Effect of diet on the cholesterol ester composition of liver and of plasma lipoproteins in the rat

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SUMMARY The relationship between the cholesterol ester composition of the liver and the plasma lipoproteins was studied in groups of rats maintained for 5–11 weeks on the following diets: (I) rat pellets, (II) rat pellets with added olive oil and cholesterol, and (III) fat-free diets containing 0.4% cholesterol.

In the control animals (Group I), the cholesterol esters of liver and d < 1.019 lipoproteins had nearly identical compositions and consisted mainly of oleate and linoleate. The d > 1.063 lipoprotein cholesterol esters were mainly linoleate and arachidonate.

In the livers of rats fed olive oil and cholesterol (Group II), the cholesterol esters contained largely oleic acid and the d 1.006–1.019 lipoproteins had a very similar cholesterol ester composition. The d > 1.063 lipoproteins had a high proportion of esters of polyunsaturated acids and oleate.

The livers of rats on the fat-free diet contained no linoleate and increased amounts of monoenoate esters as compared to the Group I control animals, and the d < 1.019 lipoproteins had a similar cholesterol ester composition. The d > 1.063 lipoproteins contained a high proportion of monoenoic acids, relatively small amounts of linoleate and arachidonate, and significant amounts of eicosatrienoate.

These results suggest that different mechanisms are involved in the formation of the cholesterol esters of the various lipoproteins.

KEY WORDS cholesterol esters · liver · plasma lipoproteins · rat · cholesterol feeding · fat-deficient diet · differential lipoprotein formation

In a previous paper we have shown that the fatty acid composition of the cholesterol esters of rat plasma is different in the various lipoprotein fractions (1). In the very low-density lipoproteins the pattern of cholesterol esters resembles that of the liver. The pattern in the high-density lipoproteins is strikingly different, with a high content of arachidonic acid and a low content of oleic acid. When a liver from an animal fed cholesterol and olive oil was perfused with normal plasma, the pattern of cholesterol esters in the very low-density lipoproteins changed and finally contained chiefly cholesteryl oleate, as did the liver that was perfused. From these studies it was suggested that the cholesterol esters of the very low-density lipoproteins are derived by a process of nonselective transfer from the liver. However, nothing could be concluded about the mechanism of the formation of the cholesterol esters in the other lipoproteins. The previous studies were acute experiments in which fatty livers were perfused. In the present study the relationship between the cholesterol ester composition of the liver and the plasma lipoproteins was compared in intact animals which had been maintained for an extended time on normal diets, diets containing added olive oil and cholesterol, and fat-free diets.

MATERIALS AND METHODS

Rats and Diets

Three groups of male Sprague-Dawley rats (Holtzman Laboratories, Madison, Wis.) were used in these studies.

Group I consisted of five animals that had been fed a normal diet of rat pellets (Rockland Farms, New City, N. Y.) for 11 weeks after weaning. Their weights ranged from 360 to 400 g with a mean of 384 g.

Group II consisted of six normal adult rats that had been raised on a normal pellet diet to a weight of about 400 g. These animals were then fed for 5 weeks a diet consisting of ground rat pellets, olive oil, and cholesterol. Their weights ranged from 500 to 600 g.

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TABLE 1 COMPOSITION OF DIETS

	Total Fat*	Cholesterol†	Fatty Acid Composition‡			
			16:0	18:0	18:1	18:2
	% by weight of diet		*% of total fatty acids*			
Group I	3.65	0.05	21.3	3.8	27.2	42.9
Group II	14.0	1.0	15.4	2.1	67.6	12.2
Group III	ca. 45	0.4	43.0	10.9	25.8	4.5

* One hundred grams of each diet was ground, and extracted and washed by the method of Folch, Lees, and Sloane Stanley (4). Aliquots of the lipid extract were then dried and weighed.

† Cholesterol analyses of the lipid extract were performed by the method of Abell, Levy, Brodie, and Kendall (7).

