British Journal of Nutrition (2004), **91**, 341–349
DOI: 10.1079/BJN20031056

Hyperlipidaemic effect of fish oil in Bio F_1B hamsters

Pujitha P. de Silva, Phillip J. Davis and Sukhinder Kaur Cheema*

Department of Biochemistry, Memorial University of Newfoundland, St John's, NL, Canada A1B 3X9

(Received 4 February 2003 – Revised 21 September 2003 – Accepted 3 November 2003)

We investigated the dietary influence of low and high levels of fish oil, supplemented with or without dietary cholesterol, on the plasma lipoprotein profile in Bio F_1B hamsters, a model susceptible to diet-induced hyperlipidaemia. The MIX diet, a diet supplemented with a mixture of lard and safflower-seed oil, was used as the control diet to maintain the saturated MUFA and PUFA levels similar to the fish-oil diet. The animals were fed the specific diets for 2 weeks and fasted for 14 h before killing. The plasma from the animals fed high levels of fish oil was milky and rich in chylomicron-like particles. The plasma total cholesterol, VLDL- and LDL-cholesterol and -triacylglycerol concentrations were significantly higher, whereas HDL-cholesterol was lower in hamsters fed fish oil compared with the MIX-diet-fed hamsters. Increasing the amount of fat in the diet increased plasma lipids in both the fish-oil- and the MIX-diet-fed hamsters; however, this hyperlipidaemic effect of dietary fat level was greater in the hamsters fed the fish-oil diet. The hepatic lipid concentrations were not dramatically different between the fish-oil-fed and the MIX-diet-fed hamsters. However, the hepatic LDL-receptor mRNA levels were significantly low in the fish-oil-fed hamsters compared with the MIX-diet-fed hamsters. Increasing the amount of fish oil in the diet further decreased the hepatic LDL-receptor mRNA expression. It is concluded that F_1B hamsters are susceptible to fish-oil-induced hyperlipidaemia, especially at high fat levels, and this increase is partially explained by the inhibition of hepatic LDL-receptor mRNA expression.

Fish oil: Bio F_1B hamsters: Lipoproteins: Chylomicrons

Epidemiological studies as well as human trials have suggested that fish oil is beneficial in lowering the risk of CHD (Bang *et al.* 1971; Harris, 1997). This is mainly attributed to the biological effects of *n*-3 PUFA, such as eicosapentaenoic and docosahexaenoic acid, found in fish oil (Kagawa *et al.* 1982; Dyerberg, 1986). The anti-atherogenic effect of these fatty acids is suggested to be due to modifications of lipid and lipoprotein metabolism (Drevon, 1992). The *n*-3 PUFA decrease plasma triacylglycerol and VLDL levels (Nestel *et al.* 1984; Wong & Nestel, 1987). This effect is due to decreased hepatic triacylglycerol synthesis (Nossen *et al.* 1986), triacylglycerol secretion (Nestel *et al.* 1984) and assembly of VLDL (Wilkinson *et al.* 1998).

The effect of *n*-3 PUFA on triacylglycerol and VLDL concentrations is well known; however, the regulation of LDL- and HDL-cholesterol concentrations by fish oil is not clear. This may be attributed to several factors, i.e. variations in individuals in human trials, lack of control of confounding factors, pre-existing dyslipidaemias etc. Normolipidaemic subjects show a reduction of plasma LDL-cholesterol and triacylglycerol concentrations following the intake of a fish-oil diet (Nestel, 1986). However, with hypertriacylglycerolaemic subjects, fish-oil supplementation causes an increase in LDL-cholesterol concentrations (Sullivan *et al.* 1986; Hsu *et al.* 2000).

Although fish-oil consumption is linked to protection against CHD by lowering plasma lipid levels, and this information is confirmed in both human and some animal models, studies in hamsters show opposite results (Surette *et al.* 1992; Lin *et al.* 1995; Kubow *et al.* 1996; Lu *et al.* 1996). These investigators reported a hyperlipidaemic effect of fish oil in Golden Syrian hamsters, which was more obvious in hamsters fed a fish-oil diet supplemented with cholesterol. A similar effect was observed with an *n*-6 PUFA diet supplemented with cholesterol; however the effect of the fish-oil diet given along with cholesterol was significantly higher compared with *n*-6 PUFA diets (Lu *et al.* 1996). The hyperlipidaemic effect of fish oil was dependent on the level of dietary cholesterol (CHOL) and *n*-3 PUFA content (Surette *et al.* 1992).

Hamsters have circulating LDL levels and show responses to dietary fats that are comparable with humans (Spady & Dietschy, 1985, 1988; Ohtani *et al.* 1990). The Golden Syrian hamster is extensively used for the study of diet-induced regulation of lipoprotein metabolism as the lipoprotein profile of hamsters is more similar to humans than mice and rats. Another hamster strain that is highly susceptible to diet-induced hyperlipidaemia is the Bio F_1B hamster. The F_1B hamster is a hybrid strain that develops aortic atherosclerotic lesions at much lower CHOL concentrations (Kowala *et al.* 1991), and is

Abbreviations: CHOL, dietary cholesterol; DL, dietary fat level; DT, diet type; MIX diet, mixed diet (supplemented with lard and safflower-seed oil); RBC, erythrocyte.

* **Corresponding author:** Dr Sukhinder Kaur Cheema, fax + 1 709 737 2422, email skaur@mun.ca

suggested to develop type 2 diabetes mellitus on high-fat diets. However, there is very little information available on the diet-induced regulation of lipoprotein metabolism in Bio F_1B hamsters. Whether fish oil, along with low and high levels of cholesterol, induces hyperlipidaemia in F_1B hamsters, in a similar way to the Golden Syrian hamster, is not known; nor are the mechanisms involved known.

The present study was designed to investigate the changes in circulating plasma cholesterol levels in Bio F_1B hamsters over a range of fish-oil and cholesterol intake. The effect of fish-oil and cholesterol consumption on the hepatic lipids and LDL-receptor mRNA expression was also determined to investigate the possible mechanisms involved. Our results show that fish oil causes hyperlipidaemia in F_1B hamsters, and this effect is partially explained due to the inhibition of hepatic LDL-receptor gene expression.

