Effects of dietary cholesterol and fatty acids on plasma cholesterol level and hepatic lipoprotein metabolism

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Abstract The effects of dietary cholesterol and fatty acids on the plasma cholesterol level and rates of very low density lipoprotein (VLDL) cholesterol secretion and low density lipoprotein (LDL) transport through LDL receptors in the liver of the hamster were investigated. Increases of plasma VLDL- and LDL-cholesterol levels and VLDL-cholesterol secretion from hepatocytes were observed in animals fed a diet enriched with 0.1% cholesterol for 2 weeks in comparison with animals fed a control diet. The addition of dietary palmitic acid accelerated the effect of dietary cholesterol on plasma VLDL- and LDL-cholesterol levels and VLDL-cholesterol secretion from hepatocytes. Dietary linoleic acid accelerated the effect of dietary cholesterol on VLDL-cholesterol secretion from hepatocytes and diminished the effect on the plasma LDL-cholesterol level. Hepatic LDL receptor activity was considerably suppressed by a control diet containing 0.05% cholesterol and a further small suppression was induced by a diet enriched with 0.1% cholesterol with or without 5% palmitic acid. However, dietary linoleic acid diminished the effect of dietary cholesterol on the suppression of hepatic LDL receptor activity. These results suggest that dietary palmitic acid augments the effect of dietary cholesterol in elevating the plasma LDL-cholesterol level through acceleration of VLDL-cholesterol secretion from the liver, and that dietary linoleic acid diminishes the effect of dietary cholesterol in elevating the plasma LDL-cholesterol level by preventing the suppression of hepatic LDL receptor activity induced by cholesterol. —**Ohtani, H., K. Hayashi, Y. Hirata, S. Dojo, K. Nakashima, E. Nishio, H. Kurushima, M. Saeki, and G. Kajiyama.** Effects of dietary cholesterol and fatty acids on plasma cholesterol level and hepatic lipoprotein metabolism. *J. Lipid Res.* 1990. **31:** 1413–1422.

Supplementary key words plasma LDL-cholesterol • hepatic VLDL secretion • hepatic LDL receptor

There is known to be a close relationship between plasma cholesterol level and the occurrence of coronary heart disease (1, 2). Therefore, for the prevention of atherosclerotic disease, it is important to reduce the plasma cholesterol level. It has been reported that the relative content of saturated and polyunsaturated fatty acids in the diet affects the plasma level of cholesterol (3–8). Generally, polyunsaturated fatty acids are known to reduce the plasma cholesterol when exchanged for saturated fatty acids. There are several explanations with regard to the mechanism by which fatty acids regulate the plasma level of cholesterol such as redistribution of cholesterol between plasma and tissue pools (9), a change of lipoprotein composition (10), a change in LDL production (11, 12), and a change in clearance rate of LDL (13). The plasma level of LDL-cholesterol is kept constant by a balance in the rates of production and removal of this lipoprotein from the circulation (14). LDL-cholesterol is produced from VLDL, which is secreted from the liver, and a relatively large proportion of plasma LDL-cholesterol removal is mediated by hepatic LDL receptors (15–18). Therefore, it is suggested that the liver may play an important role in changing the plasma LDL-cholesterol level induced by alteration of fatty acids in the diet. Recently, Spady and Dietschy (19, 20) reported that dietary saturated triacylglycerol suppressed receptor-dependent LDL transport in the liver of hamsters, and suggested that dietary saturated triacylglycerol plays an important role in suppressing hepatic LDL receptor activity and elevating the plasma LDL-cholesterol level. The present study using male hamsters was undertaken to investigate the role of liver and the mechanism by which cholesterol and fatty acids in the diet regulate the level of plasma LDL-cholesterol both in vivo and in vitro. Especially, for the investigation of alterations in the rate of VLDL synthesis and hepatic LDL receptor activity, which were induced by a diet supplemented with cholesterol and fatty acids, we used primary cultured hepato-

Abbreviations: LDL, low density lipoprotein; VLDL, very low density lipoprotein; HDL, high density lipoprotein; SDS, sodium dodecyl sulfate; FBS, fetal bovine serum; LPDS, lipoprotein-deficient serum; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A.

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cytes prepared from hamsters fed various diets. Linoleic acid (5%, w/w) or palmitic acid (5%, w/w) was added to the diet in free fatty acid form instead of triacylglycerol, because these fatty acids are major components of dietary fatty acids and their fatty acid chain-length and numbers and positions of double bonds are defined.