‡ Methyl esters of the total fatty acids were formed by the method of Stoffel, Chu, and Ahrens (11).

Group III comprised animals fed fat-deficient diets for 11 weeks after weaning. The cholesterol was added to the diet to hasten the onset of the fat-deficiency syndrome (2). The diet was obtained from General Biochemical Co., Inc., Chagrin Falls, Ohio. Two groups, each comprising 10 rats, were maintained on the diet. In Group III A, three rats died and the weights of the survivors ranged from 160 to 270 g (mean 224 g). In Group III B two rats died and the weights of the survivors ranged from 160 to 270 g (mean 202 g). Both groups were similar with respect to the extent of essential fatty acid deficiency as judged by the presence of lesions on the tails and paws (3).

The lipid composition of the diets fed the various groups are shown in Table 1. All rats were allowed food and water ad lib. At the end of the feeding period the animals were anesthetized with ether and killed by exsanguination.

Lipid Analyses

The livers from each group of animals were combined and homogenized with water in a Waring Blendor. Lipids from an aliquot of the homogenate were extracted by the method of Folch, Lees, and Sloane Stanley (4). The blood from the rats in each group was pooled and plasma lipoproteins were separated by the method of Havel, Eder, and Bragdon (5). Three fractions were obtained: very low-density lipoproteins ($d < 1.019$), low-density lipoproteins (d 1.019–1.063), and high-density lipoproteins ($d > 1.063$).[1] In one study (Group II) native plasma was centrifuged at 30,000 × *g* for 30 min and the fraction of $d < 1.006$ was separated (6). Aliquots of plasma, the lipoprotein fractions, and the liver lipid extracts were taken for cholesterol determinations (7). The remainder of the lipoprotein fractions was extracted by the method of Folch et al. (8).

[1] The high density lipoproteins have densities between 1.063 and 1.21. However, in these studies separation at the latter density was not performed since the $d > 1.21$ fraction contains virtually no cholesterol esters (5).

The liver and plasma lipoprotein lipids were separated on silicic acid columns (100–200 mesh Unisil, Clarkson Chemical Co., Williamsport, Pa.). After elution of hydrocarbons and cholesterol esters with 6% benzene in hexane and 18% benzene in hexane, respectively (9), chloroform was added to the column to remove the remaining neutral lipids. As tested by thin-layer chromatography (10), cholesterol esters were present only in the 18% benzene in hexane fraction, which contained no other lipids.

Methyl esters were prepared by the method of Stoffel, Chu, and Ahrens (11) and were purified by thin-layer chromatography (hexane–diethyl ether–acetic acid 83:16:1).

Gas–liquid chromatographic analyses were performed on a Model 10 Barber Colman instrument with argon as carrier gas and an ionization detector (0.056 mc radium source). Separations were carried out on 6 ft × 6 mm i.d. columns packed with 16.1% ethylene glycol succinate polyester on 80–100 mesh Gas Chrom P (Applied Science Laboratories, Inc., State College, Pa.). The columns were operated at 178° and at 30 psi inlet pressure. Quantitative results with National Heart Institute Fatty Acid Standards A and D and Applied Science Laboratories, Inc. quantitative methyl ester mixtures K-108 (16:0, 18:0, 18:1, 18:2, 18:3), H-105 (18:0, 18:1, 18:2, 18:3), and two different preparations of H-104 (14:0, 16:0, 18:0, 20:0, 22:0, 24:0) agreed with the stated composition data with a relative error of less than 5% for the major components (>10% of the total mixture) and 5–12% for the minor components (<10% of the total mixture). For identification of all fatty acids except the eicosatrienoic acids, retention times (relative to methyl stearate) were compared to those of pure reference compounds. Samples containing eicosatrienoic acids (Group III samples) were subjected to further gas chromatographic analyses on columns packed with 10% Apiezon L on 80–100 mesh Gas Chrom P (6 ft × 6 mm i.d., operated at 220°). The identity of eicosatrienoic acids was established by their position on a log–log plot of

relative retention times on ethylene glycol succinate polyester versus relative retention times on Apiezon L (12, 13). Further confirmation of the identity of the eicosatrienoic acids was established by comparing their retention times on ethylene glycol succinate polyester to published retention times (14) of known samples of 8,11,14- and 5,8,11-eicosatrienoic acids obtained on columns packed with diethylene glycol succinate polyester operated under similar conditions.

