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Characterization of lipoprotein composition in rats fed different dietary lipids and of the effects of lipoproteins upon lymphocyte proliferation

Nicola M. Jeffery, Parveen Yaqoob, David Wiggins,* Geoffrey F. Gibbons,* Eric A. Newsholme, and Philip C. Calder

*Department of Biochemistry, University of Oxford, South Parks Road, Oxford, United Kingdom; *Metabolic Research Laboratory, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Woodstock Road, Oxford, United Kingdom*

Weanling Lewis rats were fed for 10 weeks on a low fat (2.5% by weight; LF) diet or on diets containing 20% by weight of hydrogenated coconut oil (HCO), olive oil (OO), safflower oil (SO), evening primrose oil (EPO), or menhaden (fish) oil (MO); all other components of the diets were identical. The chylomicron (CM), very low density lipoprotein (VLDL), low density lipoprotein (LDL), and high density lipoprotein (HDL) fractions were isolated from the serum. The serum from MO-fed animals had lower LDL and HDL cholesterol concentrations than the serum from animals fed each of the other diets. The apolipoprotein A-1 concentration in the HDL fraction from animals fed the MO diet was also low. The serum from HCO-fed animals had a higher CM triacylglycerol concentration than serum from animals fed each of the other diets. The serum from OO-fed animals had a higher VLDL triacylglycerol concentration than serum from animals fed each of the other diets. The concentration of apolipoprotein B was also high in the VLDL fraction from OO-fed animals. The fatty acid composition of each lipoprotein fraction was affected by the nature of the lipid in the diet; the greatest effects were observed in the CM and VLDL fractions. Each lipoprotein fraction isolated from LF-fed rats inhibited mitogen-stimulated rat spleen lymphocyte proliferation in a concentration-dependent manner; LDL and HDL caused greater inhibition than CM and VLDL. Dietary lipid manipulation did not alter the inhibitory effects of any of the lipoprotein fractions upon lymphocyte proliferation, except that CM and HDL from MO-fed animals and HDL from OO-fed animals resulted in enhanced proliferation compared with either CM or HDL from animals fed the other diets. We conclude that the inhibition of lymphocyte proliferation caused by feeding rats certain dietary lipids (OO, EPO, MO) may be mediated by non-lipoprotein serum components. (J. Nutr. Biochem. 7:282–292, 1996.)

Keywords: lipoproteins; dietary lipids; fish oil; lymphocyte proliferation

Introduction

There is a link between the risk of developing coronary heart disease (CHD) and the concentration of lipids, in particular cholesterol, in the bloodstream.[1] The consumption in the diet of lipids that contain a high proportion of saturated fatty acids results in elevated blood cholesterol and triacylglycerol concentrations; thus, there is a link between saturated fat consumption and risk of developing CHD. As a result of this link, there has been much interest in the use of diets containing unsaturated, rather than saturated, fatty acids to prevent the development of CHD. However, the exact relationship between the consumption of different types of

Address reprint requests to Dr. Philip C. Calder at the Department of Biochemistry, University of Oxford, South Parks Rd, Oxford OXI 3QU United Kingdom.
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fat and blood lipid levels remains unresolved, although there have been many studies in man[2,3] and laboratory animals.[4–13] Therefore, in the current study we investigated the effects of feeding rats a low-fat diet or diets rich in saturated, monounsaturated, *n*-6 polyunsaturated, or *n*-3 polyunsaturated fatty acids (PUFAs) upon the composition of the major lipoprotein fractions.

In addition to a potential effect on blood lipid levels and CHD, dietary lipids may have immunomodulatory effects. An immunosuppressive effect of dietary fish oils, which contain high proportions of *n*-3 PUFAs, is suggested by epidemiological studies that show that populations such as Greenland Eskimos who consume large quantities of fish oils have a very low incidence of inflammatory and autoimmune disorders[14] and by clinical studies that show that fish oil supplementation has some beneficial effects in rheumatoid arthritis, multiple sclerosis, psoriasis, and prolongation of graft survival.[15] The potential clinical use of fish oils and perhaps also those containing *n*-6 PUFAs, such as evening primrose oil, which contains γ-linolenic acid, has given rise to a large number of studies investigating the effects of fatty acids and dietary oils on immune cell functions. In vitro studies have shown that unsaturated fatty acids, or triacylglycerols containing unsaturated fatty acids, inhibit a number of lymphocyte functions including proliferation,[16–23] interleukin (IL)-2 production,[19–21,24] activation marker expression,[19] degranulation of cytotoxic T-lymphocytes,[25] natural killer (NK) cell activity[26] and antigen presentation by splenocytes.[27] In studies where the effects of a range of fatty acids have been compared it has often been found that the *n*-3 PUFA eicosapentaenoic acid is the most potent inhibitor of these functions,[17–22] although other *n*-3 PUFAs and *n*-6 PUFAs are also inhibitory.[16–23,25] These in vitro studies are supported by the results of dietary studies performed in animals and man, which have shown that PUFA-rich diets, especially those containing fish oil, inhibit the proliferation of lymphocytes,[15,28–30] NK cell activity,[30,31] cytokine production,[28,32,33] and the expression of activation markers and adhesion molecules on the surface of resting and mitogen-stimulated spleen lymphocytes[30] and lymphocytes within the popliteal lymph node after the graft versus host and host versus graft reactions.[34] Some of the effects of dietary lipid manipulation on lymphocyte proliferation can be mimicked by culturing cells from chow-fed animals with serum obtained from animals fed different lipids.[35,36] This suggests that components in the serum are at least partly responsible for mediating the effects of the diet; the obvious candidates as mediators are lipoproteins. Indeed, effects of purified lipoproteins on in vitro mitogen-stimulated lymphocyte proliferation have been reported.[37–46] Some of these studies reported that all classes of lipoprotein inhibit mitogen-stimulated lymphocyte proliferation.[38,41,42] However, these studies do not agree as to which lipoproteins are the most potent inhibitors. Morse et al.,[38] Yi et al.,[41] and de Deckere et al.[45] reported that very low density lipoproteins (VLDL) were more potent than low density lipoproteins (LDL), which were more potent than high density lipoproteins (HDL) (in some of these studies HDL caused very little inhibition, if any), whereas Hsu et al.[42] found that HDL caused greater inhibition than VLDL and LDL. In contrast, Cuthbert and Lipsky[44] reported that VLDL, LDL, and HDL enhance lymphocyte proliferation, a finding supported by Xu et al.[46] for HDL and LDL. The importance of the cell culture conditions used in these experiments is apparent when the studies of Cuthbert and Lipsky[43,44] are closely scrutinised: the earlier study reported that LDL inhibited lymphocyte proliferation,[43] whereas the later study reported enhancement.[44] However, the enhancement in proliferation observed when lymphocytes were cultured in the presence of VLDL and LDL was seen only if transferrin was also present in the culture medium[44]; if transferrin was absent then VLDL and LDL caused marked inhibition of proliferation. Clearly, some of the discrepancies between the results of different studies might be due differences in the cell culture conditions used; whatever the reason, it is apparent that the exact nature of the effects of different types of lipoprotein on lymphocyte proliferation is not clear. To our knowledge, the effect of lipoproteins isolated from animals subjected to dietary lipid manipulation on the function of lymphocytes has not been investigated. Therefore, in this study we investigated the effect of lipoprotein fractions purified from the serum of rats fed various lipids on mitogen-stimulated lymphocyte proliferation. The aim of this study was first to identify the effects of lipoproteins on lymphocyte proliferation and secondly to examine whether the differential effects of dietary lipids on lymphocyte proliferation can be explained by differential effects of lipoproteins.