Materials and methods

Animals and diets

The Bio F_1B hamsters (7 weeks old) were obtained from Bio Breeders Inc. (Water Town, MA, USA) and kept on a chow diet for 1 week before feeding the specific diets. After this equilibration period, the hamsters were divided into eight groups (*n* 12) and each group was fed with one of the specified diets. The specified diets consisted of a fat-free semi-purified diet (ICN Biomedical Inc., Aurora, OH, USA) that was supplemented with either fish oil (menhaden oil; Sigma Chemical Co., St Louis, MO, USA) or a mixture of lard and safflower-seed oil in a 1·5:1 ratio (MIX diet) from a local supermarket. The composition of the diets is given in Table 1. The fat content of the diets was either 50 g/kg (low fat) or 200 g/kg (high fat). Due to the presence of cholesterol in the fish oil, the low-fat fish-oil diet contained 0·25 g cholesterol/kg, and the high-fat fish-oil diet contained 1 mg cholesterol/kg. Thus, the same amount of cholesterol was added to the low-fat and the high-fat MIX diets to keep the cholesterol content similar. To get high cholesterol concentrations, the fish-oil and MIX diets were supplemented with additional cholesterol to bring the final concentration of cholesterol to 2·5 g/kg. The highest purity grade finely powdered cholesterol (98% pure; Sigma-Aldrich, St Louis, MO, USA) was dissolved in fat and added to the diets. Lipids were extracted from the diets and the fatty acid composition was analysed using GLC (Keough & Davis, 1979). The fatty acid composition of the diets is presented in Table 2. Diets were prepared and stored at −20°C. The animals were maintained on the specific diets for 2 weeks *ad libitum* and were given fresh diets daily. Food intake was measured daily during the study period, and body weight was checked at the beginning of the study period, 1 week later and at the conclusion of the study. There was no difference in food intake and body-weight gain between the different diet groups. All animals were housed in individual cages in a single room with an enriched environment. The room was lit from 07.00 to 19.00 hours, with the temperature maintained at 21°C and humidity at 35 ± 5 %. The Institutional Animal Care Use Committee approved all experimental procedures, which are in accordance with the principles and guidelines of the Canadian Council on Animal Care.

Plasma lipid and lipoprotein profile

On day 15, after 14 h of fasting, the animals were anaesthetised and blood was collected by cardiac puncture into tubes containing EDTA (1 g/l). Blood was centrifuged at 3000 ***g*** for 10 min to separate the plasma. Plasma and erythrocytes (RBC) were collected separately. Plasma lipoprotein fractions, i.e. VLDL, LDL and HDL, were isolated by sequential density ultracentrifugation (Salter *et al.* 1998) using a TL 100 fixed angle rotor. The VLDL, LDL and HDL fractions were separated within the density

Table 1. Composition of the semi-purified diets designed for low fat and high fat level (g/kg)

	Low-fat diet*				High-fat diet†			
	Fish oil		MIX‡		Fish oil		MIX‡	
Ingredients§	Low chol	High chol	Low chol	High chol	Low chol	High chol	Low chol	High chol
Casein	200	200	200	200	200	200	200	200
DL-Methionine	3	3	3	3	3	3	3	3
Sucrose	500	500	500	500	305	305	305	305
Maize starch	146	146	146	146	190	190	190	190
Vitamin mix‖	11	11	11	11	11	11	11	11
Mineral mix‖	40	40	40	40	40	40	40	40
Fibre¶	50	50	50	50	50	50	50	50
Fat	50	50	50	50	200	200	200	200
Chol	0·25	2·5	0·25	2·5	1	2·5	1	2·5

Chol, cholesterol.
* Semi-purified diet designed for a 50 g/kg fat level.
† Semi-purified diet designed for a 200 g/kg fat level.
‡ Control diet supplemented with a mixture of lard and safflower-seed oil.
§ Ingredients were from ICN Biomedicals, Cleveland, OH, USA.
‖ Supplied in quantities adequate to meet requirements (National Research Council, 1995).
¶ Cellulose was supplied as Alphacel non-nutritive bulk (ICN Biomedicals Inc., Aurora, OH, USA).

Table 2. Fatty acid composition of the diets (g/kg)*

Fatty acids	Fish oil	MIX†
14:0	96	10
16:0	193	193
16:1*n*-7	131	20
18:0	38	100
18:1	138	310
18:2*n*-6	27	340
18:3*n*-3	45	30
18:4*n*-3	34	ND
20:1*n*-9	16	ND
20:4*n*-6	10	ND
20:5*n*-3	129	ND
22:5*n*-3	24	ND
22:6*n*-3	120	ND
ΣSFA	320	300
ΣMUFA	280	320
ΣPUFA	380	370
Σ*n*-3 PUFA	350	30
Σ*n*-6 PUFA	30	340

ND, not detected; SFA, saturated fatty acids.
* Lipids were extracted from the diets and fatty acid composition was determined by GLC.
† Control diet supplemented with a mixture of lard and safflower-seed oil.

ranges of $<1{\cdot}006$, $1{\cdot}006-1{\cdot}060$ and $>1{\cdot}060$ g/ml respectively. The hamsters fed the high-fat fish-oil diet had milky plasma and contained chylomicron-like particles, which were isolated from the plasma by spinning at 15 500 ***g*** for 20 min at 12°C. The remaining lipoproteins were then separated as described earlier. The individual lipoprotein fractions were stored at 4°C. Lipids were extracted from RBC and liver samples as described previously (Yokode *et al.* 1990). The fatty acid composition of the RBC was analysed using GLC (Keough & Davis, 1979). The fatty acid composition of the RBC was reflective of the dietary fatty acid composition, indicating that the length of the experimental feeding period was sufficient to induce significant changes in the fatty acid composition of the tissues (data not shown).

The plasma, liver and individual lipoprotein fractions were analysed for total cholesterol and total triacylglycerol concentrations using cholesterol assay kit no. 402 and triacylglycerol assay kit no. 344 (Sigma Diagnostics Inc., St Louis, MO, USA). Free cholesterol was assayed in the plasma, liver and individual lipoprotein fractions using a free cholesterol assay kit (Wako Chemicals, Richmond, VA, USA). Cholesteryl ester concentration was determined by subtracting the free cholesterol concentration from total cholesterol concentration (Friedewald *et al.* 1972).

Hepatic low-density lipoprotein-receptor mRNA expression

Total RNA from hamster livers was isolated using the Fast-RNA Kit (Qbiogene, Carlsbad, CA, USA) and stored at −20°C. Hepatic LDL-receptor mRNA abundance was determined by reverse transcription and *in vitro* PCR amplification. Complementary DNA was synthesised from total liver RNA (2 μg) using Superscript™ II reverse transcriptase and used as templates for *in vitro* DNA amplification. LDL-receptor (Bennett *et al.* 1995) and β-actin mRNA sequences were simultaneously amplified using hamster-specific primers. No amplification products were detectable in the absence of reverse transcriptase. The total number of cycles for each PCR reaction was chosen to remain within the exponential phase of the reaction. All PCR reactions were performed in triplicate, and the products were resolved on 1·2 % agarose gel. The representative bands were quantified using a gel documentation system. The amount of LDL-receptor mRNA was normalised to β-actin mRNA content and expressed as relative units.

Statistical analysis

The effect of diet type (DT), dietary fat level (DL) and CHOL was assayed using three-way ANOVA, and a Tukey's *post hoc* test was used to test significant differences revealed by the ANOVA. Values are group means and standard deviations (n 12); differences were considered statistically significant if the associated P value was $<0{\cdot}05$ (Steel & Torrie, 1980).