The distribution of the fatty acids was calculated from the area of the peaks, determined by triangulation. The relative differences between replicate analyses were less than 5% for the major components (>10% of the total mixture) and 5–12% for the minor components (2–10% of the total mixture).

RESULTS

Table 2 shows the cholesterol concentrations in liver and plasma lipoproteins of the rats on the three diets. Compared to the control animals (Group I), the rats on the olive oil–cholesterol diet (Group II) had a large increase in the concentration of liver cholesterol. The animals on the fat-free cholesterol diet (Groups III A and III B) showed moderate increases.

In the control animals most of the plasma cholesterol was in the d >1.063 lipoproteins, while the d < 1.019 fraction contained only about 10% of the total amount. On the other hand, the rats fed olive oil and cholesterol (Group II) had an elevated plasma cholesterol concentration, and the increase was almost exclusively in the very low-density lipoproteins. The rats on the fat-free diet had slightly elevated cholesterol levels, and the rise was solely in the d < 1.019 fraction.

Plasma triglyceride concentrations were also determined. In Group II they were about twice the control value (39 mg/100 ml plasma), and in Groups III A and B were about half the control value.

The fatty acid compositions of the cholesterol esters of liver and of the lipoprotein fractions of the three dietary groups are shown in Tables 3–6. The data for the control animals are tabulated in Table 3. The major liver cholesterol ester fatty acids were oleic, linoleic, and palmitic. Cholesterol esters of the d < 1.019 lipoproteins had very similar percentages of the same fatty acids. Even in the case of the acids present in minor concentrations, i.e., myristic, palmitoleic, stearic, and arachidonic, the distribution in liver and the d < 1.019 lipoproteins was nearly identical. Characteristic of the liver and d < 1.019 lipoprotein cholesterol esters are the relatively high concentrations of oleic acid and the low concentrations of arachidonic acid. This is in contrast to the d > 1.063 cholesterol esters, in which nearly half of the fatty acids were arachidonic, and oleic acid comprised only about 5% of the total. The d 1.019–1.063 lipoprotein cholesterol esters also had a fatty acid composition which was different from that of liver and the d < 1.019 fractions, and which was intermediate between that of the d < 1.019 and the d > 1.063 fractions.

Table 4 has corresponding data for the animals fed the olive oil–cholesterol diet. Of the liver cholesterol esters about 75% were oleate and 20% linoleate with only minor amounts of the other fatty acids. The cholesterol esters of the d < 1.006 and the d 1.006–1.019 lipoproteins were very similar in composition but they were different from the cholesterol esters of the liver, which con-

TABLE 2 CHOLESTEROL CONCENTRATION OF THE LIVERS AND PLASMA LIPOPROTEINS

	Group I	Group II	Group III	
	(5)*	(6)	A (7)	B (8)
	mg/g			
Liver	1.44	25.4	4.18	4.83
	mg/100 ml plasma†			
Lipoproteins				
d < 1.006	—	10.8	—	—
d < 1.019	4.6	125.9‡	28.0	59.3
d 1.019–1.063	12.2	15.6	6.3	5.6
d > 1.063	30.3	20.7	28.5	19.1
Sum	47.1	173.0	62.8	84.0
Total plasma cholesterol§	43.2	207.7	68.2	91.3

* Numbers in parentheses are the number of animals in each group.