Materials and methods

Animals and diets

Weanling male Lewis rats (aged 3 weeks, weighing between 65g and 85g) were obtained from Harlan-Olac, Bicester, Oxon., England. The animals were housed in groups of six for a period of 10 weeks before killing, during which time they were allowed access, ad libitum, to water and to one of six experimental diets. Animals were fed either a low fat (LF) diet or one of five high-fat diets (purchased from ICN Biomedicals, Aurora, OH USA). The LF diet contained 2.5% by weight of corn oil and the high fat diets contained 20% by weight of the lipid under study, plus 1% corn oil to prevent essential fatty acid deficiency. The high fat diets contained either 20% hydrogenated coconut oil (HCO), 20% olive oil (OO), 20% safflower oil (SO), 20% evening primrose oil (EPO), or 20% menhaden (fish) oil (MO). The oils used for the production of these diets were obtained from Capital City Products, Columbus, OH USA (corn oil, HCO, OO); California Oils, Richmond, CA USA (SO); Zapeta Haynie, Reedville, VA, USA (MO); and Scotia Pharmaceuticals, Guildford, Surrey, England (EPO). The composition of the diets is shown in *Table 1*. Lipid was extracted from the diets using chloroform/methanol (2:1 v/v); the fatty acid composition was analyzed by gas chromatography (see below) and is shown in *Table 2*. Details of food intake and weight gain of animals fed these diets have been published elsewhere.[13,29] Animals were sacrificed by exposure to a rising concentration of CO_2. All procedures involving animals were approved under the Animals (Scientific Procedures) Act 1986 by the Home Office.

Chemicals

Chemicals were obtained from the sources described elsewhere.[15,17,47,48] In addition, potassium bromide was purchased from BDH, Poole, Dorset, England and polyclonal antibodies to human apolipoprotein (Apo) B and Apo A-1 were purchased from Boehringer Mannheim, Lewes, Sussex, England.

Table 1 Composition of the diets used

Component (g/kg)	Diet					
	LF	HCO	OO	SO	EPO	MO
High nitrogen casein	200	200	200	200	200	200
Sucrose	295.8	295.8	295.8	295.8	295.8	295.8
Corn starch	200	200	200	200	200	200
Non-nutritive bulk	215	50	50	50	50	50
Mineral mix[a]	40	40	40	40	40	40
DL-α-Tocopherol Acetate[b]	1.2	1.2	1.2	1.2	1.2	1.2
Vitamin mix[c]	10	10	10	10	10	10
Methionine	3	3	3	3	3	3
Corn oil	25	10	10	10	10	10
HCO	0	200	0	0	0	0
OO	0	0	200	0	0	0
SO	0	0	0	200	0	0
EPO	0	0	0	0	200	0
MO	0	0	0	0	0	200
Cholesterol	0.001	0.004	0.005	0.001	0.003	0.006

[a]AIN-76 containing per kg: 500 g calcium phosphate dibasic, 74 g sodium chloride, 220 g potassium citrate monohydrate, 52 g potassium sulphate, 24 g magnesium oxide, 3.5 g manganese carbonate, 6 g ferric citrate, 1.6 g zinc carbonate, 0.3 g cupric carbonate, 0.01 g potassium iodate, 0.01 g sodium selenite, 0.55 g chromium potassium sulphate, 118 g sucrose.
[b]250 IU vitamin E/g.
[c]Containing per kg: 1.8 g vitamin A (500,000 IU/g), 0.125 g vitamin D2 (850,000 IU/g), 45 g ascorbic acid, 5 g inositol, 75 g choline chloride, 2.25 g menadione, 5 g p-aminobenzoic acid, 4.25 g niacin, 1 g riboflavin, 1 g pyridoxine hydrochloride, 1 g thiamine hydrochloride, 3 g calcium pantothenate, 0.02 g biotin, 0.09 g folic acid. 0.00135 g vitamin B12, 833.46 g carrier dextrose.

Lipoprotein isolation

Chylomicrons (CM), VLDL, LDL, and HDL were isolated from serum by sequential flotation on increasing densities of potassium bromide as described by Havel et al.[49]; a Beckman L8-70 ultracentrifuge fitted with a 50.4 rotor was used.

Cholesterol, triacylglycerol, and apolipoprotein measurements

Cholesterol and triacylglycerol (TAG) concentrations were determined enzymatically.[50,51] The Apo B concentration of VLDL was measured using an enzyme-linked immunosorbant assay (ELISA) involving a polyclonal antibody to human Apo B and rat VLDL Apo B as a standard. The Apo A-1 concentration of HDL was measured using an ELISA involving a polyclonal antibody to human Apo A-1; in the absence of purified rat Apo A-1 to use as a standard, the results are presented as optical density values (OD).

Plasma α-tocopherol analysis

Plasma was treated and extracted as described by Desai[52] before separation and quantification of α-tocopherol by HPLC as described by Buttriss and Diplock.[53]

Lipid extraction and fatty acid composition analysis

Lipid was extracted with chloroform/methanol (2:1 v/v). Fatty acids were prepared by overnight saponification at 70°C in methanolic 0.5 M KOH. Samples were neutralised using concentrated sulphuric acid and fatty acids were extracted into ethyl acetate. After evaporation to dryness, fatty acids were prepared by reaction with an excess of diazomethane in ether. Fatty acid methyl esters (dissolved in methyl acetate) were separated by gas chromatography as described in detail elsewhere.[47,48] Fatty acids were identified by comparison with standards run previously.

Lymphocyte proliferation

Lymphocytes were prepared from the spleens of adult Lewis rats (fed on the LF diet) as described elsewhere.[15] They were cultured at 37°C in an air/CO_2 (19:1) atmosphere in 96 well micro-titre culture plates at a density of 5×10^5 cells/well and a total culture volume of 200 μl in HEPES-buffered RPMI supplemented with 2 mM glutamine, 1% (v/v) Nutridoma, antibiotics and 1 μg/ml concanavalin A (Con A). Lipoprotein fractions were added to the cultures to give the concentrations described in results. After 48 hours of culture, [6-^{3}H]thymidine was added to each well (0.2

Table 2 Fatty acid composition of the diets used

Diet	Fatty acid (mol%)											
	10:0	12:0	14:0	16:0	16:1*n*-7	18:0	18:1*n*-9	18:2*n*-6	18:3*n*-6	18:3*n*-3	20:5*n*-3	22:6*n*-3
LF	n.d.	0.1	0.9	19.3	1.7	3.6	33.4	39.9	n.d.	n.d.	n.d.	n.d.
HCO	5.0	47.3	18.2	10.5	2.2	11.0	2.4	3.5	n.d.	n.d.	n.d.	n.d.
OO	n.d.	3.0	1.5	11.1	n.d.	3.9	69.9	15.3	n.d.	n.d.	n.d.	n.d.
SO	n.d.	2.2	0.4	11.8	n.d.	4.4	20.8	60.4	n.d.	n.d.	n.d.	n.d.
EPO	n.d.	n.d.	n.d.	10.3	n.d.	3.2	15.8	68.4	5.0	n.d.	n.d.	n.d.
MO	n.d.	n.d.	10.9	24.7	16.9	4.4	13.8	5.5	n.d.	2.3	11.7	5.2

n.d., not detected.