MATERIALS AND METHODS

Chemicals

All reagents used were of the highest purity commercially available. Williams' medium E and fetal bovine serum were purchased from Flow Laboratories. Insulin, dexamethasone, trypsin inhibitor, bovine serum albumin, penicillin, and streptomycin were obtained from Sigma Chemical Co. Collagenase and *E. coli* alkaline phosphatase were obtained from Wako Pure Chemical Industries. Glucose-6-phosphate dehydrogenase was purchased from Oriental Yeast Co., Ltd. DL-3-Hydroxy-3-methyl-glutaryl coenzyme A was purchased from Sigma Chemical Co. [1-^{14}C]Acetic acid (sodium salt), DL-3-hydroxy-3-methyl-[3-^{14}C]glutaryl coenzyme A and sodium [^{125}I]iodide were obtained from Amersham.

Animals and diets

Male Golden Syrian hamsters each weighing 80 g were housed in colony cages for 2 weeks before use in experiments. The control diet used in these studies (from Oriental Kobo Co.) contained 0.05% cholesterol, 0.57% linoleic acid, 0.16% palmitic acid, 0.30% oleic acid, and 0.12% other fatty acids. The experimental diets were prepared by adding various amounts of cholesterol and/or fatty acid to this control diet as described in the legends. All experiment diets were fed ad libitum for 2 weeks and all experiments were carried out after a 12-h fast.

Preparation of lipoproteins

Hamster plasma was isolated from blood collected into tubes containing 0.15% EDTA (w/v) from normolipemic fasting hamsters fed the control diet. LDL ($1.019 < d < 1.063$ g/ml) was isolated by a modification of the procedure described by Havel, Eder, and Bragdon (21), using sequential ultracentrifugation in KBr. The lipoproteins were dialyzed at 4°C against a solution of 0.15 M NaCl and 0.2 mM EDTA (pH 7.4), sterilized by passage through a 0.45-μm filter, and stored at 4°C. LDL was radiolabeled with ^{125}I by the iodine monochloride procedure, as described previously (22). Lipoprotein-deficient serum (LPDS) was prepared from fetal bovine serum (FBS) as described previously (23). Lipoprotein concentrations are expressed in terms of protein content.

Analytical procedures

Hamster plasma was isolated as described above. VLDL ($d < 1.006$ g/ml), LDL ($1.019 < d < 1.063$ g/ml), and HDL ($1.063 < d < 1.210$ g/ml) were isolated by a modification of the procedure described by Havel et al. (21). Cholesterol concentration of each lipoprotein fraction was assayed colorimetrically as described previously (24). Cholesterol content of liver tissue and cultured hepatocytes was measured by gas–liquid chromatography as described previously (25).

Fatty acid analysis

Lipids were extracted from plasma and liver tissue and separated as described previously (26). Spots corresponding to cholesteryl ester, triglyceride, and phospholipid were visualized with I_2 vapor, scraped, and eluted with chloroform–methanol 2:1. Triglyceride and phospholipid fatty acids were methylated with 5% (w/v) methanolic HCl. Cholesteryl ester fatty acids were methylated with 5% (w/v) methanolic HCl after saponification of cholesteryl esters. Methyl esters of fatty acids were chromatographed on a 25% diethyleneglycol succinate on celite 545A (60–80 mesh) column at 180°C as described previously (27).

Assay of microsomal HMG-CoA reductase activity

Liver microsomes were prepared and HMG-CoA reductase activity was measured as described previously (28).

Preparation of cultured hepatocytes

Parenchymal hepatocytes were isolated from the liver of each hamster fed an experimental diet by in situ liver perfusion with collagenase as described previously (24). The cells were incubated in medium A (Williams' medium E containing 5% FBS, 100 IU/ml penicillin, 100 μg/ml streptomycin, 10^{-8} M insulin, 10^{-6} M dexamethasone) at 37°C in 95% air and 5% CO_2 as indicated in the figure legends, and then various activities were assayed.

Measurement of lipid secretion from cultured hepatocytes

After the preparation of hepatocytes, the cells were incubated in medium B (Williams' medium E containing 5% LPDS, 100 IU/ml penicillin, 100 μg/ml streptomycin, 10^{-8} M insulin, and 10^{-6} M dexamethasone) for 18 h. At the end of the incubation, the medium was collected and lipids secreted in the VLDL fraction ($d < 1.006$ g/ml) were separated as described previously (29). Finally, extracted cholesterol and triglyceride were dissolved in 10% Triton X-100 and the contents were determined colorimetrically as described above.

