Effects of dietary maritime pine (*Pinus pinaster*)-seed oil on high-density lipoprotein levels and *in vitro* cholesterol efflux in mice expressing human apolipoprotein A-I

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Maritime pine (*Pinus pinaster*)-seed oil contains two Δ5 unsaturated polymethylene interrupted fatty acids (all *cis*-5,9,12-18:3 and all *cis*-5,11,14-20:3 acids) one of which resembles eicosapentaenoic acid. The goal of the present study was to test whether maritime pine-seed oil consumption affects HDL and apolipoprotein (Apo) A-I levels as well as the ability of serum to promote efflux of cholesterol from cultured cells. To this end, wild type (WT) non-transgenic mice and transgenic mice expressing human ApoA-I (HuA-ITg) were fed on isoenergetic diet containing either 200 g maritime pine-seed oil/kg or 200 g lard/kg for 2 weeks. WT and HuA-ITg mice fed maritime pine-seed oil had lower cholesterol, HDL-cholesterol, LDL-cholesterol and HuA-ITg mice had lower human ApoA-I than those fed lard. The differences in cholesterol ($P < 0{\cdot}0001$) and HDL-cholesterol ($P < 0{\cdot}003$) levels between mice fed on the two diets were more pronounced in the HuA-ITg than in the WT mice. The ability of HuA-ITg serum to promote cholesterol efflux in cultured cells was greater ($P < 0{\cdot}008$) than that of WT animals. However, the maritime pine-seed oil diet was associated with lower ($P < 0{\cdot}005$) *in vitro* cholesterol efflux ability than the lard diet in both mice genotypes. This suggests a negative effect of the maritime pine-seed oil on reverse cholesterol transport. Cholesterol efflux was correlated with serum free or esterified cholesterol and phospholipid levels. The slope of the regression line was smaller in the HuA-ITg than in the WT mice indicating that overexpression of human ApoA-I reduces the negative impact of maritime pine-seed oil on cholesterol efflux. In conclusion, maritime pine-seed oil diet lowers HDL-cholesterol and diminishes *in vitro* cholesterol efflux. This potentially detrimental effect is attenuated by overexpression of human ApoA-I in mice.

Maritime pine-seed oil: Apolipoprotein A-I: Lipoproteins: Reverse cholesterol transport

Seeds from conifers such as *Pinus koraiensis*, *Pinus cembra*, *Pinus cembroides*, *Pinus edulis*, *Pinus pinea*, *Pinus sibirica* and *Pinus monophylla* are currently consumed as condiments for food preparation (Wolff & Bayard, 1995). Oils extracted from some of these seeds have significant lipid-lowering properties in rodents (Ikeda *et al*. 1992; Sugano *et al*. 1994). Earlier studies in our laboratory have shown that maritime pine (*Pinus pinaster*)-seed oil (MPSO) treatment affects lipoprotein metabolism in rats (Asset *et al*. 1999*b*) and in transgenic mice lacking apolipoprotein (Apo) E (Asset *et al*. 1999*a*) suggesting that MPSO could be an adjuvant to lipid-lowering diets in clinical practice. The most striking effect of a MPSO diet as compared with lard was to lower triacylglycerol in rats and VLDL-cholesterol levels in the ApoE-deficient mice. However, despite these favourable effects, ApoE-deficient mice were not protected against atherosclerosis (Asset *et al*. 1999*a*). Among the possible explanations of this finding, an alteration of HDL metabolism could inhibit the expected protective action of MPSO treatment. HDL are dense, protein-rich lipoproteins whose principal function is to transport cholesterol surplus from peripheral tissue to the liver for excretion in the intestinal tract. This is known as reverse cholesterol transport and prevents accumulation of cholesterol in blood

Abbreviations: Apo, apolipoprotein; MPSO, maritime pine-seed oil; WT, wild type.

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vessel walls and atherosclerosis formation. Studies in animal models (Hayes & Khosla, 1992; Wolfe *et al*. 1993; Ahn *et al*. 1994) as well as in human subjects (Hegsted *et al*. 1965, 1993; Keys & Parlin, 1966; Mata *et al*. 1992) have shown that substituting dietary saturated by polyunsaturated-enriched oils resulted in a lowering of ApoA-I and HDL levels, indicating a possible deleterious effect of polyunsaturated fats. Therefore, in the process of evaluating the potential interest of a novel oil, it is necessary to assess its impact on HDL levels.

