Harlan Olac (Bicester, Oxon., UK). Animals were allowed 2 weeks to adapt to their new environment during which time they were allowed free access to food and tap water. They were fed on a standard rodent chow (Rat and Mouse Breeding Diet 422, Pilsbury, Northants, UK) which was provided initially in a pelleted form and then in the second week as ground meal. The room was lit from 06.00 to 18.00 hours, with temperature maintained at 21° and humidity at 55 ± 10%.

Following this acclimatization period, hamsters were adapted to a semi-purified control diet (Table 1) over a further 2 weeks. This diet was mixed with ground chow in the proportion of 25 : 75 (diet : chow) and given for 2 d. The proportion of control diet to chow was then increased to 50 : 50 for a further 2 d and finally to 80 : 20. It was found that at this ratio the diet was well-tolerated by most animals. During the 2-week pre-trial period, food intake and animal weight were monitored every 2 d. Any animal that lost more than 5% of its body weight in any 2 d period or more than 10% over the whole pre-trial period was excluded from the trial. Twenty-four of the remaining animals were then randomly allocated to one of four groups (six animals per group). These animals were transferred to appropriate test diets as indicated in Table 1. Each of the four trials contained a control group which continued to consume the low-fat (50 g TO/kg) control diet. The three test diets contained 100, 150 or 200 g added fat/kg. This consisted of TO only (in the TO alone trial) or 500 g TO and 500 g TM, TP or TS/kg. Fats were added to the diet to replace the equivalent amount of energy as starch. Energy and nutrient density were maintained by increasing the cellulose content of the diet as appropriate (Table 1). The diets were mixed with rodent chow (80 : 20, diet : chow) as in the pre-trial period and animals were fed on the diets for 28 d. Food intake and animal weight were again monitored every 2 d. Any animal that lost more than 20% of its initial body weight was removed from the trial. Faeces were collected between days 24 and 27 and frozen at −30° until further analysis. On day 28, animals were fasted overnight and killed the following morning. They were anaesthetized using sodium pentabarbitone (Sagatal, 1 ml/kg) and 3–4 ml blood was collected by cardiac puncture. The liver was flushed with 10 ml ice-cold 0.15 M-NaCl, removed, immediately frozen in liquid N_2 and stored at −80° until further analysis.

Table 1. Composition of the diets*†

Added fat (g/kg)...	50 (control)	100	150	200
Starch (g/kg)	503	387	272	156
Cellulose (g/kg)	35	101	166	232

* Isoenergetic diets were formulated by replacing dietary carbohydrate with dietary fat and maintaining the energy density by increasing the cellulose (Alphacel, non-nutritive bulk) content. For details of the dietary fat, see Table 2 and p. 196.

† All diets contained (g/kg): casein 235, L-cystine 3, liver concentrate 1, sucrose 106, linseed oil 5, Hegsted salt mixture 40, vitamin mix (ICN Flow, High Wycombe, Bucks., UK) 22.

Table 2. Fatty acid composition of diets (g/100 g total fatty acids recovered)

Diet	SF	14:0	16:0	18:0	16:1	18:1	18:2	18:3
50 g TO (control)		1·8	6·8	2·4	4·1	51·6	15·1	5·7
100 g TO		2·0	5·9	2·0	4·4	55·2	13·1	3·8
150 g TO		2·1	5·5	1·8	4·6	56·6	12·3	3·0
200 g TO		2·1	5·3	1·8	4·7	57·4	11·9	2·6
50 g TO	TM	44·0	3·9	1·3				
50 g SF	TP	1·1	45·6	1·3	2·3	29·2	8·5	3·2
	TS	1·0	4·5	42·5				
75 g TO	TM	46·1	3·4	1·2				
75 g SF	TP	1·1	47·0	1·2	2·4	29·4	7·5	2·5
	TS	1·0	4·1	44·2				
100 g TO	TM	47·1	3·1	1·1				
100 g SF	TP	1·2	47·7	1·1	2·4	29·5	7·0	2·0
	TS	1·1	3·8	45·1				

SF, saturated fat; TO, triolein; TM, trimyristin; TP, tripalmitin; TS, tristearin.