μCi/well) and the cells were incubated for a further 18 hours. The cells were then harvested onto glass fibre filters and washed and dried using a Skatron Cell Harvester. Radioactive thymidine incorporation was determined by liquid scintillation counting. Results are expressed as dpm [^{3}H]thymidine incorporated/well.

Data presentation

Data are presented as mean ± SEM of the indicated number of observations. Statistical significance was determined using one-way analysis of variance followed by a least-squared difference test. A value for $P < 0.05$ was taken to indicate a statistically significant difference.

Results

Plasma α-tocopherol concentrations

Plasma α-tocopherol concentrations were higher in rats fed the high fat diets compared with those fed the LF diet (*Table 3*); they were highest in the HCO- and OO-fed animals. The HCO- and OO-fed animals also had the highest serum concentrations of cholesterol and triacylglycerol.[13] When the concentration of α-tocopherol in the plasma was expressed as a ratio of the combined concentrations of cholesterol and triacylglycerol in the serum, there were no significant differences between animals fed the high fat diets (*Table 3*). However, the relative concentration of α-tocopherol in the plasma from LF-fed rats was lower than that of rats fed the HCO, EPO, and MO diets (*Table 3*).

Lipoprotein composition

Feeding the HCO, OO, SO, or EPO diets increased the total serum cholesterol concentration compared with feeding the LF or MO diets[13]; feeding the MO diet produced a hypocholesterolaemic effect relative to feeding each of the other diets.[13] This effect is mainly due to a much lower HDL cholesterol concentration in animals fed the MO diet compared with those fed each of the other diets (*Table 4*), although LDL cholesterol concentrations were also significantly lower in MO-fed animals (*Table 4*).

The total serum TAG concentrations of animals fed the LF, SO, EPO, or MO diets were similar,[13] but feeding the HCO or OO diets caused a hypertriacylglycerolaemic effect.[13] The elevation in TAG concentration is apparent in both CM and VLDL fractions of animals fed the OO diet and in the CM fraction of animals fed the HCO diet (*Table 4*).

The Apo B concentration of VLDL isolated from animals fed the SO or EPO diets was lower than the Apo B concentration of VLDL from rats fed the OO diet (*Table 4*). The Apo A-1 concentration of HDL from MO-fed rats was lower than that of HDL from animals fed each of the other diets (*Table 4*); this was statistically significant compared with the Apo A-1 concentration of HDL from LF-, OO- or EPO-fed animals (*Table 4*).

Table 3 Plasma vitamin E concentrations*

Diet	[α-Tocopherol] (mg/l)	α-Tocopherol/ Cholesterol + Triacylglycerol†
LF	9.9 ± 0.4^{bcdef}	0.0058 ± 0.0004^{bef}
HCO	31.0 ± 4.1^{adf}	0.0095 ± 0.0011^{a}
OO	35.2 ± 5.7^{adf}	0.0091 ± 0.0015
SO	14.9 ± 2.8^{abc}	0.0076 ± 0.0012
EPO	21.7 ± 1.6^{a}	0.0109 ± 0.0015^{a}
MO	15.9 ± 2.0^{abc}	0.0107 ± 0.0016^{a}

*Data are mean ± SEM from at least four animals fed on each diet. †Indicates plasma α-tocopherol concentration (mg/l) divided by the sum of the serum cholesterol and triacylglycerol concentrations (both in mg/l); serum cholesterol and triacylglycerol concentrations have been published elsewhere.[13] Statistical significance ($P < 0.05$ at least; one-way analysis of variance) is indicated as ^{a} versus LF, ^{b} versus HCO, ^{c} versus OO, ^{d} versus SO, ^{e} versus EPO, ^{f} versus MO.

Lipoprotein fatty acid composition

The fatty acid compositions of CM, VLDL, HDL, and LDL isolated from the serum of rats fed different dietary lipids are shown in *Tables 5, 6, 7,* and *8,* respectively. These show that each lipoprotein fraction has a characteristic fatty acid composition and that the fatty acid composition of each lipoprotein fraction is influenced by the diet that the animal was fed.

As would be expected, the fatty acid composition of the CM fraction is strongly affected by that of the diet consumed by the animal (*Table 5*). For example, the proportions of lauric (12:0) and myristic (14:0) acids are very high in CM from rats fed the HCO diet. Similarly, the proportion of oleic acid (18:1*n*-9) is very high in CM from animals fed the OO diet and the proportion of linoleic acid (18:2*n*-6) is high in CM from animals fed the LF, SO, or EPO diets, each of which is rich in linoleic acid. The CM isolated from the serum of MO-fed rats are relatively rich in myristic, palmitic and palmitoleic (16:1*n*-7) acids and are relatively poor in linoleic acid; these CM are also the only ones to contain the *n*-3 PUFAs, eicosapentaenoic (20:5*n*-3), docosapentaenoic (22:5*n*-3), and docosahexaenoic (22:6*n*-3) acids. CM isolated from the serum of EPO-fed animals are the only ones to contain γ-linolenic acid; these CM also contain a high proportion of arachidonic acid (20:4*n*-6).

The fatty acid composition of the VLDL fraction is also greatly affected by that of the diet consumed by the animal (*Table 6*). For example, the proportions of lauric and myristic acids are high in VLDL from rats fed the HCO diet. Similarly, the proportion of oleic acid is very high in VLDL from animals fed the OO diet and the proportion of linoleic acid is high in VLDL from animals fed the LF, SO, or EPO diets. VLDL isolated from the serum of EPO-fed animals are the only ones to contain γ-linolenic acid; these VLDL, like those from LF-fed rats, also contain a high proportion of arachidonic acid. The VLDL isolated from the serum of MO-fed rats are relatively rich in myristic, palmitic, and palmitoleic acids and are relatively poor in linoleic acid; these VLDL are also the only ones to contain the *n*-3 PUFAs, eicosapentaenoic, docosapentaenoic, and docosahexaenoic acids.

