Decreasing dietary fat saturation lowers HDL-cholesterol and increases hepatic HDL binding in hamsters

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In order to study the mechanism by which increasing unsaturation of dietary fat lowers HDL-cholesterol levels, we studied various measures of HDL metabolism in hamsters fed with fats with different degrees of saturation. Hamsters were fed on a cholesterol-enriched (1 g/kg) semipurified diet containing 200 g/kg of maize oil, olive oil, or palm oil for 9 weeks. Increasing saturation of dietary fat resulted in increasing concentrations of total plasma cholesterol (4·29 (SD 0·51), 5·30 (SD 0·67) and 5·58 (SD 0·76) mmol/l respectively, n 12) and HDL-cholesterol (3·31 (SD 0·50), 3·91 (SD 0·12) and 3·97 (SD 0·43) mmol/l) and these concentrations were significantly higher ($P < 0.05$) in the palm-oil and olive-oil-fed hamsters compared with the maize-oil group. Total plasma triacylglycerol levels also increased with increasing fat saturation (1·01 (SD 0·59), 1·56 (SD 0·65) and 2·75 (SD 1·03) mmol/l) and were significantly higher ($P < 0.05$) in the palm-oil group compared with the olive-oil and maize-oil-fed hamsters. The three diets did not have differential effects on plasma activity levels of lecithin: cholesterol acyltransferase (LCAT) and cholesteryl ester transfer protein (CETP). Levels of phospholipid transfer protein (PLTP) tended to be higher with increasing fat saturation but this effect was not significant. The capacity of liver membranes to bind human HDL_3 was significantly higher ($P < 0.05$) in the hamsters fed with maize oil (810 (SD 100) ng HDL_3 protein/mg membrane protein, n 4) compared with those fed on palm oil (655 (SD 56) ng/mg), whereas the olive-oil group had intermediate values (674 (SD 26) ng/mg). The affinity of HDL_3 for the binding sites was not affected by the type of dietary fat. Hepatic lipase (*EC* 3.1.1.3) activity, measured in liver homogenates, increased with increasing fat saturation. We conclude that dietary maize oil, when compared with either olive oil or palm oil, may lower HDL-cholesterol concentrations by enhancing HDL binding to liver membranes.

Dietary fat: Lipoproteins: HDL binding: Hamsters

There is considerable evidence that the type of dietary fat can affect plasma cholesterol concentrations and lipoprotein metabolism. Intervention studies (McNamara, 1992; Khosla & Sundram, 1996) have indicated that high levels of saturated fatty acids in the diet are associated with increased plasma cholesterol concentrations. Replacement of saturated fat by unsaturated fatty acids, on the other hand, results in a lowering of plasma cholesterol concentrations. This plasma cholesterol lowering effect is associated with a decrease in both LDL- and HDL-cholesterol fractions (Khosla & Sundram, 1996).

Many studies have been carried out to unravel the mechanism by which dietary fatty acids can affect plasma cholesterol concentrations and lipoprotein metabolism. Frequently, animal models have been used (Khosla & Sundram, 1996), and recently, the hamster has become a popular

model to study the effects of drugs and diet on lipoprotein metabolism (Dietschy *et al.* 1993). The hamster has a lipoprotein profile that resembles more closely that of man than those of rats and mice (Bravo *et al.* 1994). In addition, the hamster, like man, has plasma cholesteryl ester transfer protein (CETP) activity (Stein *et al.* 1990). In contrast, other animal models like the pig, mouse, and rat have virtually no plasma CETP activity. Furthermore, changes in plasma lipid concentrations can be easily induced in hamsters with only small, realistic amounts of dietary cholesterol (Dietschy *et al.* 1993).

The effects of dietary fat saturation on LDL metabolism have been extensively studied in the hamster. Dietschy *et al.* (1993) reported that substitution of unsaturated for saturated fatty acids results in increased LDL-receptor activity and in a decreased LDL transport rate, which is associated with

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decreased plasma LDL-cholesterol concentrations. Recent studies in hamsters by others (Sessions & Salter, 1995) have also provided evidence of increased LDL-receptor activity due to feeding unsaturated instead of saturated fatty acids.

Limited information, however, is available on the effects of dietary fat saturation on HDL metabolism in the hamster, while the available results are not consistent. Ahn *et al.* (1994) reported that soyabean oil compared with coconut oil, given for a period of 18 weeks, resulted in reduced expression of apolipoprotein A-II which was associated with lower HDL-cholesterol concentrations and a larger proportion of small HDL particles. Similar results were reported by Trautwein *et al.* (1997). They blended different fats and obtained six diets with very different fatty acid profiles to which 4 g cholesterol/kg was added. A lowering of plasma cholesterol levels due to decreasing fat saturation was found to be associated with a lowering of HDL-cholesterol levels. Studies by Kurushima *et al.* (1995), on the other hand, indicated that replacement of dietary palmitic acid by oleic acid lowered plasma cholesterol levels but did not affect HDL-cholesterol concentrations.