Fatty acid analysis of dietary fats and oils

Chow, linseed oil, TO, TM, TP and TS (50 mg) were extracted with 2 ml chloroform–methanol (1 : 2, v/v) and 200 µl portions were taken for trans-methylation as previously described (Bennett *et al.* 1995*a*). Fatty acid methyl esters were analysed by GLC on a CP-Sil 88 capillary column (50 m × 0·25 mm; Chromopak, London, UK) in a Perkin Elmer 8300 chromatograph (Perkin Elmer, Beaconsfield, Bucks., UK). These data were then used to calculate the fatty acid composition of the individual diets (Table 2).

Separation of lipoproteins

Lipoprotein fractions were separated from plasma (normally 1 ml) by sequential preparative ultracentrifugation in a Beckman Optima TLX benchtop ultracentrifuge as previously described (Bennett *et al.* 1995*a*). VLDL, intermediate-density lipoproteins (IDL), LDL and HDL were separated within density ranges of: < 1·006, 1·006–1·02, 1·02–1·06 and > 1·06 g/ml respectively. Cholesterol and triacylglycerol concentrations in plasma and lipoprotein fractions were determined on the Cobas Mira autoanalyser (Roche Diagnostics Ltd, Welwyn Garden City, Herts., UK) using the Olympus system reagent 5000-cholesterol and triacylglycerol (GPO Trinder) kits respectively. The interassay CV for the combined separation of lipoproteins and cholesterol analysis were assessed by taking six portions of pooled hamster plasma through the whole procedure. Table 3 indicates the results of this validation for the two fractions containing the most cholesterol; LDL and HDL. As can be seen the CV were 5·5 and 3·0 % respectively and the mean recovery (i.e. the sum of all the individual fractions expressed as a percentage of the total plasma cholesterol) was 86 %. The loss in cholesterol during isolation was probably cumulative throughout the three ultracentrifugal spins. As such it was likely to have the largest effect on the two last fractions to be isolated, namely LDL and HDL. Indeed, strong, positive, linear correlations were seen between both LDL- and HDL-cholesterol and percentage recovery ($P = 0{\cdot}014$ and 0·010 respectively) and accounted for 77 and 80 % of the variation in the respective fractions. For this reason, all LDL and HDL values quoted in this paper were corrected to 100 % recovery. Table 3 indicates that such correction improved the CV for both fractions.

Table 3. Reproducibility of separation of LDL and HDL fractions of plasma*

	Mean	SD	CV
LDL (mmol/l)	0·39	0·021	5·3
HDL (mmol/l)	1·10	0·033	3·0
Recovery (%)	86	4·2	4·9
LDL (corrected)†	0·45	0·011	2·3
HDL (corrected)†	1·28	0·031	2·4

* For details of procedures, see pp. 196–197.
†Corrected to 100 % recovery.

Determination of hepatic free and esterified cholesterol and faecal cholesterol

Hepatic free and esterified cholesterol and faecal cholesterol were separated and assayed as described previously (Bennett *et al.* 1995*a*). Data for hepatic free and esterified cholesterol are presented as mg/g wet weight of liver. Data for faecal cholesterol are presented as mg produced per 3 d.

Isolation of hepatic total RNA and determination of mRNA concentrations

Total hepatic RNA was isolated by the guanidinium thiocyanate method essentially according to the method of Chomczynski & Sacchi (1987). The mRNA concentrations for hepatic ApoB, hydroxymethylglutaryl-CoA (HMG-CoA) reductase (*EC* 1.1.1.34) and LDL-receptor genes were determined by a solution hybridization/RNAse protection assay as previously described (Bennett *et al.* 1995*a*). Results were corrected for variation in the mRNA content of total RNA samples by quantitation of polyA RNA using oligo dT_{18} hybridization (Harley, 1987). All mRNA values are expressed as attomol mRNA/µg total RNA normalized to 20 ng poly A/µg total RNA. In a number of cases the amount of mRNA could not be accurately determined due to a 'smearing' of the bands on the gel. This is likely to be due to prior degradation of the RNA. The number of measurements made in each group is indicated in Table 6.