The fatty acid composition of the HDL fraction (*Table 7*) was characterized by fairly high proportions of palmitic and stearic acids and a low proportion of linoleic acid; the proportions of these fatty acids were little affected by dietary

Table 4 Composition of lipoprotein fractions*

	Fraction	Diet					
		LF	HCO	OO	SO	EPO	MO
Cholesterol (μg/ml)	CM	36 ± 5	40 ± 4^{d}	63 ± 12^{d}	21 ± 6^{bc}	41 ± 9	44 ± 9
	VLDL	47 ± 4^{b}	86 ± 7^{ad}	63 ± 10	43 ± 3^{b}	61 ± 9	71 ± 15
	LDL	130 ± 7^{f}	148 ± 25^{f}	135 ± 15^{f}	125 ± 16^{f}	137 ± 17^{f}	85 ± 16^{abcde}
	HDL	576 ± 39^{f}	776 ± 29^{f}	717 ± 31^{f}	524 ± 23^{f}	615 ± 36^{f}	317 ± 36^{abcde}
TAG (μg/ml)	CM	96 ± 20^{b}	557 ± 100^{acdef}	238 ± 70^{b}	99 ± 30^{b}	130 ± 40^{b}	117 ± 30^{b}
	VLDL	392 ± 50^{c}	388 ± 60^{c}	783 ± 130^{abdef}	315 ± 70^{c}	366 ± 40^{c}	390 ± 70^{c}
Apo B (μg/ml)	VLDL	22 ± 5	29 ± 6	35 ± 4de	20 ± 5^{c}	20 ± 3^{c}	30 ± 6
Apo A-1 (O.D.)	HDL	0.46 ± 0.06^{f}	0.37 ± 0.08^{c}	0.63 ± 0.07^{bf}	0.43 ± 0.07	0.59 ± 0.09^{f}	0.23 ± 0.02^{ace}

*Data are mean ± SEM from at least six animals fed on each diet. Statistical significance ($P < 0.05$ at least; one-way analysis of variance) is indicated as ^{a} vs. LF, ^{b} vs. HCO, ^{c} vs. OO, ^{d} vs. SO, ^{e} vs. EPO, ^{f} vs. MO.

manipulation, apart from a lower proportion of stearic acid in HDL from MO-fed animals. There were some dietary influences on the fatty acid composition of HDL. For example, the proportion of myristic acid is high in HDL from rats fed the HCO or MO diets. Similarly, the proportion of oleic acid is the highest in HDL from animals fed the OO diet. The HDL isolated from the serum of MO-fed rats contain detectable levels of *n*-3 PUFAs.

The fatty acid composition of the LDL fraction (*Table 8*) was affected to a much lesser extent by that of the diet than were the compositions of the CM and VLDL fractions. Nevertheless, there is still an influence of the diet. For example, the proportion of myristic acid is high in LDL from rats fed the HCO diet. Similarly, the proportion of oleic acid is the highest in LDL from animals fed the OO diet and the proportion of linoleic acid is highest in LDL from animals fed the LF, SO, or EPO diets. LDL isolated from the serum of EPO-fed animals are the only ones to contain γ-linolenic acid. The LDL isolated from the serum of MO-fed rats are relatively rich in myristic, palmitic, and palmitoleic acids and are relatively poor in linoleic acid; these LDL did not contain detectable levels of *n*-3 PUFAs. LDL isolated from the serum of all animals, including those fed the MO diet, contained relatively high proportions of arachidonic acid (10% of more); this proportion appears to be little affected by the nature of the diet consumed.

Effects of lipoproteins on lymphocyte proliferation

The effects of different lipoprotein fractions isolated from LF-fed animals on the proliferative response of spleen lymphocytes (isolated from LF-fed rats) to 1 μg/ml Con A are shown in *Figure 1;* the lipoproteins were included in the culture medium at concentrations between 0.5% and 5% of their concentration in serum. It was previously found that culturing lymphocytes from chow-fed rats in the presence of 2.5% or 5% unfractionated serum obtained from rats fed different types of lipid can cause inhibition of lymphocyte proliferation.[36] Both LDL and HDL caused marked concentration-dependent inhibition of Con A-stimulated lymphocyte proliferation (*Figure 1*). CM and VLDL caused some inhibition of proliferation but this was less marked (*Figure 1*). Thus, these results indicate that the order of inhibitory potencies for lipoproteins from LF-fed animals is:

$$\text{HDL} \sim \text{LDL} > \text{CM} > \text{VLDL}$$

The effect of dietary lipid manipulation upon lipoprotein-induced inhibition of lymphocyte proliferation

Once the effects of lipoproteins isolated from rats fed a LF diet were established (see previous discussion), the influence of the nature of the lipid consumed in the diet on the effect of each class of lipoprotein on spleen lymphocyte proliferation was investigated (*Figure 2*); in all cases the lymphocytes were from rats fed the LF diet.

VLDL or LDL isolated from animals fed the high fat diets suppressed lymphocyte proliferation to approximately the same extent as VLDL or LDL isolated from LF-fed animals (*Figure 2*). Thus, the effects of VLDL and LDL on lymphocyte proliferation are not greatly affected by dietary lipid manipulation.

In contrast to the observations with VLDL and LDL, there was an influence of the type of lipid fed on the subsequent effects of CM and HDL on lymphocyte proliferation. CM isolated from the animals fed the HCO, OO, SO, or EPO diets inhibited lymphocyte proliferation to approximately the same extent as that from rats fed the LF diet (*Figure 2*). However, culture of spleen lymphocytes with CM isolated from animals fed the MO diet resulted in significantly greater lymphocyte proliferation than observed for culture with CM from animals fed each of the other diets (*Figure 2*). Furthermore, whereas 2.5% CM from animals fed the LF, HCO, OO, SO, or EPO diets suppressed proliferation compared with the no lipoprotein-added control, 2.5% CM from MO-fed rats was not suppressive (*Figure 2*). At a concentration of 5%, CM from the MO-fed animals significantly enhanced lymphocyte proliferation compared with the no lipoprotein-added control and compared with CM isolated from rats fed each of the other diets; thymidine incorporation into cells cultured in the presence of 5% CM from rats fed the MO diet was 6,537 ± 768 dpm/well compared with less than 4,000 dpm/well for cells cultured in the presence of CM from rats fed the other diets.