The objective of the present study was to study further the mechanism by which increasing fat saturation lowers plasma, and particularly HDL, cholesterol concentrations. We fed hamsters on semipurified diets containing palm oil, olive oil, or maize oil and studied various measures of HDL metabolism, such as hepatic lipase (*EC* 3.1.1.3) activity, CETP activity, phospholipid transfer protein (PLTP) activity, and lecithin:cholesterol acyltransferase (LCAT) activity, as well as the capacity of liver membranes to bind HDL. It was anticipated that these measures would provide clues as to how dietary fatty acids influence HDL-cholesterol concentrations.

Materials and methods

Animals and diets

Male Golden Syrian hamsters (*Mesocritus auratus*; HsdCpb:ShGa) of 5 weeks of age were obtained from Harlan CPB, Zeist, The Netherlands, and were fed on a commercial rodent diet (RMH-B®, Hope Farms, Woerden, The Netherlands). After an adaptation period of 2 weeks, blood samples were taken for lipid measurements as described later and the hamsters were divided into three groups so that group means and distributions of total plasma cholesterol concentrations, HDL-cholesterol concentrations, and body weights were similar. Subsequently, the three groups were fed on pelleted semipurified diets (Table 1) containing either palm oil, olive oil or maize oil. The diets were given for a period of 9 weeks. Food and water were provided *ad libitum*. The hamsters were housed in groups of three or four animals in polycarbonate cages with a bedding of wood shavings. The animal room was temperature-controlled (20°) and had a 12 h light–dark cycle (light on 06.00–18.00 hours). The experimental protocol was approved by the Animal Experiments Committee of the Faculty of Veterinary Medicine, Utrecht University, The Netherlands.

Table 1. Composition of the semipurified diets*

Ingredient	Content	
	g/kg	% energy
Casein	200	17·28
Total fat	200	38·88
Maize oil	10	1·94
Maize/olive/palm oil	190	36·94
Total carbohydrates	488·15	42·16
Maize starch	244·15	21·08
Dextrose	244	21·08
Cellulose	50	
$CaCO_3$	12·4	
$NaH_2PO_4 . 2H_2O$	15·1	
$MgCO_3$	1·4	
KCl	1·0	
$KHCO_3$	7·7	
Inositol	1·25	
Mineral premix†	10	0·83
Vitamin premix‡	12	0·85
Cholesterol	1·0	
Total	1000·0	100

* The diets contained 19 370 kJ metabolizable energy/kg. The following values were used for the calculation of the energy density of the various components: protein and carbohydrates, 16·74 kJ/g; fat, 37·66 kJ/g. The diets contained 52 mg cholesterol/MJ. Maize oil contained (g/100 g total fatty acids): 13 saturated, 25 monounsaturated, and 62 polyunsaturated fatty acids; olive oil contained 14 saturated, 77 monounsaturated, and 9 polyunsaturated fatty acids; palm oil contained 51 saturated, 39 monounsaturated, and 10 polyunsaturated fatty acids. The calculated polyunsaturated : saturated fatty acid ratios of the maize-, olive- and palm-oil diets were 4·77, 0·84, and 0·26 respectively.
† Composition (mg/kg food): $FeSO_4 . 7H_2O$ 174, (Fe 35), MnO_2 79, (Mn 50), $ZnSO_4 . H_2O$ 33, (Zn 12), $NiSO_4 . 6H_2O$ 13, (Ni 3), NaF 2, (F 1), KI 0·2, (I 0·15), $CuSO_4 . 5H_2O$ 15·7, (Cu 4), $Na_2SeO_3 . 5H_2O$ 0·3, (Se 0·10), $CrCl_3 . 6H_2O$ 1·5, (Cr 0·30), $SnCl_2 . 2H_2O$ 1·9, (Sn 1), NH_4VO_3 0·2, (V 0·1), maize meal (carrier material) 9679·2.
‡ Composition (mg/kg food): thiamin hydrochloride 4, riboflavin 3, nicotinamide 20, calcium pantothenate (45 %) 17·8, pyridoxine hydrochloride 6, cyanocobalamin (purity 0·1 %) 50, choline chloride (purity 50 %) 2000, pteroylmonoglutamic acid 1, biotin 2, menadione 0·05, all-*rac*-α-tocopheryl acetate (purity 50 %) 60, retinyl acetate (150 μg/mg) 8, cholecalciferol (12·5 μg/mg) 2, maize meal (carrier material) 9826·15.

Analytical methods

Blood was collected into heparinized tubes from the retro-orbital sinus of the hamsters while they were under light diethyl ether anaesthesia. Food was always removed at 17.00 hours and any food in the cheek pouches was also removed. Blood samples were taken the next day between 09.00 and 11.00 hours. This procedure of blood sampling was used for all the time points that blood was collected. The hamsters were anaesthetized at the end of the experiment with a mixture of ketamine (140 mg/kg body weight), xylazine (24 mg/kg), and atropine (0·4 mg/kg) which was administered intraperitoneally. The hamsters were then exsanguinated from the abdominal aorta and the livers were removed.