Statistical analysis

All statistical analyses were performed using the Genstat 5 for Windows software package (Lawes Agricultural Trust, Rothamstead Experimental Station, Herts., UK). An initial one-way ANOVA was performed on the control groups from each experiment. As no statistically significant differences were seen in any of the variables measured (see p. 198) further analysis was performed on the combined test groups from each experiment. Data were analysed by two-way ANOVA with type (TO, TM, TP or TS) and amount (100, 150, 200 g/kg) of fat as factors. Tables indicate the residual degrees of freedom (df), standard error of differences of means (SED) and significances (P) for type, amount and the interaction between type and amount of fat. The effect of amount of fat was further tested for linear and quadratic regression. The effect of type of fat was further partitioned to examine the effect of: (1) TO *v.* TM + TP + TS, (2) TS *v.* TM + TP and (3) TM *v.* TP. Thus, this allowed the examination of the effect of the monounsaturated fatty acid-rich fat compared with the saturated fatty acid-rich fats, the specific effects of the stearic acid-rich fat compared to the other saturated fats and any specific differences between the myristic and palmitic acid-rich fats.

Results

Fatty acid composition of diets

Table 2 shows that within each trial the fatty acid composition of the diets containing 100, 150 and 200 g fat/kg was essentially constant. Diets containing 150 g fat/kg or less were all well tolerated by the animals with only four out of a total of seventy-two failing to thrive (one each in the control and 150 g/kg groups of the TM trial, one in the 150 g/kg group of the TP trial and one in the 100 g/kg group of the TS trial). However, the 200 g of fat/kg diets were less well tolerated with two and three animals being withdrawn from the TP and TS groups respectively due to markedly reduced food intake and resultant loss of body weight. In general, animals tended to maintain, or slightly increase, their body weight with little difference in food intake across the dietary groups. However, animals on the highest dose of TP did show a significant reduction in food intake (control: 191 (SD 7·8) g *v.* 200 g/kg: 150 (SD 22·3) g food consumed over the whole trial, $P < 0·05$).

Comparison of control groups

As indicated on p. 197, before pooling the results, a preliminary analysis of the control groups from each experiment was performed. Every variable measured was compared by one-way ANOVA. The only apparent significant differences were in percentage recovery of lipoproteins and uncorrected (for recovery) LDL and HDL. This was due to a lower mean recovery in the TP trial than in the other three. Further investigation indicated similar differences in recoveries for the test groups as well. However, when LDL and HDL were corrected for recovery, no significant difference between trials remained. Thus, for further analysis the test groups for each trial were included in a single two-way ANOVA.

Comparison of test groups

Table 4 shows plasma total cholesterol and lipoprotein cholesterol concentrations in each of the dietary test groups. Total plasma cholesterol was related to both the type and amount of dietary fat, but no interaction between

Table 4. Effect of different dietary fats on total plasma and lipoprotein cholesterol concentrations (mmol/l) in hamsters fed on diets containing 100, 150 or 200 g fat/kg, consisting of triolein (TO) alone or equal proportions of TO and trimyristin (TM), tripalmitin (TP) or tristearin (TS)

(Mean values for six animals per group and combined means for each amount and type of fat)