Results

Plasma lipids

The plasma total cholesterol, free cholesterol, cholesteryl esters and triacylglycerol concentrations of the hamsters fed the various diets are given in Fig. 1 (A)–(D). The standard deviation for some parameters was large, especially for the hamsters fed the high-fat fish-oil diets with added cholesterol. The plasma lipid concentrations dramatically increased in the fish-oil-fed hamsters; thus the samples were diluted several-fold to obtain values in the linear range, which might have caused such variation. The hamsters fed the fish-oil diet had significantly higher levels of plasma total cholesterol, free cholesterol, cholesteryl ester and triacylglycerol compared with the hamsters fed the MIX diet ($P=0{\cdot}0001$). Irrespective of the DT, the hamsters fed the high-fat diets had significantly higher levels of total cholesterol, free cholesterol, cholesteryl ester and triacylglycerol concentrations compared with the hamsters fed the low-fat diets ($P=0{\cdot}0001$). However, this hyperlipidaemic effect of DL was greater in the hamsters fed the fish-oil diet (DT × DL interaction, $P=0{\cdot}0001$).

The addition of cholesterol to the diets showed an increase in plasma total cholesterol, free cholesterol, cholesteryl ester and triacylglycerol concentrations in both the fish-oil- and MIX-diet-fed hamsters (Fig. 1 (A)–(D)). However, this increase is prominent in the fish-oil-diet-fed hamsters (DT × CHOL interaction for total cholesterol, $P=0{\cdot}0003$; free cholesterol, $P<0{\cdot}03$; cholesteryl ester, $P<0{\cdot}002$; triacylglycerol, $P<0{\cdot}03$). Furthermore, the effect of CHOL on plasma total cholesterol, cholesteryl ester, and triacylglycerol was greater in the hamsters fed the high-fat diet compared with those fed the low-fat diet (DL × CHOL interaction; $P<0{\cdot}002$, $P=0{\cdot}002$, and $P<0{\cdot}02$ respectively).

Lipoprotein lipid profile

The hamsters fed the high-fat fish-oil diets had milky plasma and contained very high levels of chylomicron-like

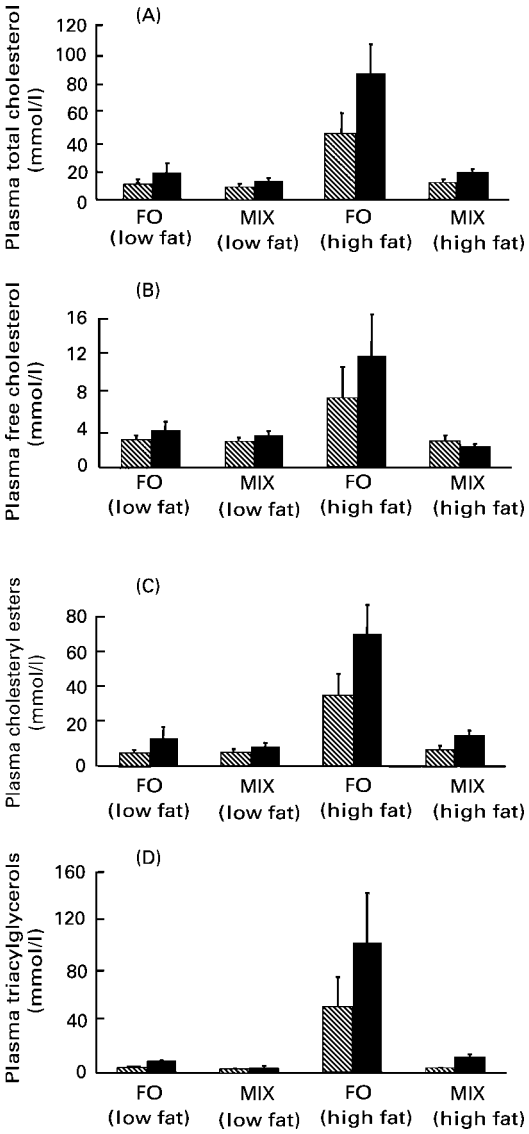

Fig. 1. Increasing the amount of fish oil (FO) and cholesterol in the diet increases plasma lipid concentrations in Bio F_1B hamsters. Hamsters were fed a FO diet or a control diet supplemented with lard and safflower-seed oil (MIX diet), at fat levels of 50 g/kg (low fat) or 200 g/kg (high fat), in the absence (▨) or presence (■) of 2·5 g cholesterol/kg. Total cholesterol (A), free cholesterol (B), cholesteryl ester (C) and triacylglycerol (D) concentrations were analysed as described on p. 343. Differences between groups were evaluated using three-way ANOVA (*n* 12). Mean values are shown, with standard deviations indicated by vertical bars.

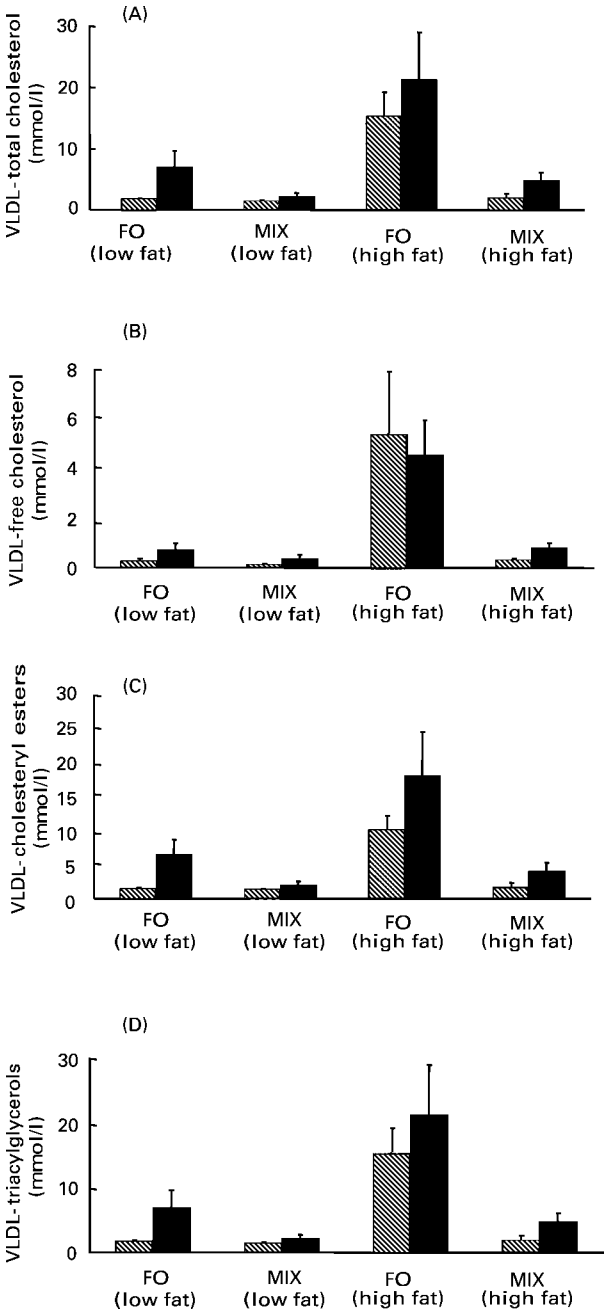

Fig. 2. Fish-oil (FO) feeding increases the VLDL-total cholesterol and -triacylglycerol concentrations in Bio F_1B hamsters. Hamsters were fed a FO diet or a control diet supplemented with lard and safflower-seed oil (MIX diet), at fat levels of 50 g/kg (low fat) or 200 g/kg (high fat), in the absence (▨) or presence (■) of 2·5 g cholesterol/kg. Total cholesterol (A), free cholesterol (B), cholesteryl ester (C) and triacylglycerol (D) concentrations were analysed as described on p. 343. Differences between groups were evaluated using three-way ANOVA (*n* 12). Mean values are shown, with standard deviations indicated by vertical bars.