Incorporation of [^{14}C]acetic acid into cholesterol by cultured hepatocytes

After the hepatocytes had been incubated for 4 h with 1 mM [^{14}C]acetic acid (1 μCi/ml) as indicated, the medium was transferred and each monolayer was washed and then dissolved in 0.1 N NaOH. [^{14}C]Cholesterol in each medium and cell monolayer was separated by thin-layer chromatography and assayed as described previously (30, 31).

Assays of surface binding and internalization of ^{125}I-labeled LDL in cultured hepatocytes

After the cells had been incubated as described in the figure legends, the growth medium was removed and each dish received 2 ml of medium B containing various concentrations of ^{125}I-labeled in the absence or presence of excess unlabeled LDL as indicated. The assays of cultured cell monolayers were performed by the methods previously described (32).

Cell solubilization, electrophoresis and blotting procedures

For blotting experiments, hepatocyte monolayers were scraped off, collected, and solubilized as described previously (30). The solubilized supernatants were subjected to SDS-polyacrylamide gel electrophoresis (4.5–18% gradient gel), and electrophoretic transfer of proteins to nitrocellulose was performed as described previously (30). Ligand-blotting was carried out in buffer containing 20 mM Tris-HCl, pH 7.4, 2 mM $CaCl_2$, 90 mM NaCl, and 5% (w/v) bovine serum albumin as a blocking agent. The concentration and specific radioactivity of the ligand used in the incubation mixtures are indicated in the legends.

Other procedures

The protein content of samples containing no Triton X-100 was determined by the method of Lowry et al. (33). The protein contents of samples containing Triton X-100 and lipoproteins were measured by a modification of the Lowry procedure as described previously (34) using bovine serum albumin as a standard.

Statistical analysis

Kruskal-Wallis and Scheffe's statistical methods were used for data obtained from more than five individuals (35, 36).

RESULTS

Effect of dietary fatty acids on plasma and hepatic esterified lipids

Fatty acid compositions of plasma and hepatic esterified lipids are shown in **Table 1** and **Table 2**. Dietary palmitic acid and linoleic acid produced marked changes in the fatty acid composition of plasma triglycerides. The relative amount of palmitic acid or linoleic acid in plasma triglycerides was increased in the animals fed diet supplemented with cholesterol and palmitic acid or diet supplemented with cholesterol and linoleic acid, respectively (Table 1). The effect of dietary linoleic acid on the hepatic lipids was also determined. There were increases in the relative amount of linoleic acid in hepatic cholesteryl ester, triglyceride, and phospholipid in the animals fed diet supplemented with cholesterol and linoleic acid (Table 2). However, there was no unique effect of dietary palmitic acid on the hepatic esterified lipids. The contents of plasma triglyceride of animals fed the control diet, control diet with 0.1% cholesterol, control diet with 0.1% cholesterol and 5% linoleic acid, or control diet with 0.1% cholesterol and 5% palmitic acid were 68.0, 89.0, 131.1, and 162.2 mg/dl, respectively. The contents of hepatic triglyceride of animals fed the control diet, control diet with 0.1% cholesterol, control diet with 0.1% cholesterol and 5% linoleic acid, or control diet with 0.1% cholesterol and 5% palmitic acid were 945, 1033, 1725, and 1624 μg/g liver tissue, respectively. These results suggest that the different diets were effective in modifying plasma and hepatic esterified lipids.

Effect of dietary fatty acids on cholesterol metabolic changes induced by cholesterol feeding in vivo

Experiments were undertaken to examine the interaction of dietary fatty acids and cholesterol in the regulation of plasma cholesterol levels. Hamsters were fed diets supplemented with 0.1% cholesterol with and without linoleic acid (5%, w/w) or palmitic acid (5%, w/w). The plasma cholesterol level increased in the animals fed cholesterol alone. By comparison the addition of linoleic acid decreased, and the addition of palmitic acid increased, the level of plasma cholesterol (**Table 3**). VLDL-cholesterol and LDL-cholesterol levels were affected by cholesterol and fatty acid feeding, but the HDL-cholesterol level was unaffected (Table 3). LDL-cholesterol level was lowest in animals fed cholesterol with linoleic acid and this contributed to the lower plasma cholesterol level induced by cholesterol feeding in animals fed linoleic acid (Table 3). Further increases in VLDL-cholesterol and LDL-cholesterol levels, which contributed to the accelerated elevation of plasma cholesterol induced by cholesterol feeding, were observed in animals fed cholesterol with palmitic acid (Table 3). After lipoprotein fractionation, not all cholesterol could be accounted for as shown in Table 3. However, the recovery rate of cholesterol was constant among the four groups of animals (75–80%). Therefore, it is possible to compare the cholesterol values in each of the lipoprotein fractions. Inasmuch as dietary cholesterol is transported into the liver via the chylomicron remnant pathway, it seems that an excess cholesterol supply from the diet induces cholesterol accumulation in the liver. The