		Amount of fat (g/kg) (A)					ANOVA		
	Type of fat (T)	100	150	200	Mean		df	SED	*P*
Total	TO	2·98	3·17	3·13	3·09	T	53	0·176	0·032
	TM	2·95	3·04	3·86	3·28	A		0·153	0·002
	TP	3·01	3·81	4·02	3·61	A × T		0·306	0·153
	TS	3·30	3·50	3·53	3·44				
	Mean	3·06	3·38	3·63	3·36				
VLDL	TO	0·53	0·60	0·75	0·63	T	51	0·069	0·004
	TM	0·34	0·48	0·75	0·52	A		0·060	0·031
	TP	0·36	0·42	0·47	0·42	A × T		0·120	0·143
	TS	0·44	0·37	0·34	0·38				
	Mean	0·42	0·47	0·58	0·49				
IDL*	TO	0·92 (0·09)	0·88 (0·09)	0·99 (0·10)	0·93	T	53	0·052	< 0·001
	TM	0·87 (0·08)	0·89 (0·08)	0·99 (0·10)	0·92	A		0·045	0·004
	TP	0·85 (0·07)	0·95 (0·09)	1·07 (0·12)	0·96	A × T		0·091	0·899
	TS	1·12 (0·15)	1·10 (0·13)	1·29 (0·20)	1·17				
	Mean	0·94	0·96	1·08	0·99				
LDL	TO	0·57	0·56	0·50	0·54	T	53	0·055	< 0·001
	TM	0·61	0·63	0·71	0·65	A		0·048	0·151
	TP	0·63	0·74	0·93	0·77	A × T		0·096	0·215
	TS	0·78	0·82	0·83	0·81				
	Mean	0·65	0·69	0·74	0·69				
HDL	TO	1·74	2·07	1·70	1·84	A	53	0·104	0·020
	TM	1·90	1·85	2·26	2·00	T		0·090	0·018
	TP	1·83	2·29	2·38	2·17	A × T		0·180	0·051
	TS	1·99	2·12	2·10	2·07				
	Mean	1·87	2·08	2·11	2·02				

IDL, intermediate density lipoprotein.
* Not normally distributed. Transformed data ($\log_{10} \times 100$) are given with actual values in parentheses.

Table 5. Effect of different dietary fats on plasma VLDL-triacylglycerol (TAG), hepatic free cholesterol (FC) and cholesteryl ester (CE) and faecal cholesterol (C) concentrations in hamsters fed on diets containing 100, 150 or 200 g fat/kg, consisting of triolein (TO) alone or equal proportions of TO and trimyristin (TM), tripalmitin (TP) or tristearin (TS)

(Mean values for six animals per group and combined means for each amount and type of fat)

		Amount of fat (g/kg) (A)					ANOVA		
	Type of fat (T)	100	150	200	Mean		df	SED	*P*
VLDL-TAG* (mmol/l)	TO	1·97 (0·97)	1·79 (0·67)	1·84 (0·72)	1·87	T	50	0·058	< 0·001
	TM	1·77 (0·60)	1·81 (0·68)	1·97 (0·97)	1·85	A		0·050	0·127
	TP	1·89 (0·82)	1·92 (1·03)	1·78 (0·67)	1·87	A × T		0·100	0·016
	TS	1·77 (0·62)	1·53 (0·38)	1·43 (0·28)	1·58				
	Mean	1·85	1·76	1·76	1·79				
Hepatic FC (mg/g)	TO	2·60	2·66	3·24	2·83	T	53	0·150	0·039
	TM	2·31	2·35	2·58	2·41	A		0·130	0·102
	TP	2·66	2·86	2·68	2·73	A × T		0·260	0·373
	TS	2·65	2·37	2·71	2·58				
	Mean	2·56	2·56	2·80	2·64				
Hepatic CE* (mg/g)	TO	2·72 (6·67)	2·99 (11·31)	3·03 (11·32)	2·91	T	53	0·127	< 0·001
	TM	2·16 (3·82)	2·63 (4·58)	2·86 (7·28)	2·55	A		0·110	0·001
	TP	2·21 (2·59)	2·59 (4·45)	2·47 (4·27)	2·42	A × T		0·220	0·444
	TS	2·06 (1·46)	2·03 (1·36)	2·46 (2·97)	2·19				
	Mean	2·29	2·56	2·70	2·52				
Faecal C (mg/3 d)	TO	3·72	3·47	3·44	3·54	T	53	0·346	< 0·001
	TM	3·32	3·31	2·61	3·08	A		0·299	0·069
	TP	4·54	4·29	4·98	4·60	A × T		0·599	0·002
	TS	6·56	9·24	6·62	7·47				
	Mean	4·53	5·08	4·41	4·67				

* Not normally distributed. Transformed data ($\log_{10} \times 100$) are given with actual values in parentheses.

the two was seen. The largest effect of type of fat in our analysis was between TO and TM + TP + TS with the unsaturated fat group being significantly lower than the combined saturated fat group ($P = 0{\cdot}018$). Plasma cholesterol increased linearly with amount of fat ($P < 0{\cdot}001$).