Plasma cholesterol (Allain *et al.* 1974) and triacylglycerol (Bucolo & David, 1973) concentrations were measured enzymically. The plasma non-HDL fraction was precipitated with phosphotungstic acid–$MgCl_2$ (Sigma Diagnostics, St Louis, MO, USA; catalogue no. 352-4) according to Weingand & Daggy (1990), and the supernatant (HDL) fraction was assayed for cholesterol. The concentration of cholesterol in the non-HDL fraction was calculated as the difference between whole plasma and HDL-cholesterol.

Lecithin: cholesterol acyltransferase activity

Plasma LCAT activity levels were determined with excess exogenous substrate composed of heat-inactivated human plasma containing [^{3}H]cholesterol as described previously (Dullaart et al. 1994). The measured activities are an estimate of LCAT mass and are independent of the endogenous lipoproteins present in each plasma sample. The activities are expressed in μmol cholesteryl ester formed/l plasma per h. A reference human plasma sample was included in each analysis.

Cholesteryl ester transfer protein activity

CETP activity in plasma was measured, using exogenous substrates, by transfer of radiolabelled cholesteryl [1-^{14}C]oleate (Amersham International, Little Chalfont, Bucks, UK; specific activity 1·85 GBq/mmol) between exogenous LDL and HDL as described previously (Groener et al. 1986). Briefly, the non-HDL fraction from plasma samples was precipitated with phosphotungstic acid–MgCl$_2$. The supernatant fraction (60 μl) was mixed with 500 nmol radiolabelled human LDL-cholesterol and 200 nmol human HDL-cholesterol in a total volume of 700 μl. 5.5-Dithiobis(2-nitrobenzoic acid) was added to inhibit the LCAT activity. The mixture was incubated for 16 h at 37°. The incubation was stopped by cooling on ice, and LDL was precipitated with 300 μl 0·1 M-sodium phosphate (pH 7·4) and 167 μl 0·1 M-MnCl$_2$ solution. The samples were centrifuged and the supernatant fraction (containing the HDL) was assayed for radioactivity. The CETP activity measured by this method is linear in time for at least 16 h and is also linearly related to the amount of supernatant fraction added. The activity is expressed as μmol cholesteryl ester transferred/l plasma per h. A reference human plasma sample was included in each analysis.

Phospholipid transfer protein activity

PLTP activity was measured in a phospholipid vesicles–HDL assay system as described previously (Speijer et al. 1991). In short, radiolabelled vesicles were prepared by mixing 10 μmol egg phosphatidylcholine with 37 kBq [^{3}H]phosphatidylcholine (L-3-phosphatidyl[N-methyl-^{3}H]-choline,1,2-dipalmitoyl; Amersham International; specific activity 3·00 TBq/mmol) and 0·1 μmol butylated hydroxytoluene. The lipids were dried under N$_2$, and 1 ml 150 mM-NaCl containing 10 mmol Tris-HCl/l and 1 mmol EDTA (pH 7·4)/l was added. The lipids were dispersed by sonicating three times for 5 min on ice with a probe-type sonifer. Plasma samples of 1 μl were incubated with radiolabelled vesicles (125 nmol phosphatidylcholine) and human HDL (160 nmol phosphatidylcholine) for 45 min at 37° in a total volume of 400 μl of 150 mM-NaCl, 10 mM-Tris-HCl, and 1 mM-EDTA (pH 7·4). The reaction was stopped on ice. A solution containing 500 mM-NaCl, 215 mM-MnCl$_2$ and 140 units heparin (300 μl) was added and mixed. This yielded final concentrations of 300 mM-NaCl, 92 mM-MnCl$_2$, and 200 units heparin/ml. The resulting precipitate was removed by centrifugation and the supernatant fraction assayed for radioactivity. The activity is expressed in mmol phosphatidylcholine transferred to HDL/l plasma per h. A human reference plasma sample was included in each analysis. This assay is specific for PLTP activity, since CETP, which does promote exchange of phospholipids between lipoproteins, shows no activity in this assay (Speijer et al. 1991).

Binding of human HDL to liver membranes

This assay measures the level of saturable, high-affinity binding sites for HDL on hepatic membranes and is comparable to the tests used by Brown & Goldstein (1986) for the assay of saturable, high-affinity LDL binding. Liver plasma membranes were prepared from the livers of four hamsters randomly selected from each dietary group. Hamster livers were immediately put on ice and stored at −70°. The preparation of liver membranes and the HDL binding studies were done as described for pig liver membranes (De Crom et al. 1989). Livers were minced on ice using surgical blades and homogenized in 0·25 M-sucrose, 1 mM-EDTA, 1 mM-benzamidine, 1 mM-phenylmethylsulfonylfluoride and 10 mM-Tris-HCl, pH 8·0 using an Ultra Turrax TP 18-10 (Janke and Kunkel KG, Staufen, Germany). Tissue debris was removed by centrifugation in a Beckman TJ-6 centrifuge (4°, 5 min, 2000 rev./min). The supernatant fraction was layered on a 410 g/l sucrose solution and centrifuged in a Beckman SW 28 rotor (Beckman, Palo Alto, CA, USA) in a Beckman ultracentrifuge (4°, 60 min, 24 000 rev./min). The white interfacial band of membranes was collected and diluted by adding an excess of homogenization buffer. Subsequently, the membranes were pelleted by centrifugation (4°, 60 min, 24 000 rev./min). The membranes were washed and resuspended in a minimal volume of homogenization buffer and stored at −70°. The final concentration of the liver membrane preparation was set at 2·2 g protein/l.

