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Interactive effects of dietary cholesterol and different saturated fatty acids on lipoprotein metabolism in the hamster

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The present study examines the interactive effects of three fatty acids: myristic, palmitic and stearic acids, with dietary cholesterol, on lipoprotein metabolism in the hamster. Each saturated fatty acid was fed at a concentration of 100 g pure synthetic triacylglycerol/kg in the presence of 100 g triolein/kg and was fed in the presence of 0·05, 1·2 or 2·4 g dietary cholesterol/kg. Dietary cholesterol increased the concentration of cholesterol in each of the major plasma lipoprotein fractions. The largest effects on VLDL and LDL were seen in the presence of tripalmitin where the increase between the lowest and highest dietary cholesterol groups were 129 % and 38 % respectively. In contrast, HDL showed the greatest change in the tristearin group when the equivalent increase was 59 %. No interactive effects of dietary cholesterol and fat were seen on hepatic mRNA concentrations for the LDL receptor, hydroxymethylglutaryl-CoA reductase or the microsomal triacylglycerol transfer protein. As the amount of cholesterol in the diet increased, large differences were seen in the storage of hepatic cholesterol ester. At the highest dietary cholesterol intake the amount of hepatic cholesterol ester was 1·7-fold higher in the animals fed trimyristin compared with those fed tripalmitin. These results suggest that, as the amount of cholesterol in the diet is increased, palmitic acid becomes more hypercholesterolaemic. This is associated with a reduced ability to store cholesterol ester in the liver.

Saturated fatty acids: Dietary cholesterol: Lipoprotein metabolism: Hamster

It has been known for many years that dietary cholesterol and dietary fatty acids can influence plasma cholesterol concentrations in man. Meta-analysis suggests a 0·07 mM rise in plasma cholesterol for each 100 mg increase in dietary cholesterol intake (Hegsted, 1986). It is well established that saturated fatty acids increase plasma cholesterol (Salter & White, 1996). This cholesterol-raising effect appears to be restricted to lauric, myristic and palmitic acids with stearic acid being essentially neutral. These first three fatty acids appear to increase cholesterol in the LDL and HDL fractions, though the effect on the former tends to be greater (Mensink & Katan, 1992). The relative potency of the individual saturated fatty acids remains to be resolved. Meta-analysis indicates that myristic acid is the most hypercholesterolaemic (Salter & White, 1996) while direct feeding studies (Tholstrup *et al.* 1994) suggest that myristic and palmitic acids have similar effects on plasma lipoprotein cholesterol concentrations. Hayes and co-workers have suggested that, in subjects with low dietary cholesterol intakes (<300 mg/d) and low plasma cholesterol concentrations (<5·2 mM), palmitic acid is neutral and that all of the cholesterol-raising influence of dietary fat is attributable to myristic acid (Hayes & Khosla, 1992; Hayes *et al.* 1995). They suggest that as dietary cholesterol intakes increase above 400 mg/d then palmitic acid may become hypercholesterolaemic. In the present study we have attempted to look at the possible interactive effects of dietary saturated fatty acids with dietary cholesterol in influencing plasma lipoprotein concentrations, using the hamster as a model species.

The hamster has been extensively used as a model system for studying lipoprotein metabolism. On a normal, low-fat, chow-based diet, HDL represents the major cholesterol-carrying lipoprotein (Sessions & Salter, 1994). However, as increasing amounts of cholesterol and saturated fatty acids are added to the diet the relative concentrations of VLDL and LDL increase (Sessions & Salter, 1994). Dietschy and co-workers have used this species to elucidate the nature of the regulation of LDL metabolism by dietary cholesterol and different fatty acids (For review see Spady *et al.* 1993). One major difference between the hamster and man is the rate of hepatic cholesterol synthesis. The absolute cholesterol synthesis rate in human subjects is about 10 mg/kg per d while that

Abbreviations: HMG-CoA, hydroxymethylglutaryl-CoA; IDL, intermediate-density lipoprotein; TM, trimyristin; TP, tripalmitin; TS, tristearin.

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for hamsters is 40 mg/kg per d (Kris-Etherton & Dietschy, 1997). Thus, it can be argued that to obtain equivalent effects of dietary cholesterol in the hamster to those seen in the human subjects much higher amounts must be fed. In the present study, three concentrations of dietary cholesterol were used specifically to elicit different responses. At 0·05 g cholesterol/kg diet, endogenous cholesterol synthesis is likely to be at a maximum. At 1·2 g/kg, endogenous synthesis should be completely replaced by the exogenous influx and at 2·4 g/kg the intake of cholesterol is approximately twice the normal endogenous synthetic capacity and would be expected to be making an appreciable contribution to plasma cholesterol concentrations.

Materials and Methods

Chemicals

All reagents were of the highest purity commercially available and solvents were of Analar 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, Taq DNA polymerase and T4 polynucleotide kinase were obtained from Boehringer Mannheim (Lewes, Sussex, UK). RPA II ribonuclease protection kits were obtained from AMS Biotechnology (Witney, Oxon., UK). Oligo deoxythymidine homopolymer (18 bases) 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). T4 DNA ligase and Superscript reverse transcriptase were obtained from Gibco BRL (Paisley, Strathclyde UK).

Corn 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 (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

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, Nottingham, Notts., UK. This was originally derived from a colony established by Harlan Olac (Bicester, Oxon., UK). Sixty 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 a standard rodent chow (Rat and Mouse Breeding Diet 422; Pilsbury, Northampton, 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°C and humidity at 55 ± 10 %.