Table 5 Fatty acid composition of CM fraction from animals fed different lipids*

Diet	Fatty acid (mol%)											
	12:0	14:0	16:0	16:1*n*-7	18:0	18:1*n*-9	18:2*n*-6	18:3*n*-6	20:4*n*-6	20:5*n*-3	22:5*n*-3	22:6*n*-3
LF	n.d.	2.1 ± 0.2^{bcf}	39.5 ± 2.3^{bcdef}	6.1 ± 0.3^{bcf}	6.5 ± 1.0^{de}	24.1 ± 0.5^{bcf}	12.9 ± 0.9^{bcdf}	n.d.	1.5 ± 0.7^{e}	n.d.	n.d.	n.d.
HCO	29.3 ± 2.1^{cef}	16.8 ± 0.5^{af}	22.4 ± 0.6^{af}	3.1 ± 0.2^{acf}	7.8 ± 0.7^{c}	13.1 ± 0.6^{acdef}	3.3 ± 0.2^{adf}	n.d.	0.9 ± 0.2^{def}	n.d.	n.d.	n.d.
OO	0.5 ± 0.1^{be}	1.2 ± 0.1^{af}	22.4 ± 1.0^{af}	2.2 ± 0.2^{abdef}	4.7 ± 0.2^{bdef}	55.1 ± 0.7^{abdef}	9.1 ± 1.0^{abdf}	n.d.	1.4 ± 0.3^{e}	n.d.	n.d.	n.d.
SO	n.d.	1.5 ± 0.2^{f}	24.9 ± 1.6^{af}	4.2 ± 0.8^{cf}	10.5 ± 1.2^{acf}	26.0 ± 2.3^{bcf}	21.4 ± 6.8^{abcf}	n.d.	3.2 ± 0.8^{be}	n.d.	n.d.	n.d.
EPO	0.9 ± 0.1^{bcf}	2.0 ± 0.5^{bcdf}	27.6 ± 2.2^{af}	4.5 ± 0.6^{cf}	11.1 ± 1.5^{acf}	20.8 ± 1.9^{bc}	12.2 ± 2.2^{bdf}	0.9 ± 0.2	9.3 ± 1.8^{abcdf}	n.d.	n.d.	n.d.
MO	0.4 ± 0.1^{be}	11.5 ± 0.7^{abcde}	36.7 ± 1.7^{abcde}	14.2 ± 0.7^{abcde}	7.2 ± 0.3^{cde}	18.7 ± 0.7^{abcd}	2.0 ± 0.2^{abcde}	n.d.	2.3 ± 0.4^{be}	0.9 ± 0	0.2 ± 0	0.2 ± 0.1

*Data are mean ± SEM from at least three animals fed on each diet. n.d. indicates not detected. Statistical significance ($P < 0.05$ at least; one-way analysis of variance) is indicated as [a] versus LF, [b] versus HCO, [c] versus OO, [d] versus SO, [e] versus EPO, [f] versus MO.

Table 6 Fatty acid composition of VLDL fraction from animals fed different lipids*

Diet	Fatty acid (mol%)											
	12:0	14:0	16:0	16:1*n*-7	18:0	18:1*n*-9	18:2*n*-6	18:3*n*-6	20:4*n*-6	20:5*n*-3	22:5*n*-3	22:6*n*-3
LF	n.d.	2.1 ± 0.3^{bcdf}	34.6 ± 1.9^{cd}	4.8 ± 0.3^{cdef}	9.5 ± 1.0^{c}	24.8 ± 0.9^{bcde}	13.7 ± 1.1^{bcdef}	n.d.	8.3 ± 0.5^{bcdf}	**n.d.**	**n.d.**	**n.d.**
HCO	4.2 ± 0.7^{cdef}	7.1 ± 0.4^{acdef}	29.3 ± 1.1^{cdf}	5.5 ± 0.3^{cdef}	9.1 ± 0.3^{c}	29.4 ± 0.7^{acdef}	6.1 ± 0.2^{acde}	n.d.	1.9 ± 0.3^{ae}	**n.d.**	**n.d.**	**n.d.**
OO	0.2 ± 0.1^{b}	1.1 ± 0.1^{abef}	20.7 ± 1.1^{abdef}	2.2 ± 0.2^{abf}	6.2 ± 0.1^{abdef}	54.5 ± 1.2^{abdef}	9.3 ± 0.1^{abef}	n.d.	1.6 ± 0.3^{ae}	**n.d.**	**n.d.**	**n.d.**
SO	0.2 ± 0.1^{b}	1.0 ± 0.1^{abef}	24.9 ± 0.9^{abcef}	1.6 ± 0.2^{abf}	8.0 ± 0.7^{c}	20.7 ± 0.5^{abcef}	38.3 ± 1.9^{abcf}	n.d.	2.4 ± 0.5^{ae}	**n.d.**	**n.d.**	**n.d.**
EPO	0.4 ± 0^{b}	1.9 ± 0.3^{bcdf}	30.8 ± 2.0^{cdf}	2.4 ± 0.4^{abf}	9.6 ± 1.0^{c}	17.1 ± 0.8^{abcdf}	30.0 ± 3.5^{abcf}	1.8 ± 0.1	7.5 ± 0.7^{bcdf}	**n.d.**	**n.d.**	**n.d.**
MO	0.4 ± 0.1^{b}	3.4 ± 0.3^{abcde}	37.5 ± 1.2^{bcde}	13.6 ± 0.6^{abcde}	8.2 ± 0.3^{c}	26.1 ± 0.5^{bcde}	5.7 ± 0.2^{acde}	n.d.	1.6 ± 0.5^{ae}	**1.7 ± 0.4**	**1.0 ± 0.3**	**1.0 ± 0.3**

*Data are mean ± SEM from at least three animals fed on each diet. n.d. indicates not detected. Statistical significance ($P < 0.05$ at least; one-way analysis of variance) is indicated as [a] versus LF, [b] versus HCO, [c] versus OO, [d] versus SO, [e] versus EPO, [f] versus MO.

Table 7 Fatty acid composition of HDL fraction from animals fed different lipids*

Diet	Fatty acid (mol%)									
	12:0	14:0	16:0	16:1*n*-7	18:0	18:1*n*-9	18:2*n*-6	20:4*n*-6	22:5*n*-3	22:6*n*-3
LF	0.6 ± 0.2	2.3 ± 0.2^{cdef}	37.9 ± 3.0	2.0 ± 0.1^{bc}	32.9 ± 1.6^{bcdf}	13.0 ± 1.7^{de}	5.0 ± 0.3^{e}	2.9 ± 0.6^{f}	n.d.	n.d.
HCO	0.5 ± 0.1	3.5 ± 0.6^{cd}	32.4 ± 3.4	1.6 ± 0.1^{af}	46.8 ± 0.8^{acdef}	8.7 ± 1.0^{cf}	4.7 ± 1.7	3.2 ± 0.7^{f}	n.d.	n.d.
OO	0.4 ± 0.1	1.4 ± 0.2^{abef}	33.2 ± 2.0^{e}	1.1 ± 0.2^{adf}	40.5 ± 0.2^{abef}	15.5 ± 1.4^{bde}	4.7 ± 1.0	6.2 ± 2.2	n.d.	n.d.
SO	0.5 ± 0.2	1.4 ± 0.2^{abef}	36.7 ± 2.2	1.7 ± 0.1^{cf}	41.3 ± 1.8^{abef}	7.7 ± 0.7^{acf}	5.5 ± 0.5	5.0 ± 0.7	n.d.	n.d.
EPO	0.6 ± 0.04	3.9 ± 0.3^{acd}	39.9 ± 1.1^{c}	1.6 ± 0.1^{f}	35.3 ± 1.4^{bcd}	8.2 ± 0.7^{acf}	3.9 ± 0.3^{a}	4.8 ± 0.8	n.d.	n.d.
MO	0.9 ± 0.3	4.0 ± 0.3^{acd}	36.9 ± 1.3	2.8 ± 0.4^{bcde}	26.8 ± 1.8^{abcde}	13.3 ± 1.1^{bde}	7.3 ± 1.8	6.2 ± 0.5^{ab}	1.3 ± 0.4	2.2 ± 1.3

*Data are mean ± SEM from at least three animals fed on each diet. n.d. indicates not detected. Statistical significance ($P < 0.05$ at least; one-way analysis of variance) is indicated as ^{a} versus LF, ^{b} versus HCO, ^{c} versus OO, ^{d} versus SO, ^{e} versus EPO, ^{f} versus MO.