particles, which were rich in triacylglycerols and cholesterol, irrespective of the absence or presence of cholesterol (data not shown). Marked differences in VLDL composition were seen in the hamsters fed the different diets (Fig. 2 (A)–(D)). There was a significant increase in VLDL-total cholesterol, -free cholesterol, -cholesteryl ester and -triacylglycerol concentrations in the fish-oil-diet-fed hamsters compared with the MIX-diet-fed hamsters. Increasing the DL from low fat to high fat resulted in a significant increase in VLDL lipid concentrations in both the fish-oil- and the MIX-diet-fed hamsters. This DL-dependent increase was greater in the fish-oil-diet-fed hamsters compared with the MIX-diet-fed hamsters (DT × DL interaction for total cholesterol, $P=0{\cdot}0001$; free cholesterol, $P=0{\cdot}0001$; cholesteryl ester, $P=0{\cdot}0001$; triacylglycerol, $P<0{\cdot}02$). Dietary supplementation of cholesterol resulted in an increase in VLDL-total cholesterol, -cholesteryl ester and -triacylglycerol concentrations in both the fish-oil- and MIX-diet-fed

hamsters. The CHOL-induced increase in VLDL-cholesteryl ester concentration was prominent in the fish-oil-diet-fed hamsters compared with the MIX-diet-fed hamsters (DT × CHOL interaction, $P<0{\cdot}006$).

The LDL from the fish-oil-diet-fed hamsters showed higher concentrations of total cholesterol, cholesteryl ester and triacylglycerol compared with the MIX-diet-fed hamsters ($P=0{\cdot}0001$) (Fig. 3 (A)–(D)). Increasing the DL of fish oil caused a significant increase in LDL-free cholesterol and -triacylglycerol (DT × DL interaction, $P<0{\cdot}0001$), whereas a significant decrease was observed for LDL-total cholesterol and -cholesteryl ester concentrations (DT × DL interaction, $P<0{\cdot}002$ and $P<0{\cdot}03$ respectively). There was no effect of DL on LDL-total cholesterol, -cholesteryl ester or -triacylglycerol concentrations in the MIX-diet-fed hamsters.

Cholesterol supplementation of the fish-oil diet resulted in an increase in LDL-cholesterol and -cholesteryl ester concentrations (DT × CHOL interaction, $P=0{\cdot}0007$ and $P=0{\cdot}0001$ respectively) (Fig. 3 (A) and (C)). The CHOL-induced increase in LDL-triacylglycerol concentration was observed only in the hamsters fed the low-fat fish-oil diet (DL × CHOL interaction, $P<0{\cdot}02$). Furthermore, the effect of CHOL was greater in the hamsters fed the low-fat fish-oil diet for total cholesterol (DT × DL × CHOL interaction, $P<0{\cdot}004$) and cholesteryl ester concentrations (DL × CHOL interaction, $P=0{\cdot}0004$).

Changes in HDL lipid composition were mainly attributed to the changes in cholesterol concentrations (Fig. 4 (A)–(D)). The fish-oil-diet-fed hamsters had significantly lower concentrations of HDL-total cholesterol, -free cholesterol and -cholesteryl ester compared with the MIX-diet-fed hamsters ($P=0{\cdot}0001$). However, there was no significant effect of fish oil on HDL-triacylglycerol concentrations. Increasing the DL resulted in a decrease in HDL-total cholesterol and -cholesteryl ester concentrations in the fish-oil-diet-fed hamsters (DT × DL interaction, $P=0{\cdot}0001$), and an increase in these parameters in the MIX-diet-fed hamsters (Fig. 4 (A) and (C)). The cholesterol supplementation of diets resulted in an increase in HDL-total cholesterol and -cholesteryl ester concentrations irrespective of the DT ($P<0{\cdot}001$).

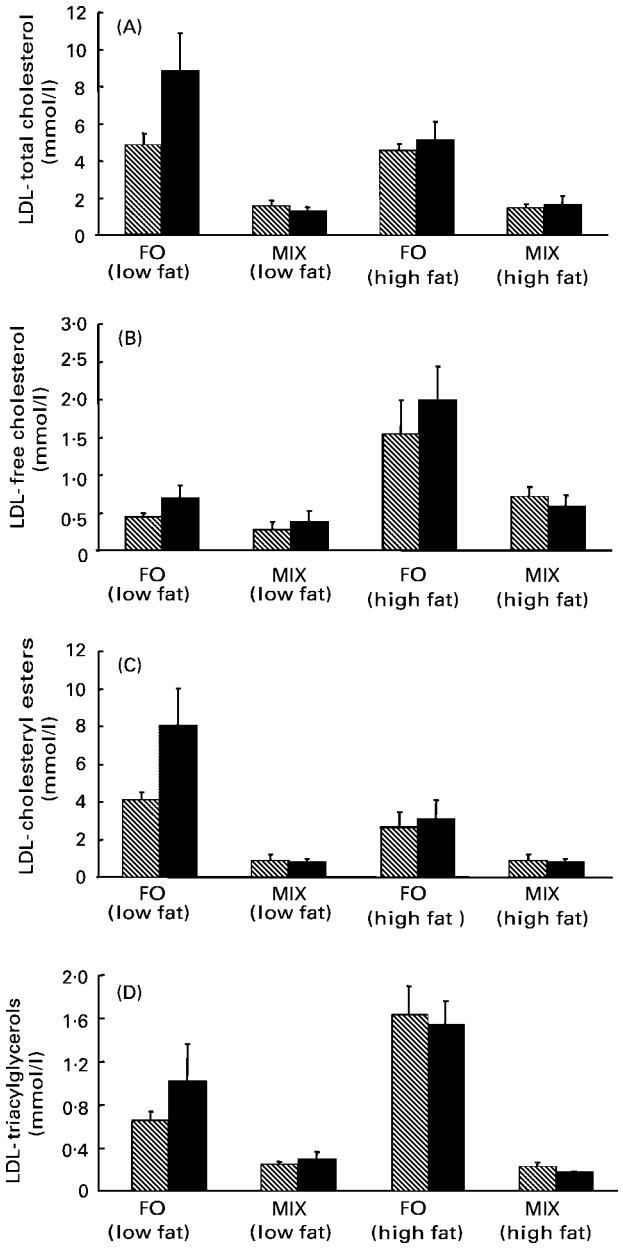

Fig. 3. The concentration of LDL lipids is higher in fish-oil (FO)-diet-fed Bio F_1B hamsters. Hamsters were fed a FO diet or a control diet supplemented with lard and safflower-seed oil (MIX diet), at fat levels of 50 g/kg (low fat) or 200 g/kg (high fat), in the absence (▨) or presence (■) of 2·5 g cholesterol/kg. Total cholesterol (A), free cholesterol (B), cholesteryl ester (C), and triacylglycerol (D) concentrations were analysed as described on p. 343. Differences between groups were evaluated using three-way ANOVA (*n* 12). Mean values are shown, with standard deviations indicated by vertical bars.