† Values in the table are means of 2–4 replicate analyses. At concentrations of <10 mg/100 ml the relative differences between replicate determinations were <10%; for concentrations >10 mg/100 ml, and for liver concentrations, the relative differences between replicate determinations were <4%.

‡ d 1.006–1.019.

§ By direct analysis of plasma.

TABLE 3 FATTY ACID COMPOSITION OF CHOLESTEROL ESTERS OF LIVER AND PLASMA LIPOPROTEINS FROM CONTROL RATS (GROUP I)

Fatty Acid*	Liver	Plasma Lipoproteins		
		d < 1.019	d 1.019–1.063	d > 1.063
	% of total fatty acids			
14:0	1.0	tr.	0.4	0.4
16:0	17.5	16.2	12.1	9.6
16:1	6.4	7.0	2.6	1.4
18:0	2.7	3.1	0.7	0.5
18:1	39.0	37.9	15.3	4.9
18:2	25.0	25.2	34.1	34.6
20:4	6.7	9.7	33.9	46.1

* Represents the major fatty acids present. Trace amounts of 18:3, 20:1, 20:3, and 20:5 as well as some unidentified acids were also observed.

TABLE 4 FATTY ACID COMPOSITION OF CHOLESTEROL ESTERS OF LIVER AND PLASMA LIPOPROTEINS FROM RATS FED OLIVE OIL–CHOLESTEROL DIET (GROUP II)

Fatty Acid*	Liver	Plasma Lipoproteins			
		d < 1.006	d 1.006–1.019	d 1.019–1.063	d > 1.063
		% of total fatty acids			
14:0	tr.	tr.	tr.	0.3	0.3
16:0	0.4	6.0	5.2	6.1	12.2
16:1	2.3	2.0	1.1	1.9	1.7
18:0	tr.	2.4	tr.	1.0	2.4
18:1	75.1	82.4	85.3	77.6	24.7
18:2	19.8	7.2	6.9	9.3	35.7
20:4	0.7	tr.	0.9	3.4	22.8

* Represents the major fatty acids present. Trace amounts of 18:3, 20:1 as well as some unidentified acids were also observed.

tained less palmitate and oleate and significantly more linoleate. As in the control animals, cholesterol esters of the liver and of the d < 1.006 and the d 1.006–1.019 lipoprotein fractions are characterized by relatively high levels of oleic acid and low levels of arachidonate. The d > 1.063 lipoprotein cholesterol esters, on the other hand, contained less oleate, and more arachidonate and linoleate than the d 1.006–1.019 fraction. The d > 1.063 fraction in the Group II rats contained a higher percentage of oleate and less arachidonate than the same fraction in Group I. The d 1.019–1.063 lipoproteins in Table 4 also had a composition which was intermediate between the other two fractions.

Tables 5 and 6 show the fatty acid composition of the cholesterol esters for Groups III A and III B respectively. The animals in these groups showed the classical signs of essential fatty acid deficiency (3). Their weight gains were less than those of the control rats and they developed dermatitis on the paws, as well as scaly, necrotic tails. In these deficient animals the liver cholesterol esters had almost no linoleate or arachidonate but had a high proportion of oleate and palmitoleate. In both groups the composition of the d < 1.019 esters was virtually the same as that of the liver cholesterol esters. However, as was seen for the other dietary groups, the d > 1.063 cholesterol esters had a remarkably different composition in that there was a greater proportion of polyunsaturated acids. In both groups linoleate and arachidonate were markedly reduced, although there was a considerably greater reduction of arachidonate in Group III B. The monoenoic acids were also elevated, although the percentage of oleate was only half that in the d < 1.019 fraction. An outstanding feature of the d > 1.063 pattern was the presence of an eicosatrienoic acid.[2] This acid was present in greatest proportion in the d > 1.063 fraction and was found only in trace amounts in the d < 1.019 fraction and liver.