Transgenic animals expressing human Apo have proved to be fruitful models for the investigation of lipoprotein metabolism (Paigen *et al*. 1994; Breslow, 1996). HuA-ITg mice are characterised by markedly increased levels of HDL (Walsh *et al*. 1989; Rubin *et al*. 1991*a*). These properties confer protection against atherosclerosis to the cholesterol-fed (Rubin *et al*. 1991*b*) or ApoE-deficient HuA-ITg mice (Plump *et al*. 1994). Thus, HuA-ITg mice appear to be a useful model to study the impact of nutritional manipulation on HDL metabolism.

The goal of the present study was to assess the effect of MPSO consumption on HDL levels in order to test whether the MPSO, like some other oils enriched in polyunsaturated fatty acids, also decreases HDL-cholesterol. Moreover, since reverse cholesterol transport is an important pathway that confers protection against atherosclerosis, the effect of a MPSO diet on cellular cholesterol efflux was determined *in vitro*.

Methods

Animals

The studies were performed with HuA-ITg mice (Rubin *et al*. 1991*a*). These mice were obtained in C57BL/6J background after multiple back-crosses (Transgenic Alliance, IFFA CREDO, L'Arbresle, France). Wild type C57BL/6J (WT) mice were used for control experiments. Before dietary studies all animals were acclimatised for 1 week under conditions of controlled temperature (20 ± 1°C) and lighting (dark from 20.00–08.00 hours) in a room of low background noise. The age of the mice was 5 months (SD 1 week).

Dietary experiments

Before each dietary experiment a blood sample was drawn for randomisation based on cholesterol levels. Mice were housed in cages (2–3 per cage) and were given free access to a fat-free semipurified diet (UAR, Villemoisson sur Orge, France) supplemented with lard (Eurogat, Saint Denis, France) or MPSO (D'ANOSTE, Vendays-Montalivet, France) at 200 g/kg for 2 weeks (Table 1). The other major nutrient components were (g/kg): carbohydrate 504, casein 180, cellulose 48, mineral mixture 56, vitamins 8. Weight gain was monitored throughout these studies. At the end of each dietary experiment, mice were food-deprived for 4 h and exsanguinated under diethyl ether anaesthesia by cardiac puncture. Experiment 1 was a three experimental factors design with ten mice per cell to compare the effect of MPSO and lard on lipid and lipoprotein variables in male and female WT and HuA-ITg mice. Experiment 2 was a three experimental factors design with three mice per cell to compare the effect of MPSO and lard on cellular cholesterol efflux in male and female WT and HuA-ITg mice.

Table 1. Fatty acid composition (g/100 g) of the lipid sources

Fatty acids	Lard	Maritime pine-seed oil
Saturated		
16:0	24	3·6
17:0	0·1	0·1
18:0	15·5	2·4
Monounsaturated		
16:1	2·6	0·2
18:1	43·2	18·1
11-20:1	0·7	1·0
Polyunsaturated		
9,12-18:2	11·6	55·9
9,12,15-18:3	0·8	1·3
11,14-20:2	nd	0·8
Δ5 olefinic		
5,9-18:2	nd	0·7
5,9,12-18:3	nd	7·1
5,11-20:2	nd	0·8
5,11,14-20:3	nd	7·1
Others	1·5	0·9

nd, not detected.

Lipid measurements

Blood was collected on EDTA or dry tubes for plasma (experiment 1) or serum (experiment 2) preparation respectively. Plasma and serum were separated by centrifugation (630 ***g***) for 20 min at 4°C. Lipids were determined enzymatically using commercially available kits for cholesterol (Cholesterol C System; Boehringer Mannheim, Mannheim, Germany), triacylglycerol (Triglycerides GPO-PAP; Boehringer Mannheim) and phospholipids (Phospholipids PAP 150; BioMérieux, Lyon, France).

Gel-filtration chromatography

Gel-filtration chromatography was performed using a Superose 6 HR 10/30 column (Pharmacia, Pharmacia LKB Biotechnology, S-751 82 Uppsala, Sweden). The gel was allowed to equilibrate with PBS (10 mM) containing 0·1 g EDTA/l and 0·1 g sodium azide/l; 150 μl plasma were eluted with the buffer at room temperature at a flow rate of 0·02 ml/min. Elution profiles were monitored at 280 nm and recorded with an analog-recorder chart tracing system (Pharmacia, Pharmacia LKB Biotechnology). The effluents were collected in 0·24 ml fractions. Cholesterol and phospholipids were measured in each collected fraction using commercially available enzymatic kits (Cholesterol C System, Boehringer Mannheim; Phospholipids PAP 150, BioMérieux).