In the HDL binding studies, we wanted to exclude any effect of the composition of the HDL particles. Therefore, we used human HDL$_3$, since human HDL$_3$ is a homogeneous, well-defined HDL fraction which can be easily prepared and stays stable for a long period of time. In addition, the plasma HDL$_3$ concentration is usually higher than that of HDL$_2$ which facilitates the preparation of larger quantities. Blood from one person was collected into tubes containing EDTA. HDL$_3$ from fresh plasma was isolated and labelled with ^{125}I exactly as described by De Crom et al. (1989). HDL binding studies with liver membranes were performed in 50 mM-Tris-HCl buffer, pH 8·0, containing 50 mM-NaCl and 20 g/l bovine serum albumin (binding assay buffer) in a final volume of 0·5 ml as described by De Crom et al. (1989). Liver membrane protein (100 μg) was incubated with ^{125}I-labelled human HDL$_3$ for 1 h at 37° in Eppendorf tubes (Eppendorf, Hamburg, Germany). We elected to use an incubation temperature of 37° since the binding capacity is considerably lower at lower temperatures (Fernandez & McNamara, 1991) and we also considered that this temperature reflected better the in vivo situation. The reaction was terminated by adding 0·4 ml cold binding assay buffer and centrifuging for 5 min in an Eppendorf 5414 centrifuge. Membrane pellets were resuspended in 0·4 ml binding assay buffer, and layered over 0·4 ml fetal calf serum and recentrifuged. The amount of radioactivity in the pellets was

determined in an LKB Wallac Ultrogamma counter (LKB Wallac, Turku, Finland). The amount of membrane-associated radioactivity bound in the presence of 25-fold excess of unlabelled ligand was regarded as nonspecific binding, and subtracted from the total binding to obtain specific binding. Scatchard plots were constructed and the maximal specific binding capacity of HDL to liver membranes (B_{max}, ng HDL protein/mg membrane protein) and the apparent affinity constant (K_d, mg HDL protein/l) were calculated from the slope and the intercepts.

Hepatic lipase activity in hamster livers

Livers were removed after exsanguination, quickly frozen in liquid N_2 and kept at $-80°$. About 100 mg liver was homogenized in 4 ml PBS, containing 5×10^3 U heparin/l, using a Polytron homogenizer (Janke and Kunkel) for 15 s. The homogenate was kept on ice and centrifuged for 2 min at $4°$ (10 000 g). Hepatic lipase activity was determined in the supernatant fraction using a gum acacia-stabilized glycerol[^{14}C]trioleate emulsion as described by Jansen & Birkenhäger (1981).

Statistics

The data were analysed by one-way ANOVA. The Bonferroni adaptation of the *t* test was used for multiple pairwise comparisons when the ANOVA test indicated a significant effect. Correlations between variables were statistically analysed with the Pearson product moment correlation test. The level of significance was preset at $P < 0.05$. Statistical analyses were done with the SigmaStat® statistical software package (Jandel Scientific, San Rafael, CA, USA).

Results

The pelleted diets were well accepted by the animals. Final body weights after 9 weeks of consuming the diets were similar in the groups fed with palm oil or maize oil, but body weights were about 10 % higher in the group fed with olive oil (Table 2). Liver weight was significantly greater in the olive-oil group than in the palm-oil group. We did not measure the food intake, but the higher body weights in the hamsters fed with olive oil may have been the result of a higher food intake. In other hamster studies in our laboratory, however, we did not observe any difference in body weights between hamsters fed on diets containing these three dietary fat types.

The hamsters fed on the diet containing maize oil had significantly lower total plasma, HDL-, and non-HDL-cholesterol concentrations than the hamsters fed on the palm- and olive-oil diets (Table 2). The animals fed on the olive-oil diet tended to have lower values than the animals fed on the palm-oil diet, but this effect was not statistically significant. There was a significant positive correlation between the concentration of total plasma cholesterol and the concentration of cholesterol in HDL or non-HDL fractions in individual hamsters (Fig. 1). Thus, changes in total plasma cholesterol concentrations were reflected in changes in both HDL and non-HDL fractions.
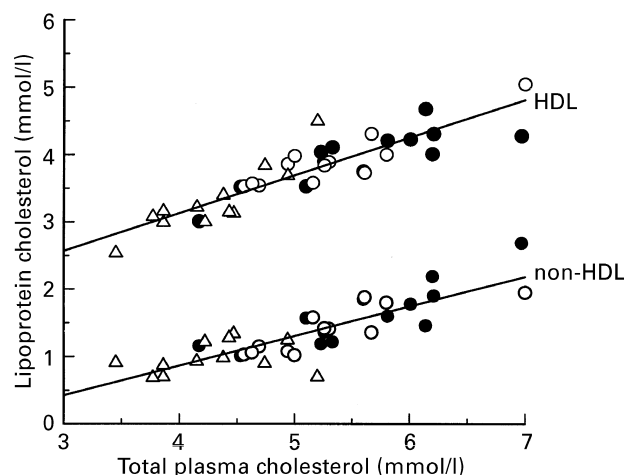

Fig. 1. Correlations between total plasma cholesterol concentration and the concentrations of HDL- and non-HDL-cholesterol fractions in hamsters fed on semipurified diets containing palm oil (●), maize oil (△) or olive oil (○). The equations of the regression lines are: HDL cholesterol $= 0.561 \times$ plasma cholesterol $+ 0.891$ (*r* 0.893, $P < 0.001$) and non-HDL-cholesterol $= 0.439 \times$ plasma cholesterol $- 0.891$ (*r* 0.841, $P < 0.001$).