HDL isolated from animals fed the HCO or EPO diets suppressed lymphocyte proliferation to a greater extent than HDL isolated from animals fed the LF diet (*Figure 2*). Furthermore, culture of lymphocytes with HDL from animals fed the OO or MO diets resulted in greater proliferation than culture with HDL from animals fed the LF diet or any of the other high fat diets (*Figure 2*). Indeed, compared with that from animals fed each of the other diets, HDL from OO- or MO-fed animals resulted in at least a 3 fold higher level of thymidine incorporation (*Figure 2*). Culture of lymphocytes with HDL from OO- or MO-fed animals resulted in enhanced proliferation compared with culture in the absence of added lipoproteins; HDL from OO- or MO-fed animals were the only lipoproteins studied that resulted in enhanced proliferation when used at a concentration of 2.5%. This enhancement of proliferation was even greater at an HDL concentration of 5%, where thymidine incorporation was 9,414 ± 248 dpm/well (HDL from OO-fed rats) and 9,513 ± 814 dpm/well (HDL from MO-fed rats). As a comparison, thymidine incorporation into lymphocytes was less than 500 dpm/well if they were cultured with 5% HDL isolated from rats fed any of the other high fat diets (data not shown). The enhancement of proliferation of lymphocytes cultured with HDL from OO- or MO-fed rats was also observed at other concentrations of Con A (0.25 and 1.5 μg/ml; data not shown).

Discussion

This study aimed to determine the effect of feeding rats diets containing lipids of markedly different fatty acid compositions upon the concentrations and compositions of the principal lipoprotein fractions and to examine the effects of these lipoproteins on lymphocyte proliferation in vitro. This was considered to be important because dietary lipids have been shown to influence lymphocyte functions[15,28–34] and it is possible that the effects of dietary lipids on lymphocytes may be exerted by one or more of the lipoprotein classes present in the circulation.

PUFAs, especially the long chain, highly unsaturated PUFAs found in fish oil, are very susceptible to oxidation. Therefore, diets containing high levels of these PUFAs should include a sufficient amount of vitamin E to protect against lipid peroxidation. The amount of vitamin E included in such diets will be greater than that normally considered to be required. The recommended level of vitamin E in the diet of rats fed standard laboratory chow is 30 IU per kg of diet. Standard laboratory chows normally contain about 2.5% fat by weight; the principal unsaturated fatty acids in such diets are linoleic (approximately 30 to 40%) and oleic (approximately 30 to 40%). In the current study, diets containing 20% fat by weight and high proportions of unsaturated fatty acids were used. The OO, SO, EPO and MO diets contained at least 10 times more fatty acid double bonds per g than the LF diet. Therefore, it was considered appropriate to use a significantly higher level of vitamin E in the diet than normally found in laboratory chow. Since vitamin E itself has potent immunomodulatory effects (for example, enhancement of lymphocyte proliferation in vitro[54,55]), it was important to ensure that all animals in the current study were fed the same level of vitamin E. For this reason, all diets used in this study contained 300 IU of

Table 8 Fatty acid composition of LDL fraction from animals fed different lipids*

Diet	Fatty acid (mol%)								
	12:0	14:0	16:0	16:1*n*-7	18:0	18:1*n*-9	18:2*n*-6	18:3*n*-6	20:4*n*-6
LF	0.9 ± 0.1^{c}	3.3 ± 0.6^{bf}	30.2 ± 3.0^{f}	1.3 ± 0.3	28.1 ± 2.8^{f}	15.1 ± 1.2^{cd}	9.0 ± 1.0^{bf}	n.d.	10.7 ± 1.4^{b}
HCO	1.7 ± 0.5^{c}	5.4 ± 0.3^{ac}	24.4 ± 0.9^{df}	1.6 ± 0.1	26.4 ± 2.5^{f}	16.4 ± 1.4^{cd}	5.7 ± 0.8^{a}	n.d.	20.7 ± 2.9^{aef}
OO	4.3 ± 0.8^{abdef}	2.8 ± 0.3^{bef}	25.8 ± 2.3^{f}	1.4 ± 0.3	25.4 ± 2.3^{f}	22.4 ± 1.7^{abdef}	5.8 ± 1.2	n.d.	10.0 ± 3.9
SO	1.1 ± 0.3^{c}	3.2 ± 1.0	32.6 ± 2.3^{def}	2.0 ± 0.2^{d}	27.6 ± 3.4^{f}	9.6 ± 1.6^{abcf}	12.4 ± 4.5	n.d.	12.1 ± 3.1
EPO	0.7 ± 0.1^{c}	3.7 ± 0.1^{bcf}	26.1 ± 1.0^{df}	1.4 ± 0.1^{e}	22.4 ± 0.4	14.4 ± 1.8^{c}	14.0 ± 5.0	4.0 ± 1.3	12.0 ± 2.1^{b}
MO	0.7 ± 0.1^{c}	5.4 ± 0.2^{ace}	40.2 ± 1.2^{abcde}	4.1 ± 1.5	19.1 ± 0.3^{abcd}	14.8 ± 0.1^{cd}	5.5 ± 0.4^{a}	n.d.	9.7 ± 0.3^{b}

*Data are mean ± SEM from at least three animals fed on each diet. n.d. indicates not detected. Statistical significance ($P < 0.05$ at least; one-way analysis of variance) is indicated as ^{a} versus LF, ^{b} versus HCO, ^{c} versus OO, ^{d} versus SO, ^{e} versus EPO, ^{f} versus MO.