Agarose-gel electrophoresis

Agarose-gel electrophoresis was performed according to Noble (Noble, 1968) with a Beckman Paragon system

(Beckman Instruments France SA, Gagny, France). Briefly, plasma (5 μl) was applied on a 0·5 % agarose gel (Paragon LIPO lipoprotein electrophoresis, Beckman Instruments France SA). Electrophoresis was performed for 30 min in a barbital buffer (pH 8·6) at 100 V. Gels were stained with Sudan Black B.

High-density lipoprotein isolation and measurements

The fraction of serum $1{\cdot}063 < d < 1{\cdot}21$ g/ml was separated by sequential ultracentrifugation using a Beckman TLA-100·4 rotor (Beckman Instruments France SA) at 480 000 ***g*** and 10°C. Serum aliquots (100 μl) were adjusted to a density of 1·063 g/ml with potassium bromide and spun for 3 h. The supernatant fraction was discarded. Then, the infranatant fraction was adjusted to a density of 1·21 g/ml and spun twice for 3 h. The resulting serum fraction $1{\cdot}063 < d < 1{\cdot}21$ g/ml was dialysed against a 10 mM-phosphate buffer containing 0·1 g EDTA/l and 0·1 g sodium azide/l. Lipids and proteins were determined enzymatically using commercially available kits for cholesterol (Cholesterol C System, Boehringer Mannheim), triacylglycerol (Triglycerides GPO-PAP; Boehringer Mannheim), phospholipids (Phospholipids PAP 150; BioMérieux) and proteins (BCA protein assay reagent; Pierce, Rockford, IL, USA).

[^{3}H]cholesterol efflux

[^{3}H]cholesterol fractional efflux was carried out essentially as previously described (de la Llera Moya *et al.* 1994). Briefly, the efflux potential of mice serum was assayed by incubating individual fresh unfrozen samples at 37°C with the [^{3}H]cholesterol-labelled Fu5AH cells and then quantifying the amount of radio-labelled cellular cholesterol released to the acceptor-containing medium. Acceptors were extensively dialysed against Eagle's minimal essential medium. Serum specimens were used at a concentration of 5 % and cells were exposed to the acceptor serum for 4 h. At least three wells of cells were incubated with each sample. Samples were diluted into Eagle's minimal essential medium just before addition to cells. The labelled cell cholesterol released was measured in a portion of the medium by using standard liquid scintillation counting. The efflux phase was ended by removing the serum-containing medium from each well. This efflux medium was collected into tubes that had been chilled in an ice-bath and centrifuged at 4°C for 5 min at 1000 ***g*** to remove any floating cells. The cell-free medium was maintained at 0°C until frozen at −70°C for storage and further analysis. At the end of the efflux period, cell monolayers were washed with PBS. The lipids were extracted by overnight incubation at room temperature with isopropanol, and cellular lipid radioactivity was measured in a portion of the extract. Fractional efflux was calculated as the amount of the label released to the medium divided by the total label in each well. To standardise the cellular response obtained with different batches of cells and labelling medium, a human serum standard prepared from a pool was always included as a test serum. Although Fu5AH cells are rat hepatoma-derived cells this model has been validated and is widely used to assess serum-mediated cholesterol efflux. The limits and advantages of this cellular model are extensively discussed in a previous paper (de la Llera Moya *et al.* 1994).

Statistical analysis

Three-way ANOVA was used to compare diet, sex and genotype effects. Whenever ANOVA was statistically significant ($P < 0{\cdot}05$), the Scheffé test was used for *post hoc* analysis. The SPSS Software release 7.5 for Windows was used (SPSS Institute Inc., Paris, France).