Further, the slopes of the regression lines for HDL and non-HDL fractions were fairly similar, indicating that a change in total plasma cholesterol was associated with equal changes in HDL- and non-HDL-cholesterol (Fig. 1). The hamsters fed with the maize oil had an average cholesterol level that was 1·29 mmol/l lower than that in the hamsters fed on the palm-oil diet, and this lowering was associated with equal decreases of HDL- (0·66 mmol/l) and non-HDL-cholesterol (0·63 mmol/l). Plasma triacylglycerol concentrations were significantly lower in the maize- and olive-oil-fed hamsters compared with the palm-oil-fed hamsters, and there was no significant difference between the maize- and olive-oil groups (Table 2).

We did not observe a significant effect of dietary fat saturation on the activity levels of plasma LCAT and CETP (Table 2). LCAT activities were virtually the same in all three dietary groups. PLTP activity, however, showed a tendency to increase with increasing fat saturation, although these effects were not statistically significant. When all the hamsters were taken together, there was a significant correlation between PLTP activity and non-HDL-cholesterol concentration (*r* 0·389, $P = 0·019$) and PLTP activity and HDL-cholesterol concentration (*r* 0·509, $P = 0·001$).

Eight hamsters from each diet group were randomly selected to measure hepatic lipase activity in quickly-frozen livers. Hepatic lipase activity was significantly higher in the hamsters fed with palm oil compared with the animals fed with maize oil (Table 2). The olive-oil-fed hamsters had intermediate activities. There was a significant correlation between hepatic lipase activity and total plasma and HDL-cholesterol concentrations in individual hamsters (Fig. 2).

Four hamsters per dietary group were used for assaying the HDL binding capacities (B_{max}) and binding affinities (K_d) of liver membranes. Maximum HDL binding was found to be significantly higher in the hamsters fed with

Table 2. Measures of cholesterol metabolism in hamsters fed on semipurified diets containing palm oil, olive oil or maize oil for a period of 9 weeks*

(Mean values and standard deviations for four, eight or twelve hamsters per group)

	Palm oil		Olive oil		Maize oil	
	Mean	SD	Mean	SD	Mean	SD
Plasma and liver variables (n 12)†						
Plasma cholesterol (mmol/l)						
Initial	3·23	0·36	3·31	0·43	3·24	0·44
Final	5·58[a]	0·76	5·30[a]	0·67	4·29[b]	0·51
HDL-cholesterol (mmol/l)	3·97[a]	0·43	3·91[a]	0·12	3·31[b]	0·50
Non-HDL-cholesterol (mmol/l)	1·61[a]	0·47	1·40[a]	0·34	0·98[b]	0·24
Plasma triacylglycerols (mmol/l)	2·75[a]	1·03	1·56[b]	0·65	1·01[b]	0·59
LCAT (μmol/l per h)	77	16	78	15	78	13
CETP (μmol/l per h)	136	24	129	15	142	21
PLTP (mmol/l per h)	6·97	1·67	6·29	1·32	5·99	1·19
Liver weight (g)	4·6[b]	0·7	5·5[a]	0·8	4·8[ab]	0·9
Liver weight (% body weight)	3·51[b]	0·27	3·85[a]	0·29	3·66[ab]	0·31
Body weight (g)						
Initial	73	8	72	8	73	7
Final	129[b]	13	141[a]	12	129[b]	17
Subgroup for determination of lipase activity (n 8)						
Plasma cholesterol (mmol/l)						
Initial	3·24	0·33	3·38	0·49	3·17	0·41
Final	5·57[a]	0·67	5·10[ab]	0·47	4·42[b]	0·47
HDL-cholesterol (mmol/l)	3·94[a]	0·42	3·75[ab]	0·18	3·38[b]	0·51
Non-HDL-cholesterol (mmol/l)	1·63[a]	0·36	1·35[ab]	0·34	1·04[b]	0·26
Hepatic lipase activity (mU/g liver)‡	148·1[a]	19·2	134·6[ab]	17·9	111·3[b]	17·7
Hepatic lipase activity (mU/mg protein)	0·62[a]	0·11	0·58[a]	0·08	0·45[b]	0·10
Subgroup for determination of binding variables (n 4)						
Plasma cholesterol (mmol/l)						
Initial	3·36	0·41	3·22	0·31	3·15	0·57
Final	5·77[a]	0·42	5·07[ab]	0·59	4·48[b]	0·55
HDL-cholesterol (mmol/l)	4·05[a]	0·27	3·75[a]	0·23	3·51[a]	0·68
Non-HDL-cholesterol (mmol/l)	1·72[a]	0·25	1·33[ab]	0·36	0·97[b]	0·27
Human HDL$_3$ binding variables						
B$_{max}$ (ng HDL protein/mg membrane protein)	655[b]	56	674[ab]	26	810[a]	100
K_d (mg HDL protein/l)	11·8	1·7	11·8	1·4	13·4	1·0

LCAT, lecithin: cholesterol acyltransferase; CETP, cholesteryl ester transfer protein activity; PLTP, phospholipid transfer protein.