TABLE 1. Effect of dietary fatty acids on plasma esterified lipids

Fatty Acid	Control	Control + 0.1% Cholesterol	Control + 0.1% Cholesterol + 5% Palmitic Acid	Control + 0.1% Cholesterol + 5% Linoleic Acid
Plasma cholesteryl ester				
16:0	14.37	13.04	17.15	12.80
16:1		5.15	6.03	4.21
18:0		2.00	7.41	2.04
18:1	28.82	28.69	29.17	20.54
18:2	55.96	50.86	39.66	59.88
18:3	0.83		0.47	
20:4		0.23	0.09	0.51
Plasma triglyceride				
16:0	32.58	32.08	43.28	28.96
16:1		4.13	1.31	4.38
18:0	5.95	4.33	4.66	2.64
18:1	39.77	40.45	37.30	28.90
18:2	18.80	17.95	12.65	34.32
18:3	1.63	1.03	0.76	0.77
20:4	1.23			
Plasma phospholipid				
16:0	38.99	38.34	30.48	37.95
16:1		0.06		
18:0	18.33	19.09	18.35	19.79
18:1	12.35	13.93	12.49	9.99
18:2	26.76	24.39	32.76	32.25
18:3		0.67	0.31	
20:4	3.54	3.49	5.58	

Groups of animals were fed diets supplemented with 0.1% cholesterol either alone or in combination with 5% linoleic acid or 5% palmitic acid. All diets were fed for 14 days. Fatty acid composition of plasma esterified lipids was determined as described in Materials and Methods. Each value represents the average of duplicate measurements. Fatty acid composition is expressed as percentage of the total recovered fatty acids.

hepatic cholesteryl ester level was increased and a dramatic suppression of HMG-CoA reductase activity was observed in animals fed cholesterol (**Table 4**). These results suggest that addition of 0.1% cholesterol to the diet is enough to induce accumulation of cholesterol in the liver through the chylomicron remnant pathway. Fatty acid feeding did not affect hepatic cholesterol accumulation or suppression of HMG-CoA reductase activity (Table 4). From these results we can conclude that the relative amount of cholesterol supply to VLDL and LDL from dietary cholesterol is increased in animals fed cholesterol, and that an excess intake of dietary cholesterol affects the plasma LDL-cholesterol level.

As we were unable to explain the mechanism by which linoleic acid lowers the LDL-cholesterol level whereas palmitic acid elevates the level in comparison with the plasma LDL-cholesterol level obtained from animals fed cholesterol alone (Table 3), further experiments were done to clarify this mechanism and to investigate whether dietary cholesterol and fatty acids influence the hepatic metabolism of cholesterol and lipoproteins.

Metabolic changes in lipoproteins and cholesterol in hepatocytes induced by dietary cholesterol and fatty acids

Hepatocytes were prepared from animals fed diets supplemented with cholesterol with and without fatty acid. The cholesteryl ester content in cultured hepatocytes from animals fed cholesterol increased, but fatty acid feeding produced no significant effect on the cholesteryl ester content (**Table 5**). Cholesterol synthesis from acetic acid in cultured hepatocytes, which reflects HMG-CoA reductase activity in hepatic microsomes, was suppressed in animals fed cholesterol (Table 5). There was a good correlation between the in vivo and in vitro data (Table 4 vs Table 5). These results suggest that cultured hepatocytes prepared from animals fed cholesterol with or without fatty acids maintain the metabolic characteristics of the liver in vivo induced by feeding cholesterol and fatty acids.

Cholesterol and triglyceride secretion in VLDL from cultured hepatocytes was slightly increased in animals fed cholesterol in comparison with the control diet. The addi-