Results

Experiment 1

Lipid and lipoprotein. Three-way ANOVA showed no significant interaction among any combination of factors and sex, i.e. sex × diet × genotype or sex × diet or sex × genotype, suggesting that male and female mice had a similar response to diet. There was, in contrast, a statistically significant interaction between genotype and diet for cholesterol ($P < 0{\cdot}0001$), phospholipids ($P < 0{\cdot}0001$) and HDL-cholesterol ($P < 0{\cdot}003$) indicating that HuA-ITg and WT mice had a different response to diet (Table 2). Cholesterol, phospholipids or HDL-cholesterol levels were lower in both WT and HuA-ITg mice supplemented with MPSO than lard. Therefore, the interaction between genotype and diet was quantitative rather than qualitative. That is, the lipid and lipoprotein differences between the MPSO and the lard group were greater in HuA-ITg than WT mice. Finally, there was a statistically significant main effect of diet for LDL-cholesterol ($P < 0{\cdot}01$) and triacylglycerol ($P < 0{\cdot}05$). MPSO-supplemented mice had lower levels of LDL-cholesterol and triacylglycerol than their respective lard-fed controls. The analyses of the electrophoretic properties of lipoproteins on agarose gel showed no major difference between diets.

High-density lipoprotein size. The cholesterol chromatography elution profile of WT mice indicated that the lowering of HDL upon MPSO treatment was similar across the HDL size range (Fig. 1). In HuA-ITg mice the decrease in HDL-cholesterol (Fig. 1) was more pronounced in the large HDL fractions than in the smaller fractions. Therefore, not only the levels but also the size distribution of HDL were affected in HuA-ITg mice with a shift toward smaller particles in the MPSO-treated animals. Similar results were obtained with measurement of phospholipids (data not shown).

Human apolipoprotein A-I in HuA-ITg mice. Two-way ANOVA indicated a statistically significant interaction ($P < 0{\cdot}05$) between sex and diet for human ApoA-I (Fig. 2). Human ApoA-I levels ($P < 0{\cdot}0001$) were lower in both male and female mice treated with MPSO than lard. The difference between dietary groups was more pronounced in males than in females suggesting a quantitative rather than a qualitative interaction.

Experiment 2

High-density lipoprotein composition. The lipid composition analysis of HDL revealed no major difference

Table 2. Plasma lipids (mmol/l) of wild type and HuA-ITg mice fed on lard or maritime pine-seed-oil diet (200 g/kg)†
(Mean values and standard deviations for ten male and ten female mice per group)

	Wild type				HuA-ITg					
	Lard		Maritime pine-seed oil		Lard		Maritime pine-seed oil		ANOVA: *P*‡	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Genotype × diet interaction	Diet main effect
Cholesterol										
Female	2·28	0·32	1·65*	0·10	5·93	0·34	4·00***	0·42	0·0001	0·0001
Male	3·31	0·54	2·40***	0·29	7·09	0·88	4·83***	0·52		
HDL-CHO§										
Female	1·52	0·23	1·00	0·11	4·28	0·62	3·29**	0·35	0·003	0·0001
Male	2·54	0·33	2·01	0·27	5·94	0·75	4·06***	0·51		
LDL-CHO§										
Female	0·51	0·11	0·37	0·08	0·96	0·69	0·50	0·09	NS	0·008
Male	0·45	0·13	0·24	0·04	0·51	0·08	0·40	0·12		
Triacylglycerol										
Female	0·38	0·11	0·33	0·13	0·40	0·09	0·40	0·08	NS	0·028
Male	0·56	0·10	0·34**	0·12	0·44	0·07	0·41	0·04		
Phospholipids										
Female	1·90	0·15	1·47	0·15	5·07	0·44	3·85***	0·37	0·0001	0·0001
Male	3·27	0·37	2·50**	0·24	7·21	0·59	5·29***	0·39		

HDL-CHO, high-density lipoprotein cholesterol; LDL-CHO, low-density lipoprotein cholesterol.
Mean values were significantly different from those of the lard-fed group: *$P < 0·05$, **$P < 0·01$, ***$P < 0·001$ (*post hoc* analysis of maritime pine-seed oil *v.* lard).
† For details of diets and procedures see pp. 254–255 and Table 1.
‡ Three-way ANOVA was used to compare means, followed by Scheffé for *post hoc* analysis. Interaction and main effects terms are those of the ANOVA.
§ HDL-CHO and LDL-CHO were determined by gel-filtration chromatography.