[a,b] Mean values within a row not sharing a common superscript letter were significantly different, $P < 0.05$.

* For details of diets and procedures, see Table 1 and pp. 152–154.

† The palm-oil group had thirteen hamsters.

‡ nmol free fatty acids formed/min per g liver.

maize oil than in those fed with palm oil. The olive-oil group had intermediate values but this effect was not significant (Table 2). There was a significant correlation between binding capacities (B$_{max}$) and total plasma and HDL-cholesterol concentrations (Fig. 3). The affinities for HDL binding (K_d) did not differ among the three dietary groups.

Discussion

Intervention studies in human subjects (McNamara, 1992; Khosla & Sundram, 1996) have indicated that unsaturated fatty acids compared with saturated fatty acids in the diet lower plasma cholesterol levels, and that this effect is associated with a lowering of both LDL- and HDL-cholesterol levels. The objective of our present study was to examine the mechanism by which unsaturated fatty acids lower plasma cholesterol and particularly HDL-cholesterol levels. We elected to use the hamster as a model for the

human situation, since the hamster has a lipoprotein profile that resembles closely that of man (Bravo *et al.* 1994) and has CETP activity (Stein *et al.* 1990) like man. The hamster has been widely used to study the effect of dietary fatty acids on lipoprotein metabolism (Dietschy *et al.* 1993; Khosla & Sundram, 1996), and the results of our studies indicate that the hamster responds to dietary fatty acids in a similar fashion to human subjects. Substitution of unsaturated fatty acids (maize oil) for saturated fatty acids (palm oil) resulted in a lowering of the total plasma cholesterol level which was associated with a lowering in both non-HDL- and HDL-cholesterol fractions (Fig. 1), as seen in studies with human subjects (Mattson & Grundy, 1985). Other studies in hamsters have also indicated that cholesterol-enriched diets containing polyunsaturated fattty acids instead of saturated fat are effective in lowering both HDL- and non-HDL-cholesterol levels (Jones *et al.* 1990; Ahn *et al.* 1994; Trautwein *et al.* 1997).
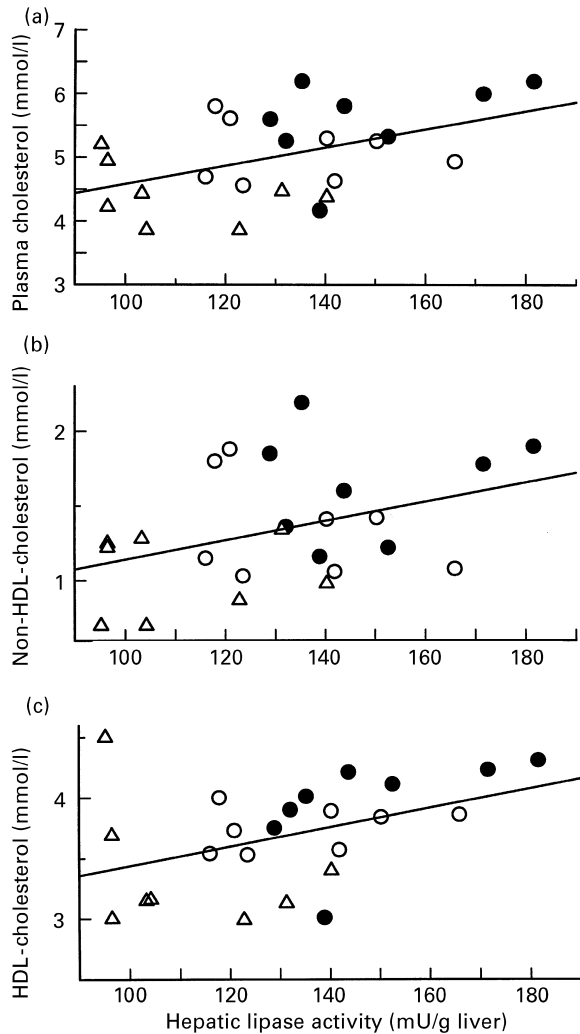
Fig. 2. Correlations between hepatic lipase activity and the concentrations of (a) total plasma cholesterol, (b) non-HDL-cholesterol, and (c) HDL-cholesterol in hamsters fed on semipurified diets containing palm oil (●), maize oil (△) or olive oil (○). The relationships are described by the equations: (a), $y=0.014x+3.133$ (r 0.475, $P=0.019$); (b), $y = 0.006x + 0.494$ (r 0.383, $P = 0.065$); (c) $y = 0.008x + 2.640$ (r 0.418, $P=0.042$).