Hepatic lipid profile

Changes in hepatic lipid composition are shown in Table 3. The hamsters fed the low-fat fish-oil diet showed lower hepatic total cholesterol, cholesteryl ester and triacylglycerol levels compared with the hamsters fed the low-fat MIX diet ($P<0{\cdot}02$). In contrast, the hamsters fed the high-fat fish-oil diet showed significantly higher levels of total cholesterol, cholesteryl esters and triacylglycerols compared with those fed the high-fat MIX diet (DT × DL interaction, $P<0{\cdot}0001$). Increasing the DL in the fish-oil-diet-fed hamsters resulted in an increase in hepatic total cholesterol, cholesteryl ester and triacylglycerol concentrations (DT × DL interaction, $P<0{\cdot}0001$), whereas no significant effect was observed in the MIX-diet-fed hamsters.

The addition of cholesterol to the diets increased the hepatic total cholesterol and cholesteryl ester concentrations ($P=0{\cdot}0004$), and this effect was more prominent in the fish-oil-diet-fed hamsters (DT × DL interaction, $P<0{\cdot}05$). The effect of CHOL on hepatic cholesteryl ester levels was greater in the hamsters fed a low-fat diet (DL × CHOL interaction, $P<0{\cdot}03$) compared with those fed a high-fat diet. Only the fish-oil-diet-fed hamsters showed a CHOL-induced elevation of hepatic triacylglycerol concentrations (DT × CHOL interaction, $P<0{\cdot}03$).

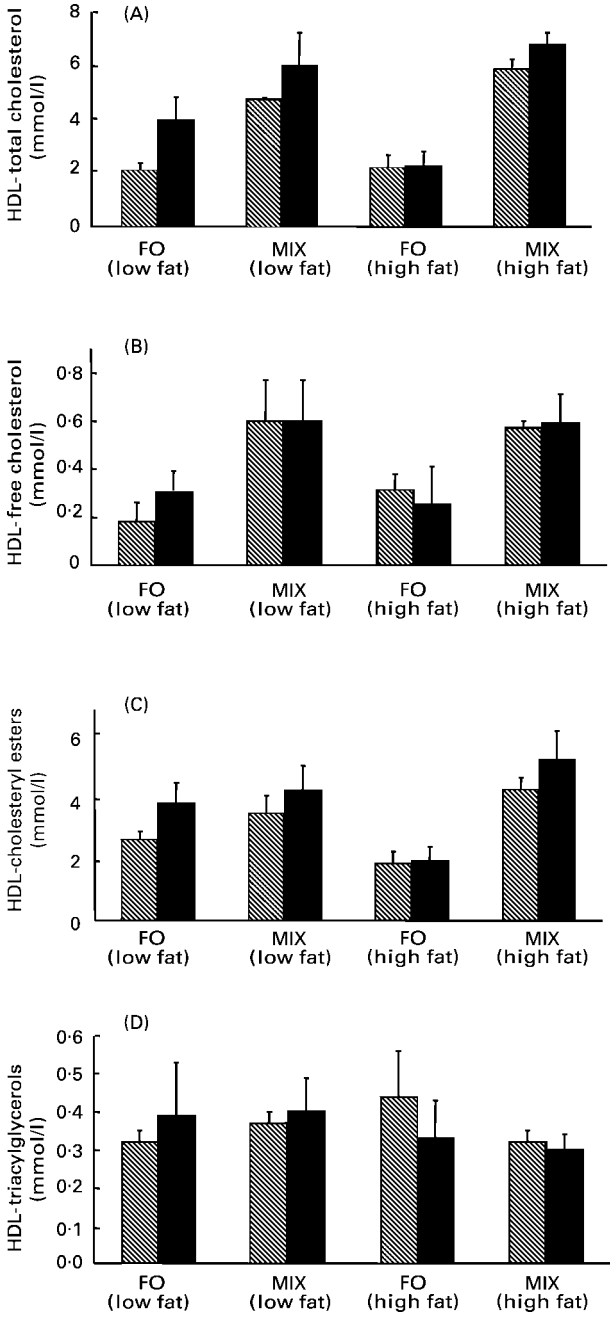

Fig. 4. Fish-oil (FO) feeding reduces HDL-cholesterol concentrations in Bio F_1B hamsters. Hamsters were fed a FO diet or a control diet supplemented with lard and safflower-seed oil (MIX diet), at fat levels of 50 g/kg (low fat) or 200 g/kg (high fat), in the absence (▨) or presence (■) of 2·5 g cholesterol/kg. Total cholesterol (A), free cholesterol (B), cholesteryl ester (C), and triacylglycerol (D) concentrations were analysed as described on p. 343. Differences between groups were evaluated using three-way ANOVA (*n* 12). Mean values are shown, with standard deviations indicated by vertical bars.

Hepatic low-density lipoprotein-receptor mRNA expression

The LDL-receptor mRNA expression from the fish-oil- and MIX-diet-fed hamsters is given in Fig. 5. The LDL-receptor mRNA expression was lower in the fish-oil-diet-fed hamsters compared with the MIX-diet-fed hamsters ($P<0·004$). Increasing the DL of fish oil further reduced the LDL-receptor mRNA expression ($P=0·001$). The addition of cholesterol to the fish-oil diet had no significant effect on LDL-receptor mRNA expression, whereas the addition of cholesterol to the MIX diet reduced the LDL-receptor mRNA expression (DT × CHOL interaction, $P<0·0001$).

Discussion

The regulation of plasma cholesterol levels by dietary fish oil is still controversial and hotly debated. Subpopulations with type 2 diabetes mellitus, and hyperlipidaemia, respond to high levels of fish oil with hypercholesterolaemia, instead of the expected hypocholesterolaemic effect. The Bio F_1B hamster, a hybrid strain, is highly susceptible to diet-induced hyperlipidaemia (Kowala *et al.* 1991), and is suggested to be a better animal model to study the dietary regulation of lipoprotein metabolism due to the similarity of the response in humans. We used this animal model to study the regulation of lipoprotein metabolism by dietary fish oil, to resolve some of the controversial issues. We investigated the effect of fish oil, rich in *n*-3 PUFA, on plasma lipoproteins, when fed at low and high levels of fat, in the absence or presence of CHOL, in Bio F_1B hamsters. The ratio of PUFA, MUFA and saturated fatty acids in the control diet was kept consistent with the fish oil; thus the main difference in the diets was the amount of *n*-6 or *n*-3 fatty acids. The results presented in the present paper are the first to demonstrate that high levels of fish oil cause hyperlipidaemia in Bio F_1B hamsters. The high-fat fish-oil diet is not physiological; however, the findings are of interest to understand the properties of fish oil and the genetics of diet-induced hyperlipidaemia.