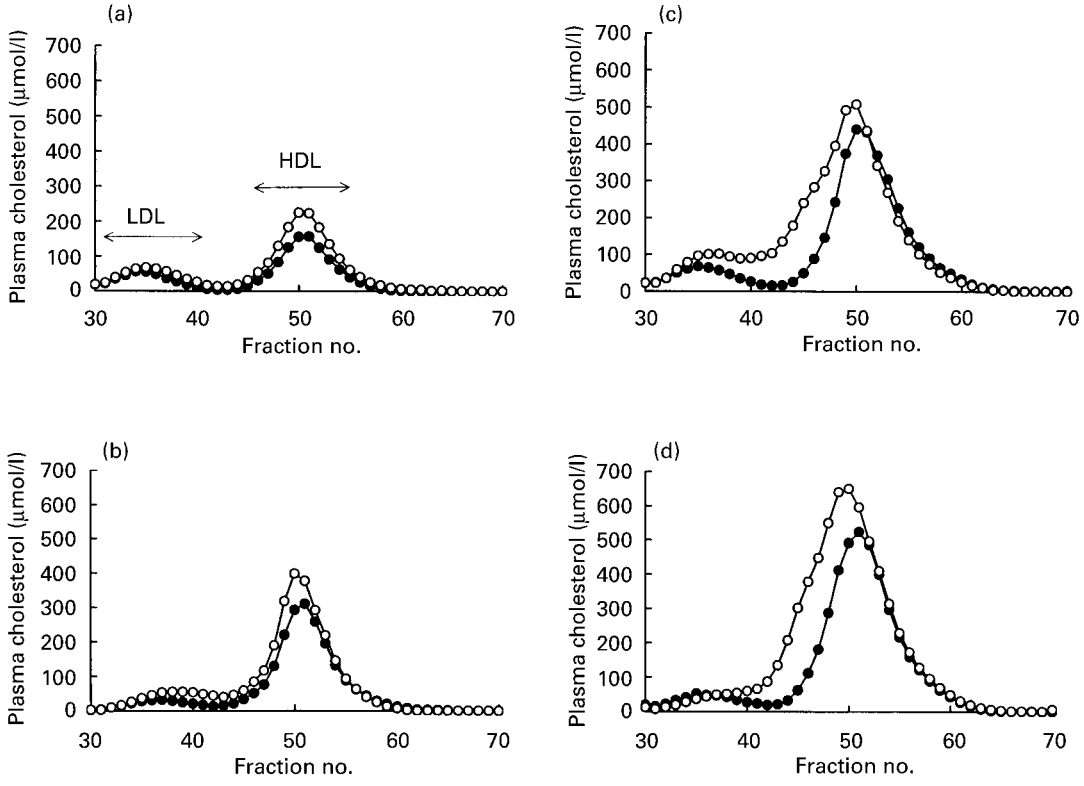

Fig. 1. Cholesterol gel-filtration chromatography profile of the LDL and HDL plasma fraction in (a, b), wild type and (c, d), HuA-ITg mice fed on (●), maritime pine-seed oil or (○), lard diet (200 g/kg diet). Pooled plasma of ten females (a, c) and ten males (b, d). For details of diets and procedures see pp. 254–255 and Table 1.

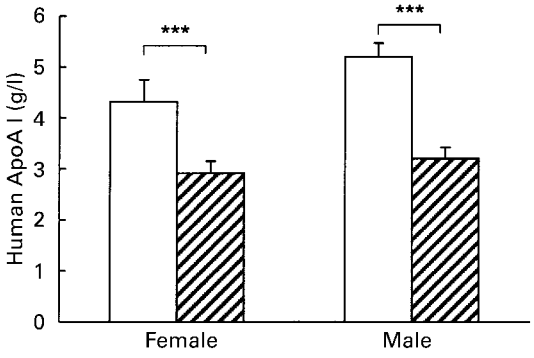

Fig. 2. Human apolipoprotein (Apo)A-I levels in HuA-ITg mice fed on ▨, maritime pine-seed oil or □, lard diet (200 g/kg diet). For details of diets and procedures see pp. 254–255 and Table 1. Values are mean for ten female and ten male mice per diet group with standard deviations represented by vertical bars. There was a statistically significant interaction of sex × diet: $P < 0{\cdot}05$ (two-way ANOVA). Mean values were significantly different: ***$P < 0{\cdot}001$ (*post hoc* analyses with the test of Scheffé).