DISCUSSION

These observations have confirmed our earlier finding that in the rat the fatty acid composition of cholesterol esters differs among the lipoprotein classes. The composition of the d < 1.019 fraction is similar to that of the liver except in the rats on the olive oil–cholesterol diet, in which the liver contains somewhat more linoleate and less oleate in its cholesterol esters than do the d < 1.006 or d < 1.019 lipoproteins. The cholesterol esters of the d > 1.063 lipoproteins are markedly different from those of the liver in that they contained a higher proportion of polyunsaturated esters. This is seen to a remarkable extent in the animals on the fat-free diets, in which the livers contained very small amounts of polyunsaturated acids while the d > 1.063 lipoproteins had appreciable amounts. The lipoproteins of d 1.019–1.063 had a composition intermediate between the d < 1.019 and the d > 1.063 lipoproteins.

These results suggest that the cholesterol esters may be incorporated into the various lipoprotein fractions by different mechanisms. The data are consistent with our hypothesis that the cholesterol esters of the d < 1.019 lipoproteins are derived from the liver by a process which is nonselective (1).

In the group fed the olive oil–cholesterol diet there are differences between the cholesterol ester composition of the liver and the d < 1.019 lipoproteins, especially with respect to linoleate. These differences may be related to the fact that in animals fed diets high in cholesterol, the cholesterol ester concentration of the liver is markedly elevated (15). Rice, Schotz, Alfin-Slater, and Deuel (16) fractionated homogenates of livers from rats fed high cholesterol diets and found that there was a relatively greater increase of cholesterol esters in the "floating layer" than in the supernatant solution or residue (cell débris, nuclei, mitochondria, and microsomes). Recent studies in our laboratory have shown that in rats on control diets the cholesterol ester composition of the d < 1.019 lipoproteins is most similar to that of the liver microsomes and mitochondria, and this is in accord with observations that the enzymes that esterify cholesterol in liver are exclusively particulate (17). It is, therefore, likely that analyses of the total liver cholesterol ester fatty acids of animals fed the olive oil–cholesterol diet rather reflect the composition of a pool from which the cholesterol esters of the d < 1.019 lipoproteins are not derived. The d < 1.019 lipoproteins turn over at such a rapid rate (18) that the cholesterol ester composition of this fraction would be governed by the availability of

[2] This acid is probably 5,8,11-eicosatrienoic acid, the major trienoic acid observed in lipids of fat-deficient rats (3).

cholesterol esters at the site of lipoprotein formation. On the other hand, the slower rate of turnover of the d > 1.063 lipoproteins would permit other processes to play a more important role.

In view of the fact that the liver contains such small amounts of cholesterol esters of polyenoic fatty acids as compared to the high-density lipoproteins, it is necessary to postulate the existence of processes capable of great selectivity. This is especially necessary with rats on fat-free diets, in which the 5,8,11-eicosatrienoic acid is present in the cholesterol esters of the liver in only trace amounts while in the d > 1.063 lipoproteins it constitutes 13–15% of the cholesterol ester fatty acids. Obviously there must be preferential incorporation of cholesterol esters of polyenoic acids into the high-density lipoproteins and this is perhaps due to specificity of the protein for these esters. However, the possibility of these esters being derived from a special pool is not excluded. Such a pool could be in the liver or in the intestine. This latter possibility has been suggested by Swell, Law, Field, and Treadwell (19), who showed that in the plasma of rats with fistulas of the thoracic duct the amounts of cholesteryl linoleate and arachidonate decreased. They concluded from their studies that lymph is an important source of plasma cholesterol esters. However, it is not known what proportion of the cholesterol esters in thoracic duct high-density lipoproteins derive from the intestine and what proportion is derived from blood plasma (20). Furthermore, approximately 10% of the thoracic duct lymph in rats is derived from the liver (21).