Following this acclimatization period, hamsters were adapted to a semisynthetic control diet (Salter *et al.* 1998) over a further 2 weeks. This diet was mixed with ground chow in the proportion 25:75 (diet:chow) and fed for 2 d. The proportion of semisynthetic 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. After this time, fifty-four of the remaining animals were randomly allocated to one of nine groups (six animals per group). These animals were transferred to appropriate test diets containing 0·05, 1·2 or 2·4 g cholesterol/kg and 200 g fat/kg which consisted of 500 g triolein/kg and 500 g either trimyristin, tripalmitin or tristearin/kg. These test diets were mixed with rodent chow (80:20 diet:chow) and animals were fed on these mixtures for 28 d. The actual experimental diets fed are termed TM, TP and TS and this refers to the 80:20 mixture of the trimyristin-, tripalmitin- and tristearin-containing test diets and chow respectively. The fatty acid and macronutrient content of each of the diets is shown in Table 1. 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°C 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 were 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°C until further analysis.

Table 1. Macronutrient content and fatty acid composition of experimental diets*

Nutrient	Experimental diet		
	TM	TP	TS
Carbohydrate (g/kg)	288	288	288
Protein(g/kg)	230	230	230
Lipid (g/kg)	174	174	174
Fatty acid composition (g/100 g)†			
14:0	47·1	1·2	1·1
16:0	3·1	47·7	3·9
18:0	1·1	1·1	45·1
16:1	2·4	2·4	2·8
18:1	29·5	29·5	29·5
18:2	7·0	7·0	7·0
18:3	2·0	2·0	2·0

TM, trimyristin; TP, tripalmitin; TS, tristearin.

* Experimental diets enriched in trimyristin, tripalmitin or tristearin consisted of semisynthetic diet–commercial chow (80:20) as outlined on p. 440. Macronutrient values are calculated from the known composition of the semisynthetic diet and the manufacturer's reported values for the commercial chow (Rat and Mouse Breeding Diet 422; Pilsbury, Northampton, Northants, UK).

† Fatty acid composition is calculated from determined values for chow, triolein, trimyristin, tripalmitin, tristearin and linseed oil and includes all fatty acids present in excess of 1 %.

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Separation of lipoproteins

Lipoprotein fractions were separated from plasma (normally 1 ml) by sequential preparative ultracentrifugation in a Beckman Optima TLX benchtop ultracentrifuge (Beckman Instruments (UK) Ltd, High Wycombe, Bucks., UK) as previously described (Bennett *et al.* 1995*a*). VLDL, intermediate-density lipoprotein (IDL) LDL and HDL were separated within density ranges of: $<1{\cdot}006$, 1·006–1·02, 1·02–1·06 and $>1{\cdot}06$ 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 (Olympus Optical Company (UK) Ltd, Southall, Middlesex, UK) and triacylglycerol (GPO Trinder) kits (Roche Diagnostics Ltd) respectively. Lipoprotein lipid concentrations were corrected for recovery as previously described (Salter *et al.* 1998).

Determination of hepatic free and esterified cholesterol and faecal cholesterol

Hepatic free and esterified cholesterol and faecal cholesterol were separated and assayed as described (Bennett *et al.* 1995*a*).

Isolation of hepatic total RNA and determination of mRNA concentrations

Total hepatic RNA was isolated by the guanidinium thiocyanate method essentially according to Chomczynski & Sacchi (1987). The mRNA concentrations for hepatic apolipoprotein B, hydroxymethylglutaryl-CoA (HMG-CoA) reductase, LDL-receptor and microsomal-triacylglycerol-transfer-protein genes were determined by a solution hybridization/RNAse protection assay as previously described (Bennett *et al.* 1995*a,b*). Results were corrected for variation in the mRNA content of total RNA samples by quantitation of polyA RNA using oligo deoxythymidine homopolymer (18 bases) hybridization (Harley, 1987). All mRNA values are expressed as attomol mRNA/μg total RNA normalized to 20 ng polyA/μg total RNA.

Statistical Analysis

All statistical analyses were performed using Genstat 5 for Windows software package (Lawes Agricultural Trust, Rothamstead Experimental Station, Herts., UK). Data were analysed by two-way ANOVA with type of fat (TM, TP and TS) and amount of cholesterol (0·05, 1·2, 2·4 g/kg) as factors. Tables indicate the residual degrees of freedom (df), standard error of the differences of means (SED) and significances (*P*) for type of fat, amount of cholesterol and the interaction between the two. Where appropriate, data were further analysed by regression analysis expressing the given variables against the amount of cholesterol consumed (mg/kg body weight per d).

Results

While all diets were generally well tolerated, five animals (out of a total of fifty-four in the trial) had to be removed before the end due to reduced food intake and resultant loss of body weight. This included one from each of the TM groups and one each from the 1·2 and 2·4 g cholesterol/kg, TP groups. No significant difference was seen in the starting weights in any of the groups (Table 2) and this represented approximately 85 % of the expected final mature body weight of this strain of hamster. No significant difference was seen in the final weights of any of the groups and overall, animals lost about 5 % of their body weight over the period of the study. This suggests that energy intake was lower than would have been expected on a chow-only diet. Food intake was greater in the animals consuming the diets containing TS compared with the other two fats. This was true at each of the dietary cholesterol concentrations and was associated with an increased output of faecal DM.

As a result of the increased food intake in the animals fed TS there was also a higher intake of dietary cholesterol in this group (Table 3). Faecal cholesterol output was also higher in the TS-fed animals but there was no significant difference when this was expressed as a proportion of the cholesterol consumed.