TABLE 2. Effect of dietary fatty acids on hepatic esterified lipids

Fatty Acid	Control	Control + 0.1% Cholesterol	Control + 0.1% Cholesterol + 5% Palmitic Acid	Control + 0.1% Cholesterol + 5% Linoleic Acid
Hepatic cholesteryl ester				
16:0	10.34	10.50	27.02	25.45
16:1				
18:0	7.84	10.49	4.79	21.49
18:1	66.98	56.50	48.92	25.88
18:2	12.68	15.55	19.25	27.16
18:3				
20:4	2.15			
Hepatic triglyceride				
16:0	25.98	38.25	38.42	29.93
16:1	6.51	2.87	4.19	2.86
18:0	13.58	4.91	4.56	3.08
18:1	36.11	31.15	32.30	22.83
18:2	14.70	21.72	19.41	40.83
18:3	2.25	1.07	1.08	0.44
20:4	0.83			
Hepatic phospholipid				
16:0	21.20	34.65	29.00	25.95
16:1			0.08	
18:0	27.12	19.94	22.14	19.37
18:1	13.91	11.88	12.78	9.83
18:2	26.49	25.06	26.17	31.63
18:3	0.55	0.03	0.33	
20:4	10.69	8.41	9.46	13.18

Groups of animals were fed diets supplemented with 0.1% cholesterol either alone or in combination with 5% linoleic acid or 5% palmitic acid. All diets were fed for 14 days. Fatty acid composition of hepatic esterified lipids was determined as described in Materials and Methods. Each value represents the average of duplicate measurements. Composition is expressed as percentage of the total recovered fatty acids.

tion of fatty acids stimulated the secretion of these lipids in VLDL and a marked stimulatory effect was observed in animals fed linoleic acid (**Table 6**). These results suggest that an overload of dietary cholesterol induces cholesterol accumulation in hepatocytes and that hepatic cholesterol accumulation stimulates cholesterol secretion in VLDL. Furthermore, the addition of fatty acids accelerates the effect of hepatic cholesterol accumulation on the secretion of both cholesterol and triglyceride in VLDL.

Uptake of LDL by the liver is another important metabolic pathway that regulates the plasma cholesterol level, and this is mediated by LDL receptors on the hepato-

TABLE 3. Effect of dietary cholesterol and fatty acids on plasma cholesterol concentrations

Diet	Total Cholesterol	VLDL-Cholesterol	LDL-Cholesterol	HDL-Cholesterol
		mg/dl		
Control	143.7 ± 4.9	23.8 ± 3.6	41.1 ± 6.6	48.2 ± 3.5
Control + 0.1% cholesterol	178.2 ± 6.0[a]	37.4 ± 7.9	50.1 ± 8.4[a]	57.5 ± 7.9
Control + 0.1% cholesterol + 5% linoleic acid	160.9 ± 10.3	42.9 ± 14.7	32.3 ± 6.0[c]	53.4 ± 5.9
Control + 0.1% cholesterol + 5% palmitic acid	224.8 ± 25.3[b,c]	51.7 ± 10.0[a]	63.7 ± 10.0[b,c]	52.3 ± 2.7

Groups of animals were fed diets supplemented with 0.1% cholesterol either alone or in combination with 5% linoleic acid or 5% palmitic acid. All diets were fed for 14 days. Each value was determined as described in Materials and Methods and represents the mean ± standard deviation for data obtained in six animals.

[a]Significantly different from control at $P < 0.05$.

[b]Significantly different from control at $P < 0.01$.

[c]Significantly different from control + 0.1% cholesterol at $P < 0.05$.

TABLE 4. Effect of dietary cholesterol and fatty acids on hepatic cholesterol content and HMG-CoA reductase activity

Diet	Free Cholesterol	Cholesteryl Ester	HMG-CoA Reductase Activity
	μg/g liver		*pmol/min/mg protein*
Control	1986 ± 351	4968 ± 1053	4.17 ± 0.18
Control + 0.1% cholesterol	2114 ± 332	13067 ± 1826[a]	1.92 ± 0.26[a]
Control + 0.1% cholesterol + 5% linoleic acid	2528 ± 328	16248 ± 2372[b]	1.77 ± 0.14[b]
Control + 0.1% cholesterol + 5% palmitic acid	2274 ± 259	12829 ± 1651[a]	1.92 ± 0.20[a]

Groups of animals were fed diets supplemented with 0.1% cholesterol either alone or in combination with 5% linoleic acid or 5% palmitic acid. All diets were fed for 14 days. Cholesterol content of liver tissue and hepatic microsomal HMG-CoA reductase activities were determined as described in Materials and Methods. Each value represents the mean ± standard deviation for data obtained in six animals.

[a]Significantly different from control at $P < 0.05$.