Previous studies have reported that fish oil induces hypercholesterolaemia in the Golden Syrian hamster, when diets were fed with moderate to high levels of cholesterol, whereas low levels of fish oil reduced plasma lipid levels in these studies (Surette *et al.* 1992; Lin *et al.* 1995; Kubow *et al.* 1996; Lu *et al.* 1996). These authors reported that the hypercholesterolaemic effect of fish oil was dependent on the cholesterol level of the diet and the DL (Surette *et al.* 1992). In the present study, fish oil, even at low levels, did not reduce plasma lipid levels compared with the MIX diet. Increasing the amount of fish oil in the diet caused severe hyperlipidaemia; however the reason for this fish-oil-induced increase in plasma lipid levels is not clear. In human studies, the circulating LDL-cholesterol concentration has been negatively correlated with hepatic LDL-receptor-binding activity and mRNA expression (Soutar *et al.* 1986; Nanjee & Miller, 1989; Wilkinson *et al.* 1998). We found that fish-oil feeding caused a significant increase in plasma LDL-cholesterol levels, and decreased the hepatic LDL-receptor mRNA expression, compared with the MIX diet. Increasing the amount of fish oil in the diet caused further inhibition of LDL-receptor mRNA abundance. These observations suggest that there is an inhibition of the removal of LDL from the circulation due to decreased hepatic LDL-receptor mediated uptake. It was interesting to note that adding cholesterol to the fish-oil diet had no inhibitory effect on

Table 3. Hepatic lipid composition of Bio F_1B hamsters fed various diets (mg/g liver)*
(Mean values and standard deviations)

		Total cholesterol		Free cholesterol		Cholesteryl ester		Triacylglycerol	
Diet type	Fat level g/kg	Mean	SD	Mean	SD	Mean	SD	Mean	SD
FO	50	$7{\cdot}86^{c}$	0·79	$1{\cdot}46^{a}$	0·21	$6{\cdot}40^{b}$	0·81	$4{\cdot}50^{b}$	0·70
FO + cholesterol	50	$10{\cdot}65^{b}$	1·51	$1{\cdot}11^{a}$	0·45	$9{\cdot}54^{a}$	1·14	$5{\cdot}96^{bc}$	1·60
MIX	50	$9{\cdot}30^{d}$	0·85	$1{\cdot}16^{ab}$	0·60	$8{\cdot}10^{c}$	1·15	$5{\cdot}98^{c}$	0·24
MIX + cholesterol	50	$10{\cdot}87^{b}$	1·30	$1{\cdot}07^{a}$	0·39	$9{\cdot}84^{a}$	1·01	$5{\cdot}93^{c}$	0·79
FO	200	$10{\cdot}00^{a}$	1·30	$0{\cdot}90^{b}$	0·23	$9{\cdot}10^{a}$	1·13	$11{\cdot}32^{a}$	2·48
FO + cholesterol	200	$11{\cdot}90^{b}$	2·30	$1{\cdot}30^{a}$	0·30	$10{\cdot}60^{a}$	2·03	$13{\cdot}00^{a}$	2·05
MIX	200	$8{\cdot}20^{cd}$	1·20	$0{\cdot}92^{ab}$	0·12	$7{\cdot}30^{c}$	1·25	$7{\cdot}90^{c}$	1·90
MIX + cholesterol	200	$8{\cdot}09^{c}$	0·90	$0{\cdot}95^{b}$	0·19	$7{\cdot}14^{c}$	0·93	$5{\cdot}60^{c}$	1·45

FO, fish oil; MIX, MIX diet (control diet supplemented with a mixture of lard and safflower-seed oil).
[a,b,c] Mean values within a column with unlike superscript letters were significantly different ($P<0{\cdot}05$).
* Animals were fed the indicated diets for 2 weeks.

LDL-receptor mRNA expression; however cholesterol caused the inhibition of LDL-receptor mRNA levels in the MIX-diet-fed hamsters. It is well established that CHOL inhibits LDL-receptor gene expression (Sudhof *et al.* 1987; Srivastava *et al.* 1995). It is possible that the lack of inhibition of LDL-receptors by cholesterol in the fish-oil-fed hamsters is a compensatory mechanism to remove excess cholesterol from the circulation.

The plasma from the hamsters fed the high-fat fish-oil diets was packed with chylomicron-like particles and very high levels of VLDL-cholesterol and -triacylglycerols. This observation clearly delineates that increases in VLDL and chylomicrons by dietary fish oil is dependent on the DL. Increased apolipoprotein B concentrations were observed by Kubow *et al.* (1996) in hamsters fed high-cholesterol menhaden oil. Thus, there is a possibility that higher VLDL-cholesterol and -triacylglycerol concentrations in fish-oil-fed hamsters are a result of increased hepatic secretion of VLDL. The hepatic triacylglycerol concentrations were significantly elevated in the hamsters fed the high-fat fish oil compared with those fed the low-fat fish oil and the MIX diet. This increase in triacylglycerols might result in an increased assembly and secretion of VLDL from the liver in the hamsters fed the high-fat fish oil (Elam *et al.* 2001; Vasandani *et al.* 2002). The hepatic total cholesterol concentration was lower in the fish-oil-fed hamsters compared with the MIX-diet-fed hamsters, indicating that an increased absorption/and or delivery of cholesterol is not the probable cause for the increased plasma lipid levels in the fish-oil-fed hamsters.

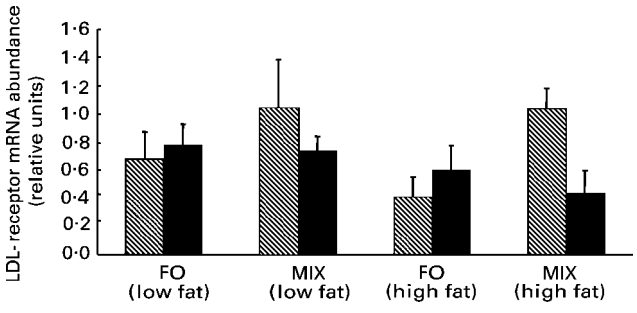

Fig. 5. Fish-oil (FO) feeding inhibits hepatic LDL-receptor gene expression. Bio F_1B hamsters were fed a FO diet or a control diet supplemented with lard and safflower-seed oil (MIX diet), at fat levels of 50 g/kg (low fat) or 200 g/kg (high fat), in the absence (▨) or presence (■) of 2·5 g cholesterol/kg. Total liver RNA was extracted, reverse transcribed and the cDNA template for hamster LDL-receptor and hamster β-actin was amplified as described on p. 343. The amounts of amplified templates were quantified and the abundance of LDL-receptor mRNA was expressed relative to β-actin mRNA. Differences between groups were evaluated using three-way ANOVA (*n* 12). Mean values are shown, with standard deviations indicated by vertical bars.