Total plasma cholesterol was influenced by both the type of saturated fat and the amount of cholesterol in the diet with a highly significant interaction between the two (Table 4). Hepatic free cholesterol increased as the amount of cholesterol in the diet increased but was not influenced by the type of fat in the diet. By contrast, there was a strong interaction between the type of fat and amount of cholesterol in the diet in their effects on hepatic cholesterol ester. Regression analysis of the amount of cholesterol consumed by each individual animal and the hepatic cholesterol ester concentration (Fig. 1) shows that as the amount of cholesterol consumed increased, the hepatic cholesterol ester concentration increased linearly for each of the dietary fat groups. However, the rate of increase in the animals fed TM was approximately 50 % greater than in those fed either of the two other fats. If the amount of cholesterol stored in the liver as cholesterol ester is expressed as a proportion of the amount consumed there is a strong interactive effect of amount of dietary cholesterol and type of fat (Table 4). In the animals fed TM this is higher than for the other two fats in the 0·05 and 2·4 g cholesterol/kg diet groups but not the 1·2 g/kg group.

VLDL-triacylglycerol was influenced by both the type of fat and amount of cholesterol in the diet but there was no interaction between the two (Table 5). In general, it increased with increasing amounts of cholesterol in the diet and tended to be lower in the TS-fed animals than in the other groups. This was despite the fact that significantly more cholesterol was consumed by the animals fed TS. In contrast, VLDL-cholesterol showed a significant interactive effect of type of dietary fat and amount of dietary cholesterol. When the relative amounts of the two VLDL lipids are considered it can be seen that as cholesterol concentration in the diet increases, the relative proportion of cholesterol in the VLDL fraction increases. VLDL in the

Table 2. Starting and final body weights, total food intake and faecal output of animals fed diets containing 200 g fat/kg consisting of 100 g triolein/kg and 100 g trimyristin, tripalmitin or tristearin/kg in the presence of different amounts of dietary cholesterol for 28 d*

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

		Amount of cholesterol (g/kg) (C)					ANOVA‡		
	Type of fat (T)	0·05	1·2	2·4	Mean		df	SED	*P*
Starting weight (g)	TM	129·8	132·5	129·1	130·5	T	40	3·24	0·210
	TP	128·6	126·0	134·2	129·6	C		3·24	0·887
	TS	129·4	125·1	120·5	125·0	T×C		5·62	0·320
	Mean	129·3	127·8	128·0	128·4				
Final weight (g)	TM	123·7	127·5	127·3	126·1	T	40	3·97	0·272
	TP	120·2	115·4	131·5	122·4	C		3·97	0·769
	TS	121·8	122·8	114·4	119·7	T×C		6·88	0·138
	Mean	121·9	121·9	124·4	122·7				
Food intake (g)	TM	3·96	4·44	4·50	4·30	T	40	0·246	<0·001
	TP	4·57	3·90	4·36	4·28	C		0·246	0·964
	TS	5·38	5·65	5·25	5·43	T×C		0·427	0·276
	Mean	4·64	4·66	4·70	4·67				
Faecal output (g)§	TM	1·19	1·34	1·22	1·25	T	40	0·082	<0·001
	TP	1·36	1·29	1·50	1·38	C		0·082	0·728
	TS	1·90	1·94	1·93	1·92	T×C		0·142	0·567
	Mean	1·48	1·52	1·55	1·52				

TM, trimyristin; TP, tripalmitin; TS, tristearin.
* For details of composition of diets see Table 1.
† In each of the TM and TP groups consuming 1·2 and 2·4 g cholesterol/kg diet only five animals completed the trial.
‡ The ANOVA table indicates the residual degrees of freedom (df), standard error of the difference (SED) and *P* value for effects of type of saturated fat (T), amount of dietary cholesterol (C) and the interaction between the two (T×C).
§ Expressed as dry matter.

animals fed TS is significantly more cholesterol-rich than that from the animals fed the other two fats.

IDL-cholesterol was highest in the TP-fed animals but was not affected by the amount of cholesterol in the diet (Table 6). A significant interaction was seen between the effects of the type of fat and amount of dietary cholesterol on LDL-cholesterol with the biggest effect of dietary fat type being seen at the highest concentration of dietary cholesterol. When VLDL-, IDL- and LDL-cholesterol are added together, i.e. non-HDL cholesterol, it can be seen that there is a highly significant interaction between type of dietary fat and amount of dietary cholesterol. Fig. 2 shows that in the presence of TP, non-HDL cholesterol shows a larger response to increasing dietary cholesterol than in the presence of the other two fats.

Independent effects of dietary cholesterol and fat on each of the hepatic mRNA measured were seen except for microsomal triacylglycerol transfer protein mRNA which was only affected by dietary cholesterol (Table 7). However, only apolipoprotein B showed an interactive effect between dietary fat and cholesterol. Hepatic LDL receptor mRNA decreased with increasing dietary cholesterol and tended to be lowest in the TP group. A similar pattern was also seen for the HMG-CoA reductase mRNA

Table 3. Cholesterol intake and faecal cholesterol output of animals fed diets containing 200 g fat/kg consisting of 100 g triolein/kg and 100 g trimyristin, tripalmitin or tristearin/kg in the presence of different amounts of dietary cholesterol*

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

		Amount of cholesterol (g/kg) (C)					ANOVA‡		
	Type of fat (T)	0·05	1·2	2·4	Mean		df	SED	*P*
Chol intake (mg/kg per d)	TM	1·8	41·8	84·6	42·7	T	40	2·17	<0·001
	TP	2·0	40·4	79·4	40·6	C		2·17	<0·001
	TS	2·0	55·7	110·0	55·9	T×C		3·76	<0·001
	Mean	1·9	46·0	91·3	46·4				
Faecal chol (mg/kg per d)	TM	10·2	13·6	12·6	12·1	T	40	1·19	<0·001
	TP	14·0	16·3	15·6	15·3	C		1·19	0·031
	TS	15·3	19·3	18·0	17·6	T×C		2·07	0·982
	Mean	13·2	16·4	15·4	15·0				
Chol excreted (%)§	TM	640	33	15	229	T	40	29·6	0·382
	TP	752	41	19	271	C		29·6	<0·001
	TS	698	35	17	250	T×C		51·3	0·594
	Mean	696	36	17	250				

Chol, cholesterol; TM, trimyristin; TP, tripalmitin; TS, tristearin.
* For details of composition of diets see Table 1.
† In each of the TM and TP groups consuming 1·2 and 2·4 g cholesterol/kg diet only five animals completed the trial.
‡ The ANOVA table indicates the residual degrees of freedom (df), standard error of the difference (SED) and *P* value for effects of type of saturated fat (T), amount of dietary cholesterol (C) and the interaction between the two (T×C).
§ Faecal cholesterol expressed as a percentage of daily intake.