Significant effects of type and amount of fat were also seen for VLDL- and IDL-cholesterol but there was no interaction. VLDL was significantly higher in the TO group compared with the combined TM + TP + TS group. There was an overall linear increase in VLDL with increasing amount of dietary fat ($P = 0{\cdot}01$) but within the saturated fats there were significant differences in this regression between the TS group and the combined TM + TP groups ($P = 0{\cdot}016$). While VLDL tended to fall with increasing amount of TS in the diet the opposite was true for TM + TP. IDL-cholesterol was found not to be normally distributed so statistical analysis was performed on transformed data ($\log_{10}(\text{IDL} \times 100)$). IDL-cholesterol concentrations increased with concentration of fat in the diet and the level was significantly higher in the TS group than in groups receiving the other saturated fatty acids ($P < 0{\cdot}001$).

LDL-cholesterol concentration was significantly affected by the type of fat but not the amount, with significant differences being seen between TO *v.* TM + TP + TS ($P < 0{\cdot}001$), TS *v.* TM + TP ($P = 0{\cdot}037$) and TM *v.* TP ($P = 0{\cdot}034$). The linear regression of amount of fat against LDL cholesterol almost attained significance ($P = 0{\cdot}054$) and there was a significant difference in the linear regressions when TO was compared with the combined saturated fatty acids ($P = 0{\cdot}047$).

An interactive effect of type and amount of fat was seen on HDL-cholesterol and when partitioned, quadratic regressions comparing TO with TM + TP + TS ($P = 0{\cdot}066$) and TM with TP ($P = 0{\cdot}059$) almost attained significance.

VLDL-triacylglycerol (Table 5) again was not normally distributed and was transformed as for IDL-cholesterol. A significant interaction between amount and type of fat was seen with significant differences in linear regression when TS was compared with TM + TP ($P = 0{\cdot}003$) and TM and TP were compared ($P = 0{\cdot}036$). In general, VLDL-triacylglycerol tended to decrease with increasing amounts of TS.

Hepatic free and esterified cholesterol were both influenced by the type of fat in the diet with the highest values in the TO group and lowest in the TS group. Hepatic cholesterol ester was again log-transformed. While no effect of amount of fat was seen on free cholesterol, the esterified form showed a strong linear relationship with the amount of fat ($P < 0{\cdot}001$). No significant interactions between amount and type of fat were seen. There was a

Table 6. Effect of different dietary fats on hepatic apolipoprotein B (ApoB), hydroxymethylglutaryl-CoA (HMG-CoA) reductase and LDL-receptor mRNA concentrations in hamsters fed on diets containing 100, 150 or 200 g fat/kg, consisting of triolein (TO) alone or equal proportions of TO and trimyristin (TM), tripalmitin (TP) or tristearin (TS)

(Values are presented as mean attomol/μg RNA normalized to 20 ng polyA/μg total RNA)

		Amount of fat (g/kg) (A)					ANOVA		
	Type of fat (T)	100	150	200	Mean		df	SED	*P*
n	TO	5	5	4*					
	TM	6	4*	4					
	TP	5	5	4					
	TS	4	5	2					
ApoB	TO	83	83	83	83	T	39	7·5	< 0·001
	TM	69	57	66	64	A		6·5	0·139
	TP	89	88	123	100	A × T		13·0	0·364
	TS	66	69	76	71				
	Mean	77	74	87	79				
HMG-CoA reductase	TO	1·00	1·36	1·27	1·21	T	41	0·10	0·214
	TM	1·17	1·24	1·07	1·16	A		0·08	0·634
	TP	1·26	1·03	0·98	1·09	A × T		0·17	0·206
	TS	1·22	1·31	1·34	1·29				
	Mean	1·16	1·23	1·17	1·19				
LDL receptor	TO	3·28	3·29	3·52	3·36	T	41	0·141	< 0·001
	TM	3·22	2·99	3·03	3·08	A		0·122	0·830
	TP	2·96	2·62	2·16	2·58	A × T		0·244	< 0·001
	TS	3·13	3·40	3·81	3·45				
	Mean	3·14	3·08	3·13	3·11				

* Each of these groups had one less determination of ApoB mRNA.

highly significant interaction however in faecal cholesterol excretion with a large difference in the quadratic regression between TS and TM + TP ($P < 0·001$).