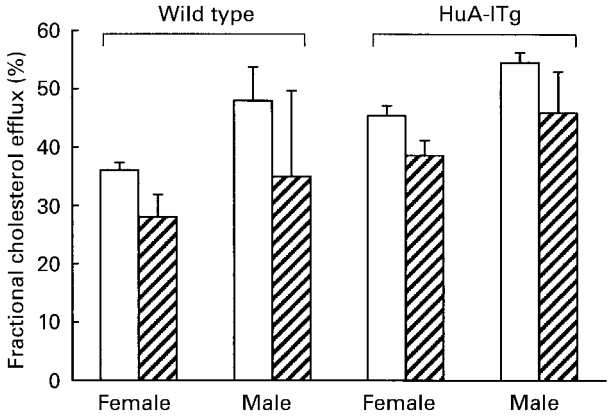

Fig. 3. Fractional cholesterol efflux in wild type and HuA-ITg mice on ▨, maritime pine-seed oil or □, lard diet (200 g/kg diet). For details of diets and procedures see pp. 254–255 and Table 1. Values are means for three female or three male mice per diet group with standard deviations represented by vertical bars. There was a main effect of diet: $P < 0{\cdot}01$ (three-way ANOVA).

between diets. In WT mice, cholesterol, phospholipids and protein contents were 19 %, 28 % and 53 % in lard-fed mice and 18 %, 29 % and 52 % in MPSO-fed mice. In HuA-ITg the values were 17 %, 33 % and 50 % and 16 %, 31 % and 53 % in the lard and MPSO group respectively. Triacylglycerol was not detected.

In vitro *cholesterol efflux*. Three-way ANOVA showed no significant interaction among any combination of factors and sex or genotype i.e. sex × diet × genotype or sex × diet or gender × genotype or genotype × diet. There was a statistically significant main effect of diet for serum free-cholesterol ($P < 0{\cdot}0001$), cholesterol esters ($P < 0{\cdot}002$) and phospholipids ($P < 0{\cdot}002$). These variables were lower in the MPSO-fed mice than in the lard-fed mice for both WT and HuA-ITg mice (Table 3).

In respect of *in vitro* cholesterol efflux, there was no evidence for a statistically significant interaction among any combination of the three factors. There was, however, a statistically significant main effect of sex ($P < 0{\cdot}008$), genotype ($P < 0{\cdot}008$) and diet ($P < 0{\cdot}05$) on cholesterol efflux (Fig. 3). *In vitro* cholesterol efflux was lower in females than in males, in WT than in HuA-ITg mice, and in the MPSO group than in the lard group. The ability of serum to promote *in vitro* cholesterol efflux was correlated to serum phospholipids and to serum free or esterified cholesterol levels (Fig. 4). The slopes of the regression lines were different between mouse genotypes. For a given difference in serum phospholipids, free or esterified cholesterol level between mice, *in vitro* cholesterol efflux difference was smaller in the HuA-ITg mice than in the WT group.

Discussion

Maritime pine seeds are harvested in France on a multi-tonne-scale for reforestation. The oil extracted from the

Table 3. Free, esterified cholesterol and phospholipids (mmol/l) of wild type and HuA-ITg mice fed on lard or maritime pine-seed-oil diet (200 g/kg)†
(Mean values and standard deviations for three male and three female mice per group)

	Wild type				HuA-ITg					
	Lard		Maritime pine-seed oil		Lard		Maritime pine-seed oil		ANOVA: *P*‡	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Genotype × diet interaction	Diet main effect
Free cholesterol										
Female	0·45	0·09	0·25	0·08	1·36	0·28	0·68	0·13	NS	0·0001
Male	0·60	0·08	0·38	0·14	1·82	0·40	0·92	0·40		
Cholesterol esters										
Female	2·28	0·13	1·54	0·13	4·31	1·25	2·53	0·58	NS	0·002
Male	2·83	0·11	1·54	0·98	5·04	0·81	3·49	1·43		
Phospholipids										
Female	2·19	0·16	1·68	0·12	4·50	0·59	3·06	0·41	NS	0·002
Male	3·12	0·26	2·11	1·06	6·79	0·78	4·38	1·65		

Mean values were significantly different from those of the lard-fed group: *$P < 0{\cdot}05$, **$P < 0{\cdot}01$, ***$P < 0{\cdot}001$ (*post hoc* analysis of maritime pine-seed oil *v.* lard; no significant differences were detected).
† For details of diets and procedures see pp. 254–255 and Table 1.
‡ Three-way ANOVA was used to compare means, followed by Scheffé for *post hoc* analysis. Interaction and main effects terms are those of the ANOVA.