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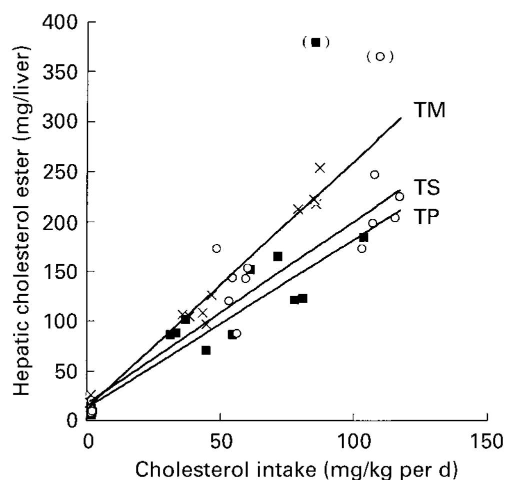

Fig. 1. Linear regression analysis of cholesterol intake *v.* hepatic cholesterol ester in hamsters fed diets containing 200 g fat/kg consisting of 100 g triolein/kg and 100 g trimyristin (TM; (×)) tripalmitin (TP; (■)) or tristearin/kg (TS; (○)) in the presence of different amounts of dietary cholesterol for 28 d. Values are shown for eighteen animals in the TS-fed group, and sixteen in the TM-fed and in the TP-fed groups. For details of the composition of the diets see Table 1. Two out-lyers, indicated in parentheses, were omitted from regression analysis. Data best fit a model in which separate lines were fitted for each fat. Equations for the lines were: TM, $y = 2{\cdot}478x + 12{\cdot}39$; TP, $y = 1{\cdot}694x + 12{\cdot}29$; TS, $y = 1{\cdot}832x + 16{\cdot}69$. Such a model accounted for 92·8 % of the variance and the standard error of observations was estimated to be 21·1. No significant difference was seen between the constants in each of the equations above. The slope of the line for animals fed TM was significantly greater than for those fed TP ($P = 0{\cdot}002$) or TS ($P = 0{\cdot}003$). No significant difference in the slope was seen between the TP and TS groups ($P = 0{\cdot}564$).

concentrations. Microsomal triacylglycerol transfer protein showed significant increases with the amount of cholesterol in the diet.

Discussion

The semisynthetic diets used in this study were designed to meet the nutritional needs of growing hamsters. However, some problems were encountered with the palatability of these diets and it was found necessary to mix the semisynthetic diets with 200 g commercial chow/kg to improve palatability. Even when this mixture was used, five animals failed to adapt to the new diet. In these animals food intake was so low that they lost more than 20 % of their initial body weight and had to be removed from the trial. The remaining animals consumed enough food to maintain their body weight at approximately 95 % of their starting weight. On a normal chow diet we would have expected the animals to gain 10–20 % during this period. Despite this there was no sign of ill health in any of the animals remaining on the trial and no evidence of any essential nutrient deficiency.

Cholesterol intake was calculated by measuring food intake and the fate of over 50 % of this intake was accounted for by measuring hepatic cholesterol and faecal cholesterol output. However, we have not determined the cholesterol content of extra-hepatic tissues nor, perhaps more importantly, the cholesterol converted to bile acids and excreted as such in the faeces. In most species, the latter accounts for an amount of cholesterol approximately equivalent to the faecal excretion of cholesterol itself (Dietschy, 1997).

One complicating factor in the present study is the difference in food intake between the TS and the TM and TP groups. Stearic acid is not absorbed as efficiently as

Table 4. Total plasma cholesterol and hepatic free and esterified cholesterol concentrations in animals fed diets containing 200 g fat/kg consisting of 100 g triolein/kg and 100 g trimyristin, tripalmitin or tristearin/kg in the presence of different amounts of dietary cholesterol*

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

		Amount of cholesterol (g/kg)(C)				ANOVA‡			
	Type of fat (T)	0·05	1·2	2·4	Mean		df	SED	*P*
Total plasma chol (mM)	TM	3·59	4·76	4·72	4·36	T	40	0·188	0·031
	TP	4·22	4·29	5·88	4·80	C		0·188	<0·001
	TS	3·31	4·20	5·50	4·34	T×C		0·326	0·006
	Mean	3·71	4·42	5·37	4·50				
Hepatic free chol (mg/liver)	TM	7·46	10·22	11·86	9·85	T	40	0·609	0·982
	TP	7·33	9·34	13·20	9·96	C		0·609	<0·001
	TS	7·63	10·40	11·58	9·87	T×C		1·055	0·434
	Mean	7·48	9·99	12·21	9·89				
Hepatic esterified chol (mg/liver)	TM	11·8	63·9	151·6	75·8	T	40	7·08	<0·001
	TP	5·6	50·8	87·5	47·9	C		7·08	<0·001
	TS	6·7	80·2	138·6	75·2	T×C		12·27	0·007
	Mean	8·0	65·0	151·6	66·3				
Chol stored (%)§	TM	213·8	43·1	49·9	102·3	T	40	8·43	<0·001
	TP	93·5	41·1	31·5	55·4	C		8·43	<0·001
	TS	89·5	42·7	39·0	57·1	T×C		14·61	<0·001
	Mean	132·8	42·3	40·1	71·6				

Chol, cholesterol; TM, trimystirin; TP, tripalmitin; TS, tristearin.
* For details of composition of diets see Table 1.
† In each of the TM and TP groups consuming 1·2 and 2·4 g cholesterol/kg diet only five animals completed the trial.
‡ The ANOVA table indicates the residual degrees of freedom (df), standard error of the difference (SED) and *P* value for effects of type of saturated fat (T), amount of dietary cholesterol (C) and the interaction between the two (T×C).
§ Amount of cholesterol within hepatic cholesterol ester expressed as a percentage of the total amount of cholesterol consumed over the trial period.