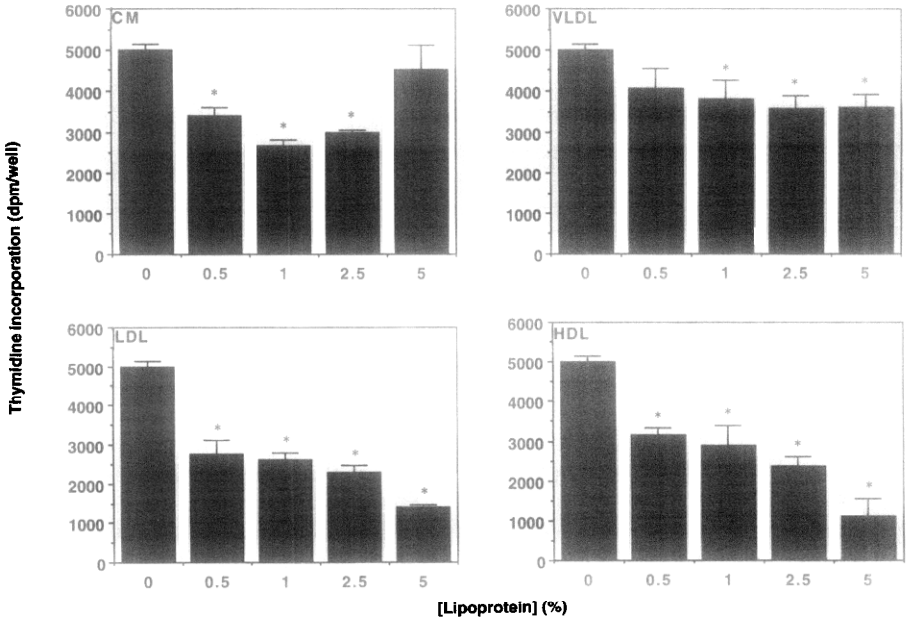

Figure 1 The effect of lipoproteins upon mitogen-stimulated spleen lymphocyte proliferation. Lipoproteins were isolated from the serum of rats fed the LF diet and were incubated with spleen lymphocytes (from LF-fed rats) and Con A (1 μg/ml); the concentration of lipoprotein used was between 0.5% and 5% of the serum concentration. Lymphocyte proliferation was measured as thymidine incorporation over the final 18 hours of a 66-hour culture period. Data (dpm thymidine incorporated/well) are mean ± SEM from three animals. Statistical significance versus no lipoprotein-added control is indicated as *.

vitamin E per kg. The α-tocopherol concentrations in the plasma of rats fed the different high fat diets were not different when the concentrations of cholesterol and triacylglycerol were taken into account (*Table 3*).

Studies involving healthy human volunteers have shown that diets containing *n*-3 PUFA decrease serum triacylglycerol levels but do not affect serum cholesterol levels, diets containing *n*-9 monounsaturated fatty acids decrease serum

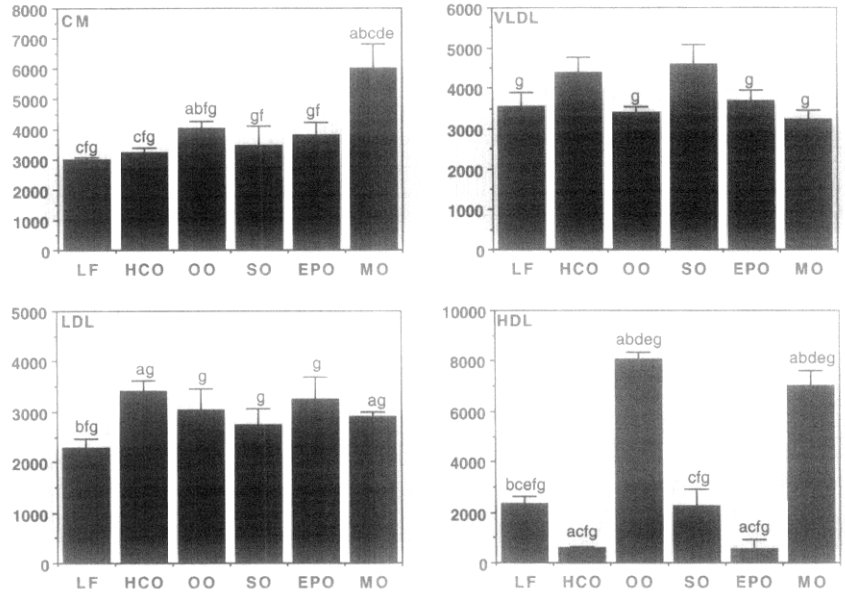

Figure 2 The effect of lipoproteins from rats fed different lipids on mitogen-stimulated lymphocyte proliferation. Lipoproteins were isolated from the serum of rats fed the diets described in Materials and Methods and were incubated with spleen lymphocytes (from LF-fed rats) and Con A (1 μg/ml); the concentration of lipoprotein used was 2.5% of the serum concentration. Lymphocyte proliferation was measured as thymidine incorporation over the final 18 hours of a 66-hour culture period. Data (dpm thymidine incorporated/well) are mean ± SEM from three animals fed on each diet. Thymidine incorporation into lymphocytes cultured in the absence of lipoprotein (i.e., the no lipoprotein-added control) was 5,004 ± 135 dpm/well. Statistical significance is indicated as a versus LF, b versus HCO, c versus OO, d versus SO, e versus EPO, f versus MO, g versus no lipoprotein-added control.

triacylglycerol levels with either a decrease or no change in serum cholesterol levels, whereas diets rich in saturated fatty acids increase serum triacylglycerol and serum cholesterol levels.[2,3] The effects of feeding rats the diets used in the current study on the serum concentrations of cholesterol and triacylglycerol have been previously reported.[13] Rats fed the HCO or OO diets had increased serum triacylglycerol concentrations compared with rats fed the LF, SO, EPO, or MO diets.[13] Thus, the MO diet failed to produce a hypotriacylglycerolaemic effect and the OO diet had a hypertriacylglycerolaemic effect. Compared with each of the other diets, the MO diet resulted in a lower serum total cholesterol concentration.[13] These observations are in contrast to those made in human studies, except where very large amounts of *n*-3 PUFA were ingested.[2] However, in accordance with these results, Høstmark et al.[12] reported lower serum triacylglycerol concentrations in rats fed on fish oil compared with those fed on coconut oil and DeSchrijver et al.[11] reported lower serum triacylglycerol concentrations in rats fed fish oil compared with those fed on beef tallow (rich in saturated fatty acids). Furthermore, both studies also reported lower serum cholesterol concentrations in the fish oil-fed rats compared with those fed coconut oil[12] or beef tallow[11]; again these findings are consistent with the results reported in our study. The apparently different effects of a fish oil-rich diet on serum cholesterol concentrations in rats and in humans may be at least partly due to the species differences in cholesterol metabolism; such differences indicate that the rat is not a good model for the investigation of lipoprotein metabolism in man.

The current study shows that the hypocholesterolaemic effect of the MO diet[13] is largely caused by a lowered level of HDL cholesterol (*Table 4*). This indicates that the MO diet may decrease the synthesis of HDL or increase its removal from the plasma. Apo A-1 is a major apoprotein component of HDL and the concentration of Apo A-1 was lower in HDL from MO-fed animals (*Table 4*). Therefore, the MO diet could affect HDL synthesis by influencing Apo A-1 availability; indeed feeding rats this diet has been shown to significantly lower Apo A-1 mRNA levels in the liver.[56] The hypertriacylglycerolaemic effects of feeding the HCO or OO diets are due to elevated levels of triacylglycerol in the CM fraction and in both the CM and VLDL fractions, respectively (*Table 4*). The effect of the HCO diet on CM triacylglycerol levels was unexpected because this diet contains a high proportion of medium chain fatty acids (*Table 2*) that might be expected to enter the hepatic portal vein directly rather than be assembled into CM. The fatty acid composition of the CM fraction from HCO-fed rats (*Table 5*) clearly indicates that medium chain fatty acids such as lauric acid (12:0) are incorporated into CM. The elevated VLDL triacylglycerol level after feeding the OO diet indicates that, in rats, oleic acid might promote hepatic VLDL synthesis.