Fig. 3. Correlations between maximum binding capacity (B_{max}) and the concentrations of (a) total plasma cholesterol and (b) HDL-cholesterol in hamsters fed on semipurified diets containing palm oil (●), maize oil (△) or olive oil (○). The relationships are described by the equations; (a), $y=-0.005x+8.635$ (r 0.645, $P=0.024$); (b), $y= -0.003x+6.026$ (r 0.647, $P=0.023$).

Olive oil tended to lower plasma cholesterol levels in our studies but this effect was not statistically significant. Trautwein *et al.* (1997) also reported that feeding hamsters with olive oil instead of palm stearin or coconut oil together with cholesterol for a period of 7 weeks did not significantly lower plasma cholesterol. Further, Trautwein *et al.* (1997) observed that changes in total plasma cholesterol levels due to different types of dietary fats, were associated with changes in both HDL- and non-HDL-cholesterol fractions, as seen in the present study (Fig. 1). Jones *et al.* (1990) and Kurushima *et al.* (1995), however, found that olive oil and oleic acid respectively, significantly lowered plasma cholesterol levels in hamsters without lowering HDL-cholesterol. We fed our hamsters on semipurified diets and similar diets were used by Trautwein *et al.* (1997). Jones *et al.* (1990) and Kurushima *et al.* (1995), on the other hand, used chow-based diets, and it has been demonstrated that these two
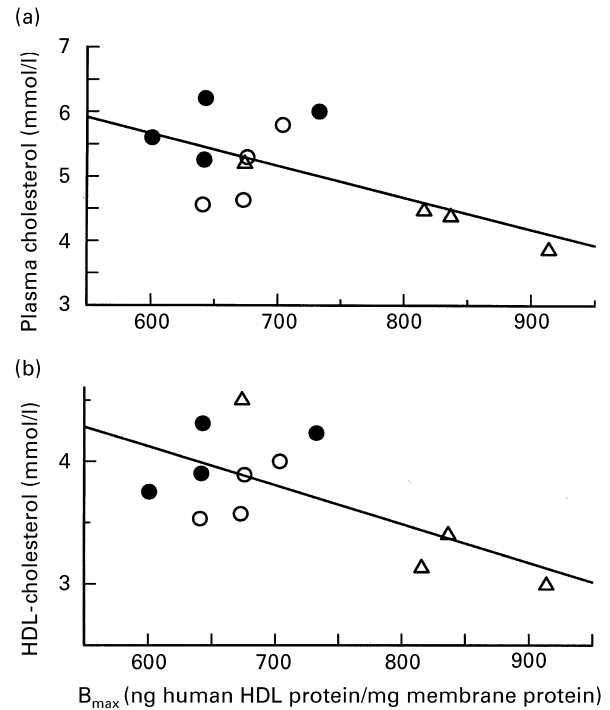
types of diets may have different effects on the lipoprotein profile in hamsters after dietary intervention (Kowala, 1993; Nicolosi *et al.* 1998). Studies in human subjects are also conflicting. Mensink & Katan (1989) reported that mono-unsaturated and polyunsaturated fatty acids were similarly effective in lowering plasma total cholesterol levels and that both also lowered HDL-cholesterol levels. Studies by Mattson & Grundy (1985), on the other hand, indicated that monounsaturated fatty acids compared with poly-unsaturated fatty acids raised HDL-cholesterol levels.

Liver weights and liver weights as a percentage of body weight were higher in the olive-oil-fed hamsters than in the hamsters fed with palm oil or maize oil, although this effect was not statistically significant for the maize-oil group. We did not measure liver cholesterol, but other studies have indicated that feeding olive oil instead of saturated or polyunsaturated fats also resulted in a higher content of liver cholesterol (Beynen, 1988). The high cholesterol content in the liver of olive-oil-fed hamsters may be related to abundant availability of oleate, the preferred substrate for acyl-CoA : cholesterol acyltransferase (*EC* 2.3.1.26), the cholesterol esterifying enzyme in the liver (Dietschy, 1998).

In the present study there was no effect of type of fat in diets containing 1 g cholesterol/kg on plasma CETP and LCAT activity levels. Similar results have been reported in other hamster studies (Ahn *et al.* 1994). Kurushima *et al.* (1995), on the other hand, reported a higher CETP activity in hamsters fed with saturated fatty acids instead of poly-unsaturated fatty acids. Other studies in hamsters also

showed that an increase in plasma cholesterol concentrations as caused by feeding high-saturated fat, cholesterol-rich diets was associated with elevated CETP levels (Stein *et al.* 1990; Quig *et al.* 1991). In human subjects, Lottenberg *et al.* (1996) reported that there was no differential effect of diets containing mono- or polyunsaturated fatty acids on CETP activity. Groener *et al.* (1991), on the other hand, reported higher CETP activities in subjects on polyunsaturated fat diets than those on monounsaturated fat diets, and other studies in human subjects (van Tol *et al.* 1995; Schwab *et al.* 1996; Lagrost *et al.* 1999) also indicated that different fatty acids in the diet such as palmitic, stearic, lauric, oleic and elaidic acids can affect CETP activity. Reports on the effects of dietary fat saturation on LCAT activity in human subjects are also conflicting. Baudet & Jacotot (1988) found a higher LCAT activity when polyunsaturated fats (sunflower oil) were given instead of saturated fats (milk fat) but they discussed that there are also studies that found the opposite effect. van Tol *et al.* (1995) reported that there was no differential effect of polyunsaturated fat (linoleic acid) and saturated fat (stearic acid) on LCAT activity in human subjects. As plasma LCAT and CETP activity levels in our studies were not significantly influenced by dietary fat saturation, the observed changes in HDL-cholesterol are unlikely to have been caused by changes in cholesterol ester synthesis and transfer.