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Table 5. VLDL-cholesterol and -triacylglycerol concentrations in animals fed diets containing 200 g fat/kg consisting of 100 g triolein/kg and 100 g trimyristin, tripalmitin or tristearin/kg in the presence of different amounts of dietary cholesterol*

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

		Amount of cholesterol (g/kg) (C)				ANOVA‡			
	Type of fat (T)	0·05	1·2	2·4	Mean		df	SED	*P*
VLDL-TAG (mM)	TM	0·85	1·18	1·26	1·10	T	40	0·129	<0·001
	TP	0·96	1·01	1·65	1·21	C		0·129	0·003
	TS	0·47	0·61	0·78	0·62	T×C		0·223	0·413
	Mean	0·76	0·94	1·23	0·97				
VLDL-chol (mM)	TM	0·59	0·89	0·87	0·78	T	40	0·080	0·039
	TP	0·61	0·76	1·40	0·92	C		0·080	<0·001
	TS	0·37	0·73	1·05	0·72	T×C		0·138	0·018
	Mean	0·52	0·79	1·11	0·81				
VLDL-TAG-chol§	TM	1·42	1·33	1·43	1·39	T	40	0·082	<0·001
	TP	1·50	1·32	1·18	1·33	C		0·082	0·004
	TS	1·23	0·85	0·72	0·93	T×C		0·143	0·152
	Mean	1·39	1·17	1·11	1·22				

TAG, triacylglycerol; TM, trimyristin; TP, tripalmitin; TS, tristearin; chol, cholesterol.
* For details of composition of diets see Table 1.
† In each of the TM and TP groups consuming 1·2 and 2·4 g cholesterol/kg diet only five animals completed the trial.
‡ The ANOVA table indicates the residual degrees of freedom (df), standard error of the difference (SED) and *P* value for effects of type of saturated fat (T), amount of dietary cholesterol (C) and the interaction between the two (T × C).
§ VLDL-triacylglycerol divided by VLDL-cholesterol.

Table 6. Intermediate-density lipoprotein, LDL, total non-HDL cholesterol and HDL concentrations in animals fed diets containing 200 g fat/kg consisting of 100 g triolein/kg and 100 g trimyristin, tripalmitin or tristearin/kg in the presence of different amounts of dietary cholesterol*

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

		Amount of cholesterol (g/kg) (C)				ANOVA‡			
	Type of fat (T)	0·05	1·2	2·4	Mean		df	SED	*P*
IDL-chol (mM)	TM	0·12	0·28	0·12	0·17	T	40	0·045	0·008
	TP	0·26	0·22	0·39	0·29	C		0·045	0·169
	TS	0·10	0·14	0·23	0·16	T×C		0·078	0·062
	Mean	0·16	0·21	0·25	0·21				
LDL-chol (mM)	TM	0·88	1·15	0·99	1·01	T	40	0·067	0·002
	TP	0·99	0·98	1·37	1·11	C		0·067	0·009
	TS	0·85	0·74	0·98	0·85	T×C		0·116	0·016
	Mean	0·90	0·96	1·11	0·99				
Total non-HDL-chol (mM)§	TM	1·59	2·32	1·99	1·96	T	40	0·118	<0·001
	TP	1·85	1·96	3·16	2·32	C		0·118	<0·001
	TS	1·32	1·61	2·25	1·73	T×C		0·203	<0·001
	Mean	1·59	1·96	2·47	2·00				
HDL-chol (mM)	TM	1·87	2·20	2·54	2·23	T	40	0·113	0·390
	TP	2·17	2·13	2·39	2·23	C		0·113	<0·001
	TS	1·85	2·31	2·94	2·36	T×C		0·195	0·043
	Mean	1·96	2·24	2·62	2·27				

IDL, intermediate-density lipoprotein; chol, cholesterol; TM, trimyristin; TP, tripalmitin; TS, tristearin.
* For details of composition of diets see Table 1.
† In each of the TM and TP groups consuming 1·2 and 2·4 g cholesterol/kg diet only five animals completed the trial.
‡ The ANOVA table indicates the residual degrees of freedom (df), standard error of the difference (SED) and *P* value for effects of type of saturated fat (T), amount of dietary cholesterol (C) and the interaction between the two (T × C).
§ Sum of VLDL-, intermediate-density lipoprotein- and LDL-cholesterol.

other shorter-chain saturated fatty acids (Mattson, 1959) which may explain the excretion of a greater DM of faeces. Animals in the TS group appear to have responded to this loss of energy by increasing their food intake. Thus, by the combination of increased food intake and increased faecal loss, their body weights have remained similar to those fed the other fats. However, they have consequently consumed more cholesterol than animals fed TM or TP (e.g. for the 2·4 g cholesterol/kg diet, animals in the TS group consumed approximately 2 mg/d (20 %) more than the other groups). This also affects other variables: for example, the daily faecal cholesterol output is higher in the TS-fed animals. However, when expressed as a proportion of that consumed, it is equivalent to the other groups. Hepatic cholesterol ester content is also affected and is probably best analysed by looking at the cholesterol intakes of the individual animals (Fig. 1).