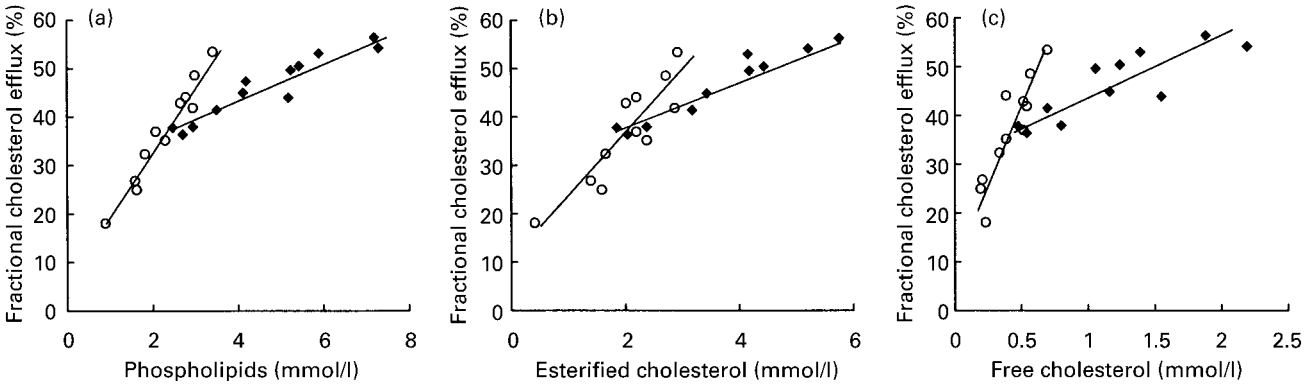

Fig. 4. Regression line between fractional cholesterol efflux and serum (a), phospholipids, (b), esterified cholesterol and (c), free cholesterol. Each line represents the regression of either wild type (○) or HuA-ITg (♦) mice fed on both maritime pine-seed oil or lard (200 g/kg diet). For details of diets and procedures see pp. 254–255 and Table 1. Values are means from three female and three male mice per diet group. Fractional cholesterol efflux *v.*: phospholipids, WT *r* 0·95, HuA-ITg *r* 0·98; esterified cholesterol, WT *r* 0·98, HuA-ITg *r* 0·87; free cholesterol, WT *r* 0·91, HuA-ITg *r* 0·85.

seeds of this pine contains an average of 16 g Δ5 unsaturated polymethylene interrupted fatty acids/100 g fat, namely of all *cis*-5,9,12-18:3 and all *cis*-5,11,14-20:3 acids that differ in fatty acid composition from previously described pine-seed oils (Wolff & Bayard, 1995). MPSO decreases plasma triacylglycerol and VLDL-cholesterol levels in rats (Asset *et al*. 1999*b*) indicating a potential interest in lipid-lowering therapy. The goal of the present study was to assess the impact of MPSO supplementation on HDL levels and cellular cholesterol efflux. To this end, WT and HuA-ITg mice were given a diet containing 200 g lard or maritime pine oil/kg for 2 weeks. The results indicated that MPSO diet lowers HDL-cholesterol levels and reduces the ability of mice serum to promote *in vitro* cholesterol efflux as compared with lard. The latter effect was attenuated by overexpression of human ApoA-I. These data suggest that the decrease in HDL levels that is observed upon MPSO treatment might be less harmful in mice with elevated levels of ApoA-I.

HDL-cholesterol levels were lower in WT and HuA-ITg mice supplemented with MPSO than with lard suggesting an unfavourable effect of MPSO treatment on HDL metabolism. The mechanism by which MPSO lowers HDL is not known. Previous studies in rodents had shown that a polyunsaturated fatty acid-enriched diet was associated with low levels of HDL-cholesterol as compared with a more saturated-fat diet (Leach & Holub, 1984). The reduction in HDL-cholesterol was attributed to either decreased production (Shepherd *et al*. 1978; Sorci-Thomas *et al*. 1989; Stucchi *et al*. 1991) or increased catabolic rates of ApoA-I (Brousseau *et al*. 1995*b*) depending on the experimental model, the type of diet and the metabolic variables that were measured (Stucchi *et al*. 1991). In these studies, hepatic ApoA-I mRNA levels were either low (Sorci-Thomas *et al*. 1989; Kushwaha *et al*. 1991; Stucchi *et al*. 1991) or not affected (Sorci-Thomas *et al*. 1992; Ahn *et al*. 1994; Brousseau *et al*. 1995*a*, *b*) so that no clear mechanism of HDL-lowering has emerged from these investigations. The MPSO diet clearly resulted in lower HDL levels in WT mice and lower HDL and ApoA-I levels in HuA-ITg mice indicating an effect independent of genotype. This result is different from the effect of fibrate, a lipid-lowering agent, which decreases HDL-cholesterol levels in WT mice but increases HDL-cholesterol and human ApoA-I in HuA-ITg mice as well as in human subjects (Berthou *et al*. 1996). Thus, if these observations can be extended to human physiology, these findings would predict a detrimental effect of MPSO diet on HDL-cholesterol and ApoA-I levels in human subjects.