Hepatic ApoB mRNA was influenced by type but not amount of dietary fat (Table 6) with the largest difference occurring between TM and TP ($P < 0·001$). There was also a significant difference in the regression of amount of TM or TP and ApoB mRNA ($P = 0·049$). By contrast hepatic HMG-CoA reductase mRNA was not influenced by either type or amount of fat. There was, however, a significant effect when TS was compared with TM + TP ($P = 0·051$). A highly significant interaction between type and amount of fat on LDL-receptor mRNA was indicated. The largest difference was seen when the linear regressions for amount of fat were compared for TS and TM + TP ($P < 0·001$).

Discussion

We have recently shown that, at low dietary cholesterol concentrations, different fatty acids have specific effects on lipoprotein metabolism in the hamster (Bennett *et al.* 1995*a*). In the current study these findings are extended to consider the dose-dependent effects of individual fatty acids. By isoenergetically replacing dietary carbohydrate with different purified fats, of known composition and structure, we have been able to increase the total amount of fat while making only very minor changes to the fatty acid composition.

Each of the saturated fats studied was fed in conjunction with TO to improve diet presentation and the absorption of the fats. It is therefore important to recognize that they may not only be having effects in their own right but also modulating any effect of oleic acid. Increasing the amount of TO alone in the diet increased hepatic cholesteryl ester concentrations and increased the plasma concentration of VLDL-cholesterol. This was associated with a decrease in the excretion of faecal cholesterol. Thus, it appears that, with increasing amounts of TO in the diet, the sequestration of cholesterol as cholesteryl ester in the liver may reduce biliary cholesterol excretion into the intestine and faeces and hence increase its secretion into the plasma as part of VLDL. When 50% of the TO in the test diets was replaced with TM then similar effects on plasma VLDL-cholesterol, hepatic cholesteryl ester and faecal cholesterol were seen as with TO alone. However, the magnitude of the changes was less and was consistent with the effect being due to the remaining TO in the diets. Thus, it appears that in this respect, myristic acid is neutral and does not influence the changes brought about by oleic acid. However, unlike TO alone, increasing amounts of TM in the diet increased plasma HDL-cholesterol concentrations. It is not clear what the mechanism of this effect is, but in monkeys fed on a low-cholesterol diet, coconut oil, which is rich in lauric and myristic acids, has been shown to decrease the fractional clearance rate of ApoA1, thus increasing plasma HDL-cholesterol and ApoA1 concentrations (Stucchi *et al.* 1991). It is also possible that TM may exert its effects on HDL-cholesterol through an inhibition of cholesteryl ester transfer protein (CETP) activity. Inhibition of CETP has been shown to increase HDL-cholesterol concentrations in hamsters (Zuckerman & Evans, 1995). Furthermore, CETP appears to be regulated by diet, with cholesterol increasing activity (Stein *et al.* 1990; Kurushima *et al.* 1995) and oleic acid partially reversing this effect (Kurushima *et al.* 1995).

It remains to be established what the effect of individual saturated fatty acids is, but an inhibition could produce the elevation in HDL-cholesterol seen in the present study.

Unlike TM, the TP diet did not lead to increases in VLDL-cholesterol, hepatic cholesteryl ester or faecal cholesterol. This suggests that dietary palmitic acid opposes some of the effects of oleic acid. TP-feeding did, however, markedly increase IDL-, LDL- and HDL-cholesterol concentrations and was also associated with significant changes in hepatic gene expression. LDL-receptor mRNA concentrations were significantly decreased by TP. Down-regulation of hepatic LDL receptors may limit cholesterol availability in the liver and hence explain why hepatic cholesteryl ester concentrations do not increase in response to the oleic acid in the diet. The mechanism whereby palmitic acid reduces the concentration of mRNA coding the LDL receptor is not clear. It has been suggested (Woollett *et al.* 1992) that saturated fatty acids are poor substrates for acyl-CoA : cholesterol acyl transferase (*EC* 2.3.1.26) and that this leads to an increase in the putative metabolically active pool of unesterified cholesterol, resulting in sterol-mediated repression of the LDL receptor and HMG-CoA reductase genes. However, this does not explain why the effect on gene expression is specific to palmitic acid, particularly as this has been shown to be a relatively good substrate for acyl-CoA : cholesterol acyl transferase (Goodman *et al.* 1964).