Cholesterol feeding is known to elicit a number of regulatory effects. It down-regulates endogenous cholesterol synthesis, down-regulates the expression of LDL receptors in the liver and increases the amount of cholesterol stored as cholesterol ester (Spady *et al.* 1993). All three of these effects can be seen in the present study. The reduction in hepatic HMG-CoA reductase and LDL

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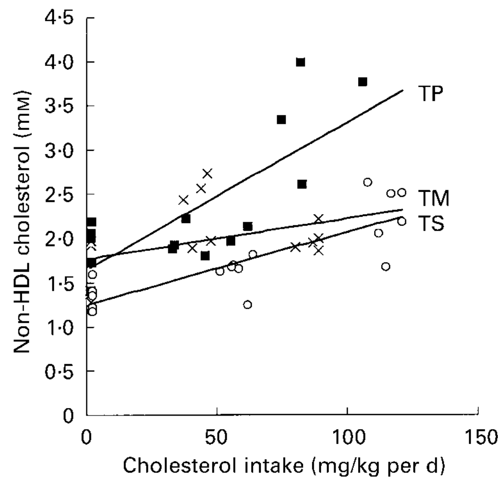

Fig. 2. Linear regression analysis of cholesterol intake *v.* non-HDL (VLDL+intermediate-density lipoprotein+LDL) cholesterol in animals fed diets containing 200 g fat/kg consisting of 100 g triolein/kg and 100 g trimyristin (TM; (×)) tripalmitin (TP; (■)) or tristearin/kg (TS; (○)) in the presence of different amounts of dietary cholesterol for 28 d. Values are shown for eighteen animals in the TS-fed group, and sixteen in the TM-fed and in the TP-fed groups. For details of the composition of the diets see Table 1. Data best fit a model in which separate lines were fitted for each fat. Equations for the curves were as follows: TM, $y = 0{\cdot}0047x + 1{\cdot}764$; TP, $y = 0{\cdot}0170x + 1{\cdot}647$; TS, $y = 0{\cdot}0086x + 1{\cdot}245$; Such a model accounted for 58·3 % of the variance and the standard error of observations was estimated to be 0·387. No significant difference was seen between the constants in the equations for TP and TM ($P = 0{\cdot}593$) or TS ($P = 0{\cdot}06$). However the constants for TM and TS were significantly different ($P = 0{\cdot}022$). The slope of the line for animals fed TP was significantly greater than for those fed TM ($P = 0{\cdot}005$) or TS ($P = 0{\cdot}024$). No significant difference in the slope was seen between the TM and TS groups ($P = 0{\cdot}280$).

receptor mRNA is likely to result in the down-regulation of cholesterol synthesis (Ness *et al.* 1994) and LDL-receptor activity (Horton *et al.* 1993) respectively. As we have shown previously (Bennett *et al.* 1995*a*; Salter *et al.* 1998), at the lowest dietary cholesterol concentration the levels of these mRNA are lowest in animals fed TP and highest in those fed TS. As the cholesterol content of the diet is increased, the levels of both mRNA species decrease in each of the dietary groups (Table 7), although the fall in LDL-receptor mRNA is substantially greater than that of HMG-CoA reductase mRNA (29 % *v.* 18 %). Since the activity of HMG-CoA reductase is likely to have decreased to a very low level (Spady & Dietschy, 1988) in the high-dietary-cholesterol groups, this may suggest regulation of cholesterol synthesis by other mechanisms in addition to changes in the steady state HMG-CoA reductase mRNA concentration. We also see a marked increase in cholesterol ester storage in each of the dietary fat groups with increasing dietary cholesterol (Table 4), which is greatest, as a proportion of the cholesterol consumed, in the TM group. It is clear that deposition as hepatic cholesterol ester is the fate of a large proportion of the cholesterol consumed, although the amount of cholesterol varies significantly between diets (Table 4). The basis for this differential effect of the individual saturated fatty acids is unclear. Dietary oleic acid promotes the storage of hepatic cholesterol ester (Sessions & Salter, 1994; Bennett *et al.* 1995*a*; Salter *et al.* 1998) while saturated fatty acids reduce cholesterol ester storage. Since the diets used in the present study contained significant amounts of oleic acid (29·5 g/100 g total fatty acids), there will be opposing effects by the oleic and saturated fatty acids in the diet on cholesterol esterification. It seems likely that myristic acid is less potent at inhibiting esterification than either palmitic or stearic acid and thus it is the effect of the oleic acid in the diet which predominates. Whether this effect is related to the relative affinity of the saturated fatty acids as substrates for esterification by acyl CoA:cholesterol acyl transferase or to a less direct effect remains to be established.

The difference in the amount of cholesterol stored appears to be the major factor in determining the differential effects of the TM and TP diets. Thus, at 1·2 g cholesterol/kg diet, when the difference in amount of cholesterol stored is relatively small, there is little difference between the TM and TP group in the cholesterol content of ‘non-HDL’ or HDL. However, in the 2·4 g cholesterol/kg diet groups, when an average of 64 mg more cholesterol ester is stored in the liver of the TM-fed animals, ‘non-HDL’ cholesterol is much higher in the TP than TM group. This effect is seen in each of the VLDL, IDL and LDL fractions (Tables 5 and 6). Thus, at high cholesterol intakes the differential hepatic cholesterol esterification results in palmitic acid becoming much more hypercholesterolaemic than myristic acid, in partial agreement with the work of Hayes and co-workers (Hayes & Khosla, 1992; Hayes *et al.* 1995).