An important function of HDL is to transport cholesterol from peripheral tissues to the liver where the cholesterol is excreted in the intestinal tract. The first step of reverse cholesterol transport involves efflux of cholesterol from peripheral cells to HDL. This transport is essential for cholesterol homeostasis in peripheral tissues and for protection against atherosclerosis. The ability of mice serum to promote *in vitro* cholesterol efflux was higher in HuA-ITg mice than in WT animals in agreement with an earlier investigation (Castro *et al*. 1997). However, *in vitro* cholesterol efflux was lower in both HuA-ITg and WT mice treated with MPSO than with lard indicating an effect independent of genotype. This finding is in agreement with previous observations which had shown a tendency (although not statistically significant) towards lower levels of *in vitro* cholesterol efflux in primates fed polyunsaturated-enriched oils instead of more saturated fats (Sola *et al*. 1993) suggesting a common effect of polyunsaturated fatty acid-enriched diets (Gillotte *et al*. 1998). The mechanism by which MPSO diminishes *in vitro* cholesterol efflux as compared with lard has not been investigated. However, the decrease in serum HDL levels certainly contributes to this effect. In rodents, HDL particles represent the vast majority of circulating lipoproteins. As in human subjects, HDL facilitate the movement of cholesterol from cultured cells to incubation media. Therefore, the lowering of HDL levels that is associated with MPSO diet as compared with lard certainly reduces the ability of serum to promote cholesterol efflux *in vitro*. On the other hand, changes in HDL lipids fatty-acid length and degree of unsaturation affect the physico-chemical properties of HDL which in turn affect the ability of HDL to promote cellular cholesterol efflux (Wassall *et al*. 1992). Overall, feeding MPSO results in decreased HDL and ApoA-I levels as well as in reduced cholesterol efflux indicating a potentially harmful effect of

MPSO on the first step of reverse cholesterol transport. This finding could explain the lack of protective effect of MPSO diet against atherosclerosis in ApoE-deficient mice (Asset *et al*. 1999*a*).

The ability of mice serum to promote *in vitro* cholesterol efflux was correlated with its concentration in free or esterified cholesterol and phospholipids. However, the relationship between serum lipids and *in vitro* cholesterol efflux was different in HuA-ITg and WT mice. The slope of the regression line was less marked in the HuA-ITg than in the WT mice. Thus, for a given decrease in serum free or esterified cholesterol or phospholipids levels between MPSO- and lard-fed mice the corresponding reduction in cholesterol efflux was less pronounced in HuA-ITg mice than in WT animal. This indicates that overexpression of ApoA-I interferes with cholesterol efflux in MPSO-fed mice and attenuates the detrimental effect of feeding a MPSO diet on cholesterol efflux *in vitro*. In a tentative extrapolation of these results to human physiology, MPSO treatment could be used in patients with elevated triacylglycerol and HDL-cholesterol levels. In this type of patient, the benefit of reducing triacylglycerol would not be hampered by a major impact on reverse cholesterol transport.

In conclusion, the results of the present study demonstrate that MPSO supplementation is associated with lower levels of HDL in HuA-ITg and WT mice as compared with lard. In human subjects, the dietary substitution of saturated fatty acids by polyunsaturated fatty acids results in decreased levels of HDL and ApoA-I. This has led to recommendation of moderate consumption of polyunsaturated fatty acids in lipid-lowering diets. The results of the present study indicate that MPSO, like other polyunsaturated fatty acid-rich oils, also decreases ApoA-I and HDL levels, indicating a potentially undesirable effect of this oil on HDL metabolism. These data suggest that a careful analysis of the impact of MPSO on HDL metabolism is necessary in human subjects before recommending its use as dietary complement to lipid disorder treatments.

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