[b]Significantly different from control at $P < 0.01$.

cytes. The sum of binding and internalization of ^{125}I-labeled LDL in cultured hepatocytes from animals fed cholesterol and linoleic acid showed a twofold increase in comparison with animals fed the control diet (**Fig. 1**). Animals fed cholesterol with or without palmitic acid produced a further small suppression of the binding and internalization of ^{125}I-labeled LDL in hepatocytes in comparison with animals fed the control diet (Fig. 1). These results suggest that LDL receptors on hepatocytes are considerably suppressed by 0.05–0.15% (w/w) dietary cholesterol and that addition of linoleic acid prevents the down-regulation of hepatic LDL receptors induced by dietary cholesterol overload. The ligand-blotting experiment also demonstrated the increase in LDL receptors on hepatocytes from animals fed cholesterol and linoleic acid (**Fig. 2**).

The plasma LDL-cholesterol levels shown in Table 3 seem to be balanced by cholesterol secretion in VLDL and uptake of LDL-cholesterol by the liver. That is, the high level of plasma LDL-cholesterol observed in animals fed cholesterol with or without palmitic acid is explained by hypersecretion of cholesterol in VLDL from hepatocytes and suppression of hepatocyte LDL receptors (Tables 3 and 6, Figs. 1 and 2), and the low level of plasma LDL-cholesterol in animals fed cholesterol and linoleic acid is due to an increase in hepatocyte LDL recpetors, which increases the uptake of LDL-cholesterol and compensates for the hypersecretion of cholesterol from the liver (Tables 3 and 6, Figs. 1 and 2).

DISCUSSION

It is known that the level of plasma LDL-cholesterol, the rate of sterol synthesis in the liver, and lipoprotein metabolism in the hamster are similar to those in humans (19, 20). Therefore, the hamster seems a good model with which to study the regulatory mechanisms of plasma LDL-cholesterol induced by dietary lipids. Using this model, we investigated the interaction of dietary choles-

TABLE 5. Effect of dietary cholesterol and fatty acids on cholesterol content and incorporation of [^{14}C]acetic acid into cholesterol in cultured hepatocytes

Diet	Free Cholesterol	Cholesteryl Ester	[^{14}C]Acetic Acid Incorporation Into Cholesterol
	ng/mg cell protein		*dpm/mg cell protein/4 h*
Control	532	1739	14344
Control + 0.1% cholesterol	587	3522	8336
Control + 0.1% cholesterol + 5% linoleic acid	594	4563	9246
Control + 0.1% cholesterol + 5% palmitic acid	551	3378	8199

Groups of animals were fed diets supplemented with 0.1% cholesterol either alone or in combination with 5% linoleic acid or 5% palmitic acid. All diets were fed for 14 days. Hepatocytes were prepared and cultured for 24 h as described in Materials and Methods. Then cholesterol contents on cultured hepatocytes were determined as described. After incubation for 24 h in medium A, 1 mM [^{14}C]acetic acid was added to cultures of hepatocytes (1 μCi/ml medium). Four h later, [^{14}C]acetic acid incorporation into cholesterol was measured as described. Each value represents the average of duplicate incubations.

TABLE 6. Effect of dietary cholesterol and fatty acids on cholesterol and triglyceride secretions in VLDL from cultured hepatocytes

Diet	Cholesterol Secretion	Triglyceride Secretion
	ng/mg cell protein/18 h	
Control	6.04 ± 0.50	4.49 ± 0.18
Control + 0.1% cholesterol	6.49 ± 0.11	5.32 ± 0.75
Control + 0.1% cholesterol + 5% linoleic acid	11.08 ± 0.76	10.10 ± 1.74
Control + 0.1% cholesterol + 5% palmitic acid	8.39 ± 0.61	7.31 ± 0.40

Groups of animals were fed diets supplemented with 0.1% cholesterol either alone or in combination with 5% linoleic acid or 5% palmitic acid. All diets were fed for 14 days. Hepatocytes were prepared and cultured in medium B for 18 h. Cholesterol and triglyceride secretions in VLDL were measured as described in Materials and Methods. Each value represents the mean ± standard deviation for data obtained in three animals.

terol and fatty acids in the regulation of plasma LDL-cholesterol. Obvious elevation of both plasma VLDL cholesterol and LDL cholesterol levels in the animals fed a diet supplemented with cholesterol and palmitic acid was obtained in the present study, in agreement with previous data (19, 20). Spady et al. concluded that saturated triglycerides augment the effect of cholesterol in suppressing hepatic LDL receptors and elevating the level of plasma LDL-cholesterol (19, 20), and the liver is known to be an important organ for regulation of the plasma LDL-cholesterol level (15–18). To investigate the role of the liver in the regulation of plasma LDL-cholesterol, it is necessary to determine the balance between hepatic VLDL secretion and hepatic LDL degradation. Cultured hepatocytes are useful for investigating the secretion and uptake of lipoproteins, and these cells still maintain their in vivo metabolic characteristics after a short period of culture, as shown in the present and previous studies (24, 37). For these reasons, we used cultured hepatocytes for investigations of hepatic VLDL production and hepatic LDL uptake.