The fatty acid composition of each lipoprotein fraction was modified according to that of the lipid consumed in the diet; the greatest effects were seen in the CM and VLDL fractions, whose fatty acid compositions differed significantly between animals fed different lipids (*Tables 5, 6*). Because CM transport triacylglycerol of dietary origin, the marked effects of dietary lipids on the fatty acid composition of this fraction are not surprising. VLDL contain triacylglycerol that is synthesised in the liver (although some VLDL is released from absorptive cells of the small intestine); the fatty acid components of these triacylglycerols could be derived from de novo synthesis but this is unlikely in animals fed a diet that includes 20% fat. Alternatively, the fatty acids found in the triacylglycerols of VLDL could come from other lipoproteins (e.g., chylomicron remnants) or free fatty acids taken up by the liver. The fatty acid composition of lipoprotein-free serum from these animals, which presumably represents that of the non-esterified fatty acids, strongly reflects the fatty acid composition of the diets themselves,[36] as does the fatty acid composition of the adipose tissue.[13] Thus, if the fatty acids of the VLDL triacylglycerols are derived from circulating non-esterified fatty acids, it is no surprise that the fatty acid composition of VLDL is strongly influenced by diet.

The fatty acid compositions of the LDL and HDL fractions are less markedly affected by diet than the CM and VLDL fractions; however a number of differences were observed (*Tables 7, 8*). Because the fatty acids found in HDL and LDL are likely to be components of either phospholipids or cholesterol esters, it is possible that the fatty acid composition of these lipoproteins are less sensitive to dietary manipulation than the lipoprotein fractions responsible for triacylglycerol transport. However, it is likely that after 10 weeks on a particular dietary regime, the fatty acid precursors used for the synthesis of phospholipids will reflect those present in the diet. Thus, the observed diet-induced changes in the fatty acid compositions of HDL and LDL are not unexpected.

Lymphocytes are thought to possess receptors for various types of lipoprotein[41,46]; in addition lymphocytes possess lipoprotein lipase activity[23] and so can remove fatty acids from circulating lipoproteins. A number of studies have previously investigated the effect of purified lipoproteins upon lymphocyte proliferation.[37–46] Several of these studies agree that VLDL and LDL inhibit lymphocyte proliferation,[37–43,45] although there are also reports of enhancement by these lipoproteins.[44,46] Similarly, although some studies report inhibition of lymphocyte proliferation by purified HDL,[38,42] other studies show little effect[41,45] or enhancement.[44,46] When surveying the previous studies, it is apparent that culture conditions are important in determining the overall effect observed. In the current study, identical conditions were used to investigate the effects of each lipoprotein; the culture medium was serum-free but included albumin, transferrin, and insulin at appropriate concentrations. We found that each class of lipoprotein can inhibit rat spleen lymphocyte proliferation in response to Con A (*Figure 1*), and that LDL and HDL have more potent effects than CM or VLDL. Thus, the current results agree with the previous studies that show potent inhibition of lymphocyte proliferation by LDL[37–40,43] and HDL.[42] Discrepancies between the results presented here and those of some previous studies may relate to differences in the cell culture conditions used; it is apparent that the outcome of lymphocyte proliferation assays is affected by the types of growth factors that are present,[44] the presence or absence of serum,[36] the origin and concentration of any serum used,[15] and the concentration of lipoprotein used (*Figure 1*).

Whether the type of lipid consumed in the diet can influence the effect of different lipoproteins on lymphocyte proliferation has not been previously investigated. The results of the current study show that fish oil feeding can alter the effects of CM or HDL on lymphocyte proliferation and that OO feeding can alter the effects of HDL (*Figure 2*). Whereas CM and HDL from rats fed a LF diet or high fat diets rich in saturated or *n*-6 PUFAs inhibit lymphocyte proliferation, CM or HDL from MO-fed rats enhance lymphocyte proliferation (*Figure 2*). Feeding the OO or MO diets has been shown to significantly inhibit lymphocyte functions[15,28–33] and we speculated that the effects of these dietary lipids might be mediated by lipoproteins. Therefore, the observation that some lipoprotein fractions from OO- or MO-fed rats markedly enhance lymphocyte proliferation was surprising. Nevertheless, it was a consistent observation.

The effects of lipoproteins on lymphocyte proliferation indicate that they contain components that are suppressive; these may be lipids or apolipoproteins. Because fatty acids[16–22,24,25] and triacylglycerols[23,26,27] inhibit lymphocyte functions in vitro, it would not be surprising if lipid components of lipoproteins were also inhibitory. However, in vitro studies indicate that *n*-3 PUFAs are the more inhibitory than other types of fatty acids[17–22] and so it might be expected that lipoproteins from animals fed the MO diet would be more suppressive than those from animals fed the other diets. This was found not to be the case; VLDL and LDL from MO-fed animals have the same effect as those from animals fed the other diets, whereas HDL and CM from MO-fed animals are enhancing compared with those from animals fed the other diets. The stimulatory effects of CM and HDL from MO-fed animals indicates that these lipoproteins contain lower levels of suppressive factors and/or higher levels of stimulatory factors than those from animals fed the other diets. Both CM and HDL contain Apo A-1, and MO feeding resulted in a significantly lower HDL Apo A-1 concentration compared with feeding the other diets. Thus, Apo A-1, or apolipoproteins in general, might be suppressive.

A further explanation for the enhancement of lymphocyte proliferation observed after culture with CM and HDL from MO-fed rats and HDL from OO-fed rats is that the lymphocytes might have efficiently taken up *n*-3 PUFAs or oleic acid from these lipoproteins. This would lead to replacement of arachidonic acid in lymphocyte phospholipids by the *n*-3 PUFAs or oleic acid. As a result, lower levels of PGE_2 would be produced by these cells; PGE_2 is known to inhibit lymphocyte proliferation.[18] Alternatively, these lipoproteins might be enriched in α-tocopherol, which is known to enhance lymphocyte proliferation.[54,55] The α-tocopherol contents of the various lipoprotein fractions were not determined in this study.

The findings of this study suggest that the inhibitory effects of the MO diet, and also of the OO and EPO diets,[15,29–31] on lymphocyte proliferation may not be mediated by lipoproteins. Furthermore, when whole serum is used in cell culture, the stimulatory effect of CM and HDL in the serum of MO-fed rats (and of HDL in the serum of OO-fed rats) must be overridden by a suppressive effect of at least one other serum component. This may be VLDL or LDL. In addition, lipoprotein-free serum from rats fed the HCO, OO, EPO, or MO diets was found to suppress Con A-stimulated proliferation of spleen lymphocytes.[36] This suggests that a non-lipoprotein component of serum is inhibitory. This may be non-esterified fatty acids [*n*-3 PUFAs are potent inhibitors of lymphocyte function][17–22] or a non-lipid component. The nature of the suppressive non-lipoprotein factor warrants further investigation.

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