The effects of dietary stearic acid also appear to be dependent on the cholesterol content of the diet. As we have shown previously (Bennett *et al.* 1995*a*; Salter *et al.* 1998), plasma cholesterol concentrations are lower in animals fed a TS rich, low cholesterol diet than in corresponding TM or TP groups with the most marked effect being in the VLDL fraction where cholesterol, and particularly triacylglycerol, concentrations are reduced compared with animals fed the other two fats (Table 5). As cholesterol is added to the diet at 2·4 g/kg, plasma cholesterol rises to a similar level as in the TP groups but the distribution between the fractions is somewhat different, namely ‘non-HDL’-cholesterol is lower but HDL-cholesterol higher than in the TP group (Table 6). Further, the relative enrichment of the VLDL fraction in cholesterol also increases, consistent with an inhibition of VLDL-triacylglycerol formation due to partitioning of stearic acid into phospholipid rather than triacylglycerol (Bruce & Salter, 1996). These results are in disagreement with those of Hassel *et al.* (1997) who used diets enriched in different saturated fatty acids derived from natural fat sources and found no difference in the VLDL- or HDL-cholesterol concentrations at 0·05 g cholesterol/kg diet. However, the relative enrichment of the diets with the individual fatty acids was not as great as in the present study with the stearic acid-rich diet containing almost equal amounts of palmitic acid.

Saturated fatty acids and cholesterol show clear interactive effects on total plasma cholesterol, VLDL-, IDL- and LDL-cholesterol, implying common mechanisms for their effects. Besides events acting through VLDL secretion,

Table 7. Hepatic apolipoprotein B, microsomal triacylglycerol transfer protein, LDL receptor and hydroxymethylglutaryl CoA reductase mRNA concentrations in animals fed diets containing 200 g fat/kg consisting of 100 g triolein/kg and 100 g trimyristin/kg, tripalmitin or tristearin/kg in the presence of different amounts of dietary cholesterol*†

(Mean values for *n* hamsters per group and combined means for each type of fat and amount of cholesterol‡)

		Amount of cholesterol (g/kg) (C)					ANOVA§		
	Type of fat (T)	0·05	1·2	2·4	Mean		df	SED	*P*
n	TM	5	4	5					
	TP	6	4	5					
	TS	5	5	5					
ApoB	TM	79·9	110·9	84·4	91·7	T	36	6·69	0·043
	TP	112·3	107·0	102·6	107·5	C		6·69	0·009
	TS	79·5	120·4	119·1	106·3	T×C		11·59	0·015
	Mean	90·7	112·8	102·0	101·8				
MTP	TM	3·14	3·54	3·86	3·52	T	38	0·160	0·222
	TP	2·81	3·07	3·83	3·24	C		0·160	<0·001
	TS	2·96	3·63	3·68	3·42	T×C		0·277	0·461
	Mean	2·97	3·41	3·79	3·39				
LDLr	TM	2·53	1·80	1·28	1·87	T	38	0·128	<0·001
	TP	1·98	1·54	1·17	1·56	C		0·128	<0·001
	TS	3·67	3·02	2·61	3·10	T×C		0·221	0·712
	Mean	2·73	2·12	1·69	2·18				
HMG-CoA	TM	1·15	1·17	0·98	1·10	T	38	0·058	<0·001
	TP	0·96	0·73	0·73	0·81	C		0·058	0·004
	TS	1·36	1·17	1·15	1·22	T×C		0·101	0·448
	Mean	1·16	1·02	0·95	1·04				

TM, trimyristin; TP, tripalmitin; TS, tristearin; ApoB, apolipoprotein B; MTP, microsomal triacylglycerol transfer protein; LDLr, LDL receptor; HMG, hydroxymethylglutaryl.

* For details of composition of diets see Table 1.

† Results are expressed in attomol/μg RNA normalized to 20 ng polyA/μg RNA.

‡ Data points are missing where animals are removed from the trial or RNA was found to be degraded.

§ The ANOVA table indicates the residual degrees of freedom (df), standard error of the difference (SED) and *P* value for effects of type of saturated fat (T), amount of dietary cholesterol (C) and the interaction between the two (T × C).

these could include effects on LDL-receptor and cholesterol synthesis. Dietschy and co-workers suggest that dietary fatty acids modulate the effect of dietary cholesterol by changing the balance between cholesterol in the hepatic cholesterol ester pool and that in a putative regulatory pool (Spady *et al.* 1993). This in turn may regulate the expression of the LDL-receptor and other cholesterol-sensitive genes, thereby influencing lipoprotein metabolism. However, whereas saturated fatty acids and cholesterol show highly significant separate effects on LDL-receptor and HMG-CoA reductase mRNA levels there is no evidence of interaction between fats and cholesterol in their effects on these variables (Table 7). This is consistent with fat and cholesterol acting on the level of each mRNA by independent mechanisms rather than through a common regulatory cholesterol pool.

HDL-cholesterol was also affected by both the cholesterol content and the type of saturated fatty acid present in the diet. There was a strong interactive effect of dietary cholesterol content and type of saturated fat in the diet on HDL-cholesterol. Plasma HDL-cholesterol concentrations may be regulated by the rate of HDL production, the transfer of cholesterol to the HDL fraction, from either extra-hepatic tissues or other lipoproteins, or the rate of removal of HDL via specific receptors (scavenger receptor-B1) in the liver. However, hepatic expression of scavenger receptor-B1 and the flux of HDL-cholesterol to the liver are not affected by increasing the cholesterol and saturated fatty acid content of the diet (Wollett *et al.* 1997). Thus, the differences are likely to be as a result of an increased flux of cholesterol into the HDL fraction. By contrast, polyunsaturated fatty acids have been shown to cause increased expression of hepatic scavenger receptor-B1 (Spady *et al.* 1999). As considerably more cholesterol was associated with the HDL fraction (approximately 50 % of the total) in these hamsters than would be expected in human subjects (typically 20 %) it is important to note that changes in HDL metabolism may have an impact on cholesterol metabolism as a whole and the metabolism of the less dense lipoproteins.