In this study we obtained two major results. First, cholesterol and triglyceride secretions in VLDL from cultured hepatocytes prepared from animals fed a diet high in cholesterol were increased; moreover, the addition of linoleic acid or palmitic acid stimulated this lipid secretion. This stimulation of cholesterol output in VLDL from hepatocytes seemed attributable to the hepatic accumulation of dietary cholesterol, because HMG-CoA reductase activity and cholesterol synthesis from [^{14}C]acetic acid were decreased and the cholesterol content was increased in hepatocytes from animals fed a diet high in cholesterol. The stimulatory effect of fatty acids on hepatic VLDL secretion has already been reported (38–40). Therefore, the hypersecretion of cholesterol and triglyceride in VLDL observed in hepatocytes from animals fed cholesterol along with palmitic acid or linoleic acid is consistent with previous data. A previous study has reported that the hepatic cholesteryl ester content of animals fed cholesterol with coconut oil was lower than that of animals fed cholesterol with or without safflower oil (20). This result is different from our present data, since the hepatic cholesterol content of animals fed a high-cholesterol diet was not influenced by the addition of a large amount of fatty acid, as shown in Tables 4 and 5. We are unable to explain this discrepancy. Second, the activity of hepatic LDL receptors measured as the increased amount of ^{125}I-labeled LDL binding and internalization was increased in

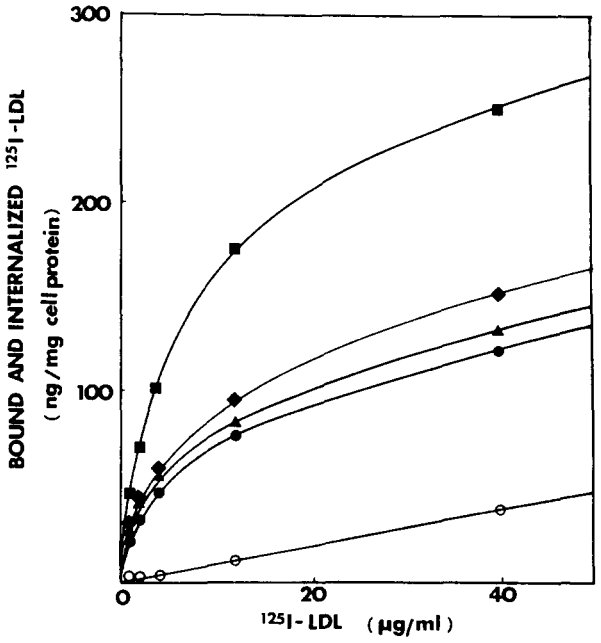

Fig. 1. Surface binding and internalization of ^{125}I-labeled LDL in cultured hepatocytes. Groups of animals were fed diets supplemented with 0.1% cholesterol either alone or in combination with 5% linoleic acid or 5% palmitic acid. All diets were fed for 14 days. Hepatocytes were prepared and cultured in medium A for 24 h. Then surface binding and internalization of ^{125}I-labeled LDL in hepatocytes were assayed as described in Materials and Methods. The specific activity of ^{125}I-labeled was 100 cpm/ng protein; ♦, control diet; ▲ control diet supplemented with 0.1% cholesterol; ●, control diet supplemented with 0.1% cholesterol and 5% palmitic acid; ■, control diet supplemented with 0.1% cholesterol and 5% linoleic acid; ○, nonspecific binding and internalization.