Increased concentrations of chylomicrons in the hamsters fed the high-fat fish oil also raise the possibility of suppressed lipoprotein lipase activity. Huff *et al.* (1993) have previously reported a fish-oil-induced reduction of post-heparin plasma lipoprotein lipase and hepatic triacylglycerol lipase activity. A recent study by McAteer *et al.* (2003) has shown that CHOL almost completely inhibits post-heparin lipoprotein lipase activity in Bio F_1B hamsters, compared with DNSI hamsters. These authors also reported chylomicron-like particles in cholesterol-fed F_1B hamsters and these hamsters showed significantly higher atherosclerotic lesion formation. Although we did not measure lipoprotein lipase activity in the present study, it is probable that the inhibition of lipoprotein lipase activity plays an important role in causing hyperlipidaemia in hamsters fed high-fat fish oil. Another possibility for the increased VLDL concentrations in fish-oil-fed hamsters could be due to competition between VLDL and chylomicrons for lipoprotein lipase (Harris, 1989). Future studies will concentrate on some of these possibilities to explore the mechanisms involved.

The hamsters fed the fish-oil diet had lower HDL-cholesterol compared with the hamsters fed the MIX diet. This parallels other human, and animal-feeding trials where fish-oil consumption caused reductions in HDL-cholesterol concentrations (Childs *et al.* 1990; Jones *et al.* 1990; Fincham *et al.* 1991). The HDL-cholesterol concentrations are mainly regulated by the reverse cholesterol transport pathway and cellular cholesterol and phospholipid efflux (Dietschy, 1997; Bruce *et al.* 1998). HDL acquires triacylglycerol from VLDL and LDL in exchange for cholesteryl esters via the reverse cholesterol transport pathway and acquires cholesterol and phospholipids from peripheral

cells. A reduction of HDL-cholesterol concentrations, while no change in triacylglycerol concentrations, implicates that the fish-oil-induced effect might be attributed to a decreased efflux of cholesterol and phospholipids from peripheral cells rather than an increased reverse cholesterol transport.

Previous reports have linked hyperlipidaemia with increased serum concentration of lipid peroxidation products (Vladimirov *et al.* 1980; Yagi, 1987). Fish oil is rich in *n*-3 PUFA that are efficiently incorporated into membrane phospholipids, displacing *n*-6 PUFA. The *n*-3-rich membranes are more prone to oxidative stress (Leibowitz *et al.* 1990). It was recently found that vitamin E supplementation of a fish-oil diet prevents lipid peroxidation and thus reduces fish-oil-induced hyperlipidaemia in hamsters (Kubow *et al.* 1996). We did not measure lipid peroxidation and oxidative stress in the present study; however these possibilities cannot be excluded.

In summary, the changes in plasma lipoproteins with *n*-3 PUFA feeding in F_1B hamsters are not consistent with the hypolipidaemic properties of fish oil observed in human trials and in other animal models. Fish oil at high fat levels and increasing cholesterol concentrations was previously reported hypercholesterolaemic in the Golden Syrian hamster; however, this is the first report on the effect of fish-oil feeding on the plasma lipoprotein profile and hepatic LDL-receptor mRNA expression in F_1B hamsters. The compensatory mechanism to prevent the down regulation of the LDL-receptor mRNA expression by cholesterol in fish-oil-fed hamsters is interesting and might shed some new light in maintaining whole-body cholesterol homeostasis. Whether the F_1B hamster proves to be a good model to study diet-induced hyperlipidaemia, and to investigate the molecular mechanisms involved, is not clear. The F_1B hamster is suggested to develop type 2 diabetes mellitus on high-fat diets; whether high levels of fish oil induce type 2 diabetes in this particular animal model is yet another question that needs to be addressed. We are currently investigating whether high levels of fish oil cause alterations in plasma glucose and insulin levels in F_1B hamsters, and whether these changes correlate with the plasma lipoprotein profile. The findings will help in understanding whether fish-oil therapy is appropriate to lower plasma lipid levels under certain pathophysiological conditions.

Acknowledgements

This research was supported by a grant from the Natural Sciences & Engineering Research Council (grant no. 217451-00) and the Canadian Innovation Fund. S. K. C. holds a New Investigator Award from the Canadian Institutes of Health Research. We thank Amanda Bennett and Stephanie Tucker for technical support.

References

Bang HO, Dyerberg J & Hjorne N (1971) Fatty acid composition of food consumed by Greenland Eskimos. *Acta Med Scand* **200**, 69–73.

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

Bruce C, Chouinard RA & Toll AR (1998) Plasma lipid transfer proteins, high density lipoproteins, and reverse cholesterol transport. *Annu Rev Nutr* **18**, 297–330.

Childs MT, King IB & Knopp RH (1990) Divergent lipoprotein responses to fish oils with various ratios of eicosapentaenoic acid and docosapentaenoic acid. *Am J Clin Nutr* **52**, 632–639.

Dietschy JM (1997) Theoretical consideration of what regulates low-density-lipoprotein and high-density-lipoprotein cholesterol. *Am J Clin Nutr* **65**, 1581S–1589S.

Drevon CA (1992) Marine oils and their effects. *Nutr Rev* **50**, 38–45.

Dyerberg J (1986) Linolenate derived polyunsaturated fatty acids and prevention of atherosclerosis. *Nutr Rev* **44**, 125–134.

Elam MB, Wilcox HG, Cagen LM, Deng X, Raghow R, Kumar P, Heimberg M & Russell JC (2001) Increased hepatic VLDL secretion, lipogenesis and SREBP-1 expression in the corpulent JCR:LA-cp rat. *J Lipid Res* **42**, 2039–2048.

Fincham JE, Gouws E, Woodroof CW, *et al.* (1991) Atherosclerosis. Chronic effects of fish oil and a therapeutic diet in non-human primates. *Arterioscler Thromb* **11**, 719–732.

Friedewald WT, Levy RI & Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. *Clin Chem* **18**, 499–502.

Harris WS (1989) Fish oil and plasma lipid and lipoprotein metabolism in humans: a critical review. *J Lipid Res* **30**, 785–807.

Harris WS (1997) n-3 fatty acids and serum lipoproteins: human studies. *Am J Clin Nutr* **65**, Suppl. 5, 1645S–1654S.

Hsu HS, Lee YT & Chen MF (2000) Effect of n-3 fatty acids on the composition and binding properties of lipoproteins in hypertriglyceridemic patients. *Am J Clin Nutr* **71**, 139–146.

Huff MW, Telford DE, Edmonds BW, McDonald CG & Evans AJ (1993) Lipoprotein lipases, lipoprotein density gradient profile and LDL receptor activity in miniature pigs fed fish oil and corn oil. *Biochim Biophys Acta* **1210**, 113–122.