In conclusion, our data suggest that there is an interactive effect between different dietary saturated fatty acids and dietary cholesterol in their effects on lipoprotein metabolism. The major site of this interaction appears to be at the level of storage of hepatic cholesterol ester and is only seen at relatively high dietary cholesterol intakes. In the presence of significant amounts of dietary oleic acid, myristic acid tends to favour the esterification of cholesterol compared with palmitic or stearic acids. The magnitude of this effect is so great that it has a major impact on cholesterol balance in the animals and significantly alters the metabolic fates of the cholesterol. These effects only appear to be significant at cholesterol intakes above the endogenous synthetic capacity.

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References

Bennett AJ, Billett MA, Salter AM, Mangiapane EH, Bruce JS, Anderton KL, Marenah CB, Lawson N & White DA (1995*a*) Modulation of hepatic apolipoprotein B, 3-hydroxy-3-methyl-glutaryl-CoA reductase and low density lipoprotein receptor mRNA and plasma lipoprotein concentrations by defined dietary fats. *Biochemical Journal* **311**, 167–173.

Bennett AJ, Billett MA, Salter AM & White DA (1995*b*) Regulation of hamster hepatic microsomal triglyceride transfer protein mRNA levels by dietary fat. *Biochemical and Biophysical Research Communications* **212**, 473–478.

Bruce JS & Salter AM (1996) Metabolic fate of oleic acid, palmitic acid and stearic acid in cultured hamster hepatocytes. *Biochemical Journal* **316**, 847–852.

Chomczynski P & Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. *Analytical Biochemistry* **62**, 156–159.

Dietschy JM (1997) Theoretical considerations of what regulates low-density-lipoprotein and high-density-lipoprotein cholesterol. *American Journal of Clinical Nutrition* **65,** Suppl., 1581S–1589S.

Harley CB (1987) Hybridisation of oligo(dT) to RNA on nitrocellulose. *Gene Analysis Techniques* **4**, 17–22.

Hassel CA, Mensing EA & Gallaher DD (1997) Dietary stearic acid reduces plasma and hepatic cholesterol concentrations without increasing bile acid excretion in cholesterol-fed hamsters. *Journal of Nutrition* **127**, 1148–1155.

Hayes KC & Khosla P (1992) Dietary fatty acid thresholds and cholesterolemia. *FASEB Journal* **6**, 2600–2607.

Hayes KC, Pronczuk A & Khosla P (1995) A rationale for plasma cholesterol modulation by dietary fatty acids: modelling the human response in animals. *Journal of Nutritional Biochemistry* **6**, 188–194.

Hegsted DM (1986) Serum-cholesterol response to dietary cholesterol: a re-evaluation. *American Journal of Clinical Nutrition* **44**, 299–305.

Horton JD, Cuthbert JA & Spady DK (1993) Dietary fatty acids regulate hepatic low density lipoprotein (LDL) transport by altering LDL receptor protein and mRNA levels. *Journal of Clinical Investigation* **92**, 743–749.

Kris-Etherton PM & Dietschy J (1997) Design criteria for studies examining individual fatty acid effects on cardiovascular disease risk factors: human and animal studies. *American Journal of Clinical Nutrition* **65,** Suppl., 1590S–1596S.

Mattson FH (1959) The absorbability of stearic acid when fed as a simple or mixed triglyceride. *Journal of Nutrition* **69**, 338–342.

Mensink RP & Katan MB (1992) Effect of dietary fatty acids on serum lipids and lipoproteins. A meta-analysis of 27 trials. *Arteriosclerosis and Thrombosis* **12**, 911–919.

Ness GC, Eales S, Lopez D & Zhao Z (1994) Regulation of 3-hydroxy-3-methyl-glutaryl-CoA reductase gene expression by sterols and nonsterols in rat liver. *Archives of Biochemistry and Biophysics* **308**, 420–425.

Salter AM, Mangiapane EH, Bennett AJ, Bruce JS, Billett MA, Anderton KL, Marenah CB, Lawson N & White DA (1998) The effect of different dietary fatty acids on lipoprotein metabolism: concentration-dependent effects of diets enriched in oleic, myristic, palmitic and stearic acids. *British Journal of Nutrition* **79**, 195–202.

Salter AM & White DA (1996) Effects of dietary fat on cholesterol metabolism: regulation of plasma LDL concentrations. *Nutrition Research Reviews* **9**, 241–257.

Sessions VA & Salter AM (1994) The effects of different dietary fats and cholesterol on serum lipoprotein concentrations in hamsters. *Biochimica et Biophysica Acta* **1211**, 207–214.

Spady DK & Dietschy JM (1988) Interaction of dietary cholesterol and triglycerides in the regulation of hepatic low density lipoprotein transport in the hamster. *Journal of Clinical Investigation* **81**, 300–309.

Spady DK, Kearney DM & Hobbs HH (1999) Polyunsaturated fatty acids upregulate hepatic scavenger receptor B1 (SR-B1) expression and HDL cholesteryl ester uptake in the hamster. *Journal of Lipid Research* **40**, 1384–1394.

Spady DK, Woollett LA & Dietschy JM (1993) Regulation of plasma LDL-cholesterol levels by dietary cholesterol and fatty acids. *Annual Review of Nutrition* **13**, 355–381.

Tholstrup T, Marckmann P, Jespersen J, Vessby B, Jart A & Sandström B (1994) Effect on blood lipids, coagulation, and fibrinolysis of a fat high in myristic acid and a fat high in palmitic acid. *American Journal of Clinical Nutrition* **60**, 919–925.

Woollett LA, Kearney DM & Spady DK (1997) Diet modification alters plasma HDL cholesterol concentrations but not the transport of HDL cholestryl esters to the liver in the hamster. *Journal of Lipid Research* **38**, 2289–2302.

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