It is also possible that the cholesterol esters in the high-density lipoproteins could be formed in the plasma, where esterification of free cholesterol has been shown to occur when plasma is incubated in vitro at 37° (22–25). Glomset has shown that the increase in ester cholesterol is accompanied by a decrease in lecithin (26). The fatty acid composition of the newly formed cholesterol esters was found to be very similar to that of the fatty acids on carbon-2 of lecithin, which are largely polyunsaturated, and Glomset has shown that these acids are incorporated into the newly formed cholesterol esters by transesterification. Such a mechanism could account for the high degree of unsaturation of the cholesterol esters of the high density lipoproteins. With the very low density lipoproteins, this mechanism would play a lesser role because of their rapid rate of turnover (18). However, Goodman and Shiratori, in studies of the turnover of different cholesterol esters in rat liver and plasma (27) point out that if such a transesterification reaction did occur one might expect to find cholesterol esters of polyunsaturated fatty acids of higher specific activity in the plasma than the liver. This was not observed in their studies.

It should be noted that in humans the cholesterol ester composition of the various lipoprotein fractions is quite similar (28), in contrast to our findings in the rat. Moreover, Goodman has shown (29) that, in humans, the individual cholesterol esters in whole plasma have the same rate of turnover. Within each lipoprotein class, the individual cholesterol esters also turn over at the same rate, but this rate differs from one lipoprotein class to another. Goodman and Shiratori have shown (27) that in the rat the individual cholesterol esters of plasma turn over at different rates. We have recently found that in the rat the cholesterol esters turn over at different rates in the different lipoproteins but that within a single lipoprotein class, in contrast to the human, the individual cholesterol esters turn over at different rates (Gidez, unpublished

TABLE 5 Fatty Acid Composition of Cholesterol Esters of Liver and Plasma Lipoproteins from Rats Fed Fat-Free Cholesterol Diet (Group III A)

Fatty Acid*	Liver	Plasma Lipoproteins		
		d <1.019	d 1.019–1.063	d >1.063
		% of total fatty acids		
14:0	0.3	0.3	0.5	0.4
16:0	15.8	16.9	13.6	7.3
16:1	18.0	17.2	18.3	20.3
18:0	2.5	2.0	2.0	0.5
18:1	61.8	62.7	51.2	31.2
18:2	0.3	tr.	2.0	4.6
20:1	tr.	tr.	0.4	1.2
20:3†	tr.	tr.	4.6	14.9
20:4	tr.	tr.	6.1	17.1

* Represents the major fatty acids present. Trace amounts of 18:3, 20:3 (probably $\Delta^{8,11,14}$), as well as some unidentified acids were also observed.

† Probably $\Delta^{5,8,11}$.

TABLE 6 Fatty Acid Composition of Cholesterol Esters of Liver and Plasma Lipoproteins from Rats Fed Fat-Free Cholesterol Diet (Group III B)

Fatty Acid*	Liver	Plasma Lipoproteins		
		d <1.019	d 1.019–1.063	d >1.063
		% of total fatty acids		
14:0	0.5	0.4	1.4	0.4
16:0	15.1	14.2	20.7	6.5
16:1	18.7	23.5	16.3	32.8
18:0	1.4	1.5	5.2	0.2
18:1	61.4	57.9	45.4	30.3
18:2	0.5	0.4	2.9	4.3
20:1	0.3	tr.†	0.5	1.3
20:3†	0.3	tr.	2.9	12.8
20:4	0.2	0.3	2.6	9.4

* Represents the major fatty acids present. Trace amounts of 18:3, 20:3 (probably $\Delta^{8,11,14}$), as well as some unidentified acids were also observed.

† Probably $\Delta^{5,8,11}$.

experiments). These differences are undoubtedly related to the differences in cholesterol composition of the rat lipoproteins and suggest that the mechanism of formation of plasma cholesterol esters may differ between humans and rats.

In view of these findings it is apparent that in studies of the metabolism of the cholesterol esters of rat plasma, valuable information may be concealed or lost unless individual lipoproteins are isolated.

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