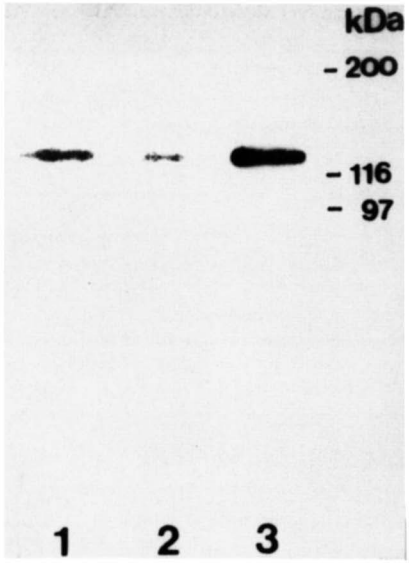

Fig. 2. Ligand-blotting analysis of LDL receptors in cultured hepatocytes. Groups of animals were fed diets supplemented with 0.1% cholesterol either alone or in combination with 5% linoleic acid or 5% palmitic acid. All diets were fed for 14 days. Hepatocytes were prepared and cultured in medium A for 24 h, and a ligand-blotting experiment was carried out as described in Materials and Methods. For this, 200 μg of protein in detergent extracts from hepatocytes was electrophoresed. ^{125}I-labeled LDL concentration used in this experiment was 10^6 cpm/ml (300 cpm/ng protein); lane 1, control diet supplemented with 0.1% cholesterol; lane 2, control diet supplemented with 0.1% cholesterol and 5% palmitic acid; lane 3, control diet supplemented with 0.1% cholesterol and 5% linoleic acid.

animals fed linoleic acid, and this elevation of LDL receptor activity seemed to be due to an increase in receptor number, judging from ligand-blotting analysis. In the present study, the control diet contained 0.05% cholesterol, 0.57% linoleic acid, 0.16% palmitic acid, and 0.3% oleic acid. Therefore, it is suggested that a considerable suppression of hepatic LDL receptors is induced by a diet containing 0.05% cholesterol, since we could not obtain further large suppression of hepatic LDL receptors in animals fed a diet high in cholesterol (0.15%, w/w) with or without palmitic acid (5.16%, w/w). In contrast, a diet high in linoleic acid (5.57%, w/w) prevented the suppression of hepatic LDL receptors induced by dietary cholesterol (Figs. 1 and 2). Spady et al. reported that saturated triglycerides augmented the effect of cholesterol in suppressing hepatic LDL receptor activity (19, 20). Although we did not obtain such a result, the effect of linoleic acid in diminishing the effect of cholesterol on the suppression of hepatic LDL receptors was revealed. These results suggest that the high levels of plasma LDL-cholesterol observed in animals fed cholesterol and palmitic acid are dependent on the hypersecretion of both cholesterol and triglyceride in VLDL from the liver, and that the low level of plasma LDL-cholesterol induced by dietary linoleic acid is due to increased activity of hepatic LDL receptors, which are spared from the suppressive effect of dietary cholesterol.

There is a discrepancy in the regulation of HMG-CoA reductase and LDL receptor activities in liver from animals fed cholesterol with linoleic acid. In spite of a high content of hepatic cholesterol and obvious suppression of hepatic HMG-CoA reductase activity, the hepatic LDL receptor activity was rather increased in animals fed cholesterol with linoleic acid in comparison with control animals (Tables 4 and 5, Fig. 1). This result suggests that fatty acids, especially linoleic acid, independently influence the regulatory pathway of LDL receptors and HMG-CoA reductase activity by cholesterol. It has been proposed that enhanced uptake of dietary cholesterol through the chylomicron remnant pathway leads to expansion of the regulatory pool of sterol, which suppresses the activities of both the rate-limiting enzymes involved in cholesterol biosynthesis and the LDL receptors, and expands the pool of cholesteryl esters (41). According to this hypothesis, dietary fatty acids may alter the hepatic response to dietary cholesterol through interaction with the regulatory pool of sterol. Furthermore, previous data have suggested that the metabolic process of hepatic cholesterol synthesis and LDL receptors are regulated independently (42). There are many unclear areas to be resolved in order to clarify the precise mechanism by which dietary sterol and fatty acids regulate the hepatic LDL receptor pathway. At present, the mechanism by which hepatic LDL receptors are increased in animals fed a diet high in linoleic acid is not clear. Furthermore, the percentage of VLDL that is converted to LDL may be under separate regulation. Thus, this is complex issue and it may not necessarily follow that the excessive secretion rates of VLDL brought about by cholesterol and triacylglycerol in this study are actually reflected by an increased rate of LDL production in the live animal. However, we can conclude that the increase in the number of hepatic LDL receptors contributes to the lower plasma LDL-cholesterol level, because no difference was observed in other parameters that contribute to the regulation of plasma cholesterol, such as the secretion of cholesterol.

It seems difficult to envisage the exact mechanism by which saturated and polyunsaturated fatty acids in the diet regulate the level of plasma LDL-cholesterol in vivo as described above. Also, we did not calculate the rates of production and removal of this class of lipoproteins in the steady state in vivo. However, the present study on metabolic changes in cholesterol and lipoproteins in the liver induced by dietary cholesterol and fatty acids provided some answers regarding the regulatory mechanism of the plasma cholesterol level induced by dietary lipids.

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