The TP diet also increased hepatic ApoB gene expression, confirming our previous findings (Bennett *et al.* 1995*a*). We have also recently shown (Bennett *et al.* 1995*b*) that TP-feeding leads to a dose-dependent increase in hepatic microsomal triacylglycerol transfer protein (MTP) mRNA concentrations. Diet-induced changes in MTP mRNA in the hamster have previously been shown to be associated with changes in MTP protein concentration and transfer activity (Lin *et al.* 1994). The MTP is thought to be involved in the transfer of triacylglycerol from its site of synthesis, on the smooth endoplasmic reticulum, to the site of VLDL assembly. The increase in MTP mRNA associated with TP-feeding correlated positively with LDL-cholesterol concentrations (Bennett *et al.* 1995*b*). Thus, concomitant increases in ApoB and MTP synthesis would be expected to result in increased VLDL secretion which, in the presence of reduced LDL receptor activity, could lead to the increase in LDL concentrations observed.

Like the TM-rich diet, TP also increased plasma HDL-cholesterol concentration. Again the mechanism for this increase remains to be established.

It has been suggested for many years that stearic acid is not as hypercholesterolaemic as other long-chain saturates and as such was not included in several attempts to quantify the effects of saturated fats on plasma cholesterol, such as those by Keys *et al.* (1965) and Hegsted *et al.* (1965). More recently it has been included as a slightly hypocholesterolaemic effector in an attempt to update such equations (Yu *et al.* 1995). The present study confirms that stearic acid has a neutral effect. None of the major lipoprotein fractions showed an increased cholesterol content with increasing amounts of TS in the diet and like palmitic acid, stearic acid appeared to inhibit any TO-related increase in hepatic cholesteryl ester or VLDL-cholesterol. No effect of TS was seen on hepatic gene expression. The reason for the apparently neutral effect of stearic acid has been the subject of considerable research. The digestion and absorption of stearic acid is less than shorter chain saturates but the magnitude of this effect is relatively small (Denke & Grundy, 1991). We have shown (Bennett *et al.* 1995*a*) that, when fed in combination with TO, in excess of 95 % of the stearic acid is absorbed and thus poor absorption is unlikely to explain the effects seen in the present study. Other workers have suggested that stearic acid may be extensively desaturated to oleic acid and that this is the reason for its effects (Denke & Grundy, 1991). However, the extent to which this occurs is again unlikely to explain the current findings (Emken, 1994; Bruce & Salter, 1996). Furthermore, if any significant desaturation occurred the characteristic oleate-induced accumulation of cholesteryl ester in the liver would have been expected in our experiments. We have shown that stearate is preferentially directed towards the synthesis of phospholipid rather than triacylglycerols in hamster hepatocytes (Bruce & Salter, 1996) and fatty acid analysis of hepatic lipids in the present study showed that stearate was more readily incorporated into phospholipid than myristate or palmitate (results not shown). Such a change in membrane phospholipids may influence VLDL secretion, reducing the amount of cholesterol secreted through this route. It is also of note that TS-feeding was associated with an increase in faecal cholesterol excretion and no increase in hepatic cholesterol stores. This has also been reported by other workers (Imaizumi *et al.* 1993) and may be one explanation for the relative hypocholesterolaemic effect of stearic acid compared with other long chain saturates.

The present study shows that, even under dietary conditions where cholesterol intake is very low, saturated fatty acids exert differential, dose-dependent effects on cholesterol and lipoprotein metabolism. It is apparent that some of these effects are due to actions at the level of mRNA by changes in gene expression or message stability. The possibility that fatty acids themselves might have direct effects on gene expression (Amri *et al.* 1994; Gearing *et al.* 1994; Jump *et al.* 1994) opens a potentially new and exciting area of research.

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