Plasma PLTP activity levels were measured using excess exogenous substrates. To our knowledge, this is the first report on PLTP activity levels in animals fed on different types of fat. The PLTP activities were not significantly different for the three different dietary fatty acid profiles but there was a tendency for increasing PLTP activity with increasing fat saturation. Moreover, when all the hamsters were taken together, there was a significant positive correlation between PLTP activity and HDL- as well as non-HDL-cholesterol levels. In human subjects, it has been reported that various dietary fatty acids like palmitic, lauric, and oleic acid can affect PLTP activities and that there was also a positive correlation between HDL-cholesterol and PLTP activities (Lagrost *et al.* 1999). Two possible functions of PLTP in lipoprotein metabolism have been proposed. First, PLTP may act in the transfer of surface fragments, formed during lipolysis of triacylglycerol-rich lipoproteins to HDL (Tall *et al.* 1985). Second, PLTP is active in HDL subfraction conversion (Jauhiainen *et al.* 1993). Isolated HDL_3 is converted by purified PLTP to bigger HDL_2-like HDL, with the concomitant formation of small pre-β-HDL (von Eckardstein *et al.* 1996).

Hepatic lipase activity was significantly higher in the hamsters fed with palm oil when compared with the maize-oil group. Hepatic lipase plays a role in the conversion of LDL and HDL subfractions (Syvanne & Taskinen, 1997). Studies in human subjects have shown that hepatic lipase activity and HDL-cholesterol concentrations are inversely related (Bensadoun & Berryman, 1996). In the present study with hamsters, however, there was a positive correlation between HDL-cholesterol concentration and hepatic lipase activity. Similarly, Jansen *et al.* (1989) described in the hamster an increase in liver-bound hepatic lipase activity after feeding cholesterol, which in the hamster is usually associated with increases in both total plasma cholesterol and HDL-cholesterol levels. Further, it is known that addition of VLDL- and LDL-cholesterol to the perfusate may increase hepatic lipase levels in a recirculating liver perfusion system (Ebert *et al.* 1993). Rabbits respond to cholesterol feeding by elevation of hepatic lipase activities (Meijer *et al.* 1993). These data are consistent with our observation that in the hamsters fed with palm oil, high plasma non-HDL-cholesterol concentrations coincided with high hepatic lipase activities. We suggest that increased hepatic lipase activity is secondary to the increase in non-HDL-cholesterol.

Little is known about the effects of dietary fats on HDL binding to liver membranes. Fernandez & McNamara (1991) observed that dietary unsaturated *v.* saturated fatty acids increased the binding capacity of liver membranes for HDL in the guinea-pig. We have now shown in hamsters that dietary maize oil raised B_{max} when compared with palm oil. These results indicate that there was an increase in the number of HDL binding sites on the liver membranes.

HDL plays an important role in reverse cholesterol transport (Swenson, 1992), and an increase in HDL binding to liver membranes may stimulate this process. Early kinetic studies in human subjects indeed suggested an increased transport rate of HDL cholesteryl esters when saturated fat in the diet was replaced by unsaturated fat (Nestel, 1970). Thus, the observation that maize oil compared with palm oil significantly enhanced HDL binding to liver membranes could explain why dietary maize oil lowers HDL-cholesterol levels in the hamster.

We used the hamster as a model to study the effects of dietary fat saturation on plasma cholesterol and HDL metabolism. Eventually, this model is meant to obtain information about the human situation. It should be stressed, however, that there may be differences in responses to dietary fats when compared with human subjects and one should be careful in extrapolating results obtained in hamsters to man. For instance, in our present study there was a significant lowering in plasma triacylglycerol levels with decreasing fat saturation, whereas no such findings are reported in man (Khosla & Sundram, 1996). Further, we found in hamsters a positive correlation between HDL-cholesterol level and hepatic lipase activity, whereas in human subjects a negative correlation has been reported (Bensadoun & Berryman, 1996). These different responses may reflect a difference in lipid metabolism between human subjects and hamsters. In addition, there may also be differences in response between various strains of hamsters (Trautwein *et al.* 1993) and the effects may also be modulated by the type of diets used, i.e. semipurified *v.* chow-based diets (Kowala, 1993; Nicolosi *et al.* 1998). Differences between hamster strains and types of diet may also explain why there were no effects of fat saturation on CETP activity in our studies and the studies of Ahn *et al.* (1994), while there were effects in other studies (Kurushima *et al.* 1995).

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