Jones PJH, Ridgen JE & Benson AP (1990) Influence of dietary fatty acid composition on cholesterol synthesis and esterification in hamsters. *Lipids* **25**, 815–820.

Kagawa Y, Nishizawa M, Zuzuki M, *et al.* (1982) Eicosapolyenoic acids of serum lipids of Japanese islands with low incidence of cardiovascular diseases. *J Nutr Sci Vitaminol* **28**, 441–453.

Keough KMW & Davis PJ (1979) Gel to liquid-crystalline transitions in water dispersions of saturated mixed-acid phosphatidylcholines. *Biochemistry* **18**, 1453–1459.

Kowala MC, Nunnari JJ, Durham SK & Nicolosi RJ (1991) Doxazosin and cholestyramine similarly decrease fatty streak formation in the aortic arch of hyperlipidemic hamsters. *Atherosclerosis* **91**, 35–49.

Kubow S, Goyette N, Kermasha S, Stewart-Phillip J & Koski KG (1996) Vitamin E inhibits fish oil-induced hyperlipidemia and tissue lipid peroxidation in hamsters. *Lipids* **31**, 839–847.

Leibowitz BE, Hu ML & Tappel AL (1990) Lipid peroxidation in rat tissue slices: effect of dietary vitamin E, corn oil-lard and menhaden oil. *Lipids* **25**, 125–129.

Lin MH, Lu SC, Hsieh JW & Huang PC (1995) Lipoprotein responses to fish, coconut and soybean oil diets with and without cholesterol in the Syrian hamster. *J Formos Med Assoc* **94**, 724–731.

Lu SH, Lin MH & Huang PL (1996) A high cholesterol (n-3) polyunsaturated fatty acid diet induces hypercholesterolemia

more than a high cholesterol (n-6) polyunsaturated fatty acid diet in hamsters. *J Nutr* **126**, 1759–1765.
McAteer MA, Grimsditch DC, Vidgeon-Hart M, Benson GM & Salter AM (2003) Dietary cholesterol reduces lipoprotein lipase activity in the atherosclerosis-susceptible Bio F_1B hamster. *Br J Nutr* **89**, 341–350.
Nanjee MN & Miller NE (1989) Human hepatic low-density lipoprotein receptors: associations of receptor activities *in vitro* with plasma lipid and apolipoprotein concentrations *in vivo*. *Biochim Biophys Acta* **1002**, 245–255.
National Research Council (1995) *Nutrient Requirements of Laboratory Animals*, 4th revised ed., pp. 11–79. Washington, DC: The National Academy Press.
Nestel PJ (1986) Fish oil attenuates the cholesterol induced rise in lipoprotein cholesterol. *Am J Clin Nutr* **43**, 752–757.
Nestel PJ, Connor WE, Reardon MF, Connor S, Wong S & Boston R (1984) Suppression by diets rich in fish oil or very low density lipoproteins production in man. *J Clin Invest* **74**, 82–89.
Nossen JO, Rustan AC, Gloppestad SH, Malbakken S & Drevon CA (1986) Eicosapentaenoic acid inhibits synthesis and secretion of triacylglycerol by cultured rat hepatocytes. *Biochim Biophys Acta* **879**, 56–65.
Ohtani H, Hayashi K, Hirata Y, Dojo S, Nakashima K, Nishio E, Kurushima H, Saeki M & Kajiyama G (1990) Effects of dietary cholesterol and fatty acids on plasma cholesterol level and hepatic lipoprotein metabolism. *J Lipid Res* **31**, 1413–1422.
Salter AM, Mangiapane EH, Bennett AJ, Bruce JS, Billett MA, Anderton KL, Marenah CB, Lawson N & White DA (1998) The effect of different fatty acids on lipoprotein metabolism: concentration-dependent effects of diets enriched in oleic, myristic, palmitic, and stearic acids. *Br J Nutr* **79**, 195–202.
Soutar AK, Harders-Spengel K, Wade DP & Knight BL (1986) Detection and quantification of low density lipoprotein (LDL) receptors in human liver by ligand blotting, immunoblotting and radioimmunoassay. LDL receptor protein content is correlated with plasma LDL cholesterol concentration. *J Biol Chem* **261**, 17127–17133.
Spady DK & Dietschy JM (1985) Dietary saturated triacylglycerols suppress hepatic low density lipoprotein receptor activity in the hamster. *Proc Natl Acad Sci USA* **82**, 4526–4530.
Spady DK & Dietschy JM (1988) Interaction of dietary cholesterol and triglycerides in the regulation of hepatic low density lipoprotein transport in the hamster. *J Clin Invest* **81**, 300–309.
Srivastava RA, Ito H, Hess M, Srivastava N & Schonfeld G (1995) Regulation of low density lipoprotein receptor gene expression in HepG2 and Caco2 cells by palmitate, oleate and 25-hydroxycholesterol. *J Lipid Res* **36**, 1434–1446.
Steel RGD & Torrie JH (1980) *Principles and Procedures of Statistics: a Biometrical Approach*. New York: McGraw-Hill Book Co.
Sudhof TC, Van der Wwesthuyzen DR, Goldstein JL, Brown MS & Russell DW (1987) Three direct repeats and a TATA-like sequence are required for regulated expression of the human low density lipoprotein receptor gene. *J Biol Chem* **262**, 10773–10779.
Sullivan DR, Sanders TAB, Trayner IM & Thompson GR (1986) Paradoxical elevation of LDL apoprotein B levels in hypertriglyceridaemic patients and normal subjects ingesting fish oil. *Atherosclerosis* **61**, 129–134.
Surette ME, Whelan J, Lu GP, Broughton KS & Kinsella JE (1992) Dependence on dietary cholesterol for n-3 polyunsaturated fatty acid-induced changes in plasma cholesterol in the Syrian hamster. *J Lipid Res* **33**, 263–271.
Vasandani C, Kafrouni AI, Caronna A, Bashmakov Y, Gotthardt M, Horton JD & Spady DK (2002) Upregulation of hepatic LDL transport by n-3 fatty acids in LDL receptor knockout mice. *J Lipid Res* **43**, 772–784.
Vladimirov YA, Olenew VI, Suslove TB & Chememesina ZP (1980) Lipid peroxidation in mitochondrial membrane. *Adv Lipid Res* **17**, 173–247.
Wilkinson J, Higgins JA, Fitzimmons C & Bowyer DE (1998) Dietary fish oils modify the assembly of VLDL and expression of the LDL receptor in rabbit liver. *Arterioscler Thromb Vasc Biol* **18**, 1490–1497.
Wong S & Nestel PJ (1987) Eicosapentaenoic acid inhibits the secretion of triacylglycerol and of apoprotein B and the binding of LDL in Hep G2 cells. *Atherosclerosis* **64**, 139–146.
Yagi K (1987) Lipid peroxides and human diseases. *Chem Phys Lipids* **45**, 337–351.
Yokode M, Hammer RE, Ishibashi S, Brown MS & Goldstein JL (1990) Diet induced hypercholesterolemia in mice: prevention by overexpression of LDL receptors. *Science* **250**, 1273–1275.