Cholesteryl ester turnover in human plasma lipoproteins during cholestyramine and clofibrate therapy

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ABSTRACT The effects of cholestyramine and of clofibrate on the turnover rates of individual cholesteryl esters in whole human plasma and in each of the three classes of plasma lipoproteins have been studied. Four hyperlipidemic patients (two under treatment with each of the two drugs) were injected intravenously with cholesterol-^{14}C, and serial plasma samples were collected after 3–4 hr, 8 hr, 24 hr, and 4–5 days. The plasma samples were separated into three classes of lipoproteins by ultracentrifugation. The cholesteryl esters and free cholesterol were isolated from each sample, and the specific radioactivity of the free and esterified cholesterol was determined. The specific radioactivity of each individual cholesteryl ester was then determined for each sample, by separately measuring the distribution of cholesterol mass and of radioactivity among four different cholesteryl ester groups, namely the saturated, mono-, di-, and tetra-unsaturated esters.

In all subjects the plasma cholesteryl esters were metabolically heterogeneous, and could be divided into three pools corresponding to the three classes of plasma lipoproteins. High density lipoprotein ($d > 1.063$) cholesteryl esters showed the greatest fractional turnover rate, and low density lipoprotein (d 1.019–1.063) cholesteryl esters showed the smallest fractional turnover rate. In each subject the cholesteryl ester composition of the three classes of plasma lipoprotein was almost identical. Within each lipoprotein, and in whole plasma, all the different individual cholesteryl esters were found to turn over at the same fractional rate. In all respects these results were similar to those previously obtained with normal subjects. The results suggest that neither drug has a strongly selective effect on the turnover of one particular cholesteryl ester, or on the turnover or composition of the cholesteryl esters in one particular plasma lipoprotein.

SUPPLEMENTARY KEY WORDS man · hyperlipidemia

PLASMA ESTERIFIED cholesterol consists of a mixture of several different esters distributed among several plasma lipoproteins. Because of this, there are many possibilities for the heterogeneous metabolism of plasma cholesteryl esters. For example, the metabolism of one ester, such as cholesteryl palmitate, may differ from the metabolism of another ester such as cholesteryl linoleate. Furthermore, the metabolism of the same or of different esters within different lipoproteins may not be the same.

It was previously reported (1) that, in two normal subjects injected with mevalonate-^{14}C, the fractional turnover rate of different cholesteryl esters was the same, both in whole plasma and in each of three classes of plasma lipoproteins. In contrast, the fractional turnover rate of cholesteryl esters differed from lipoprotein to lipoprotein, with the greatest turnover rate occurring in cholesteryl esters of the high density lipoproteins, and the smallest turnover rate in the low density (d 1.019–1.063) lipoprotein cholesteryl esters. It was concluded that under normal conditions metabolic heterogeneity among plasma cholesteryl esters in man exists between the different plasma lipoproteins, rather than between the different esters in whole plasma or in a given lipoprotein. This conclusion was confirmed by Nestel, Couzens, and Hirsch, who obtained similar findings in subjects with both normal and high plasma cholesterol levels who had been injected with cholesterol-^{14}C (2). These workers (2) also examined the effect of diet

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins.

on the turnover of individual cholesteryl esters, using diets rich in carbohydrate, saturated fat, or safflower oil. Although the composition of the plasma cholesteryl esters was altered by changing the diet, under each condition the fractional turnover rates of the different individual cholesteryl esters were all the same.

We now report the results of studies of the turnover of individual cholesteryl esters in different plasma lipoproteins in patients being treated for hyperlipidemia with either cholestyramine or clofibrate. These two drugs were selected because cholestyramine greatly increases the total body turnover of cholesterol (3) but has very little effect on the plasma cholesteryl ester composition,[1] whereas clofibrate markedly alters the composition of the plasma cholesteryl esters (4, 5).[1] The results demonstrate that neither drug appears to have a clearly selective effect on the turnover of one particular cholesteryl ester or of the cholesteryl esters in one particular class of lipoprotein.

METHODS

Four volunteer male subjects, whose ages are given in Table 1, participated in these studies. Two of the subjects (JP and LP) had mild type II hyperlipoproteinemia, and two (RL and AM) had type IV hyperlipoproteinemia (6). The subjects ate their usual diets during the period of study. Subjects JP and LP were being treated with cholestyramine (Questran; Mead Johnson & Co., Evansville, Ind.), 12 g/day, and subjects RL and AM were being treated with clofibrate (Atromid-S; Ayerst Laboratories, New York,) 2 g/day, at the time of study. Each subject had been under treatment for at least 2 months (range 2–6 months) at the time of study and had achieved a lowered and stable level of serum lipids as a result of therapy (see Table 1).

Cholesterol-4-^{14}C (New England Nuclear Corp., Boston, Mass.; specific radioactivity 56 μc/μmole) was added to normal control serum and prepared for injection as previously described (3). Subjects JP and LP were each injected intravenously with serum containing 27 μc, and subjects RL and AM (in a later study) were injected with 15 μc of cholesterol-^{14}C. Serial blood samples were collected after 3–4 hr, 8 hr, 24 hr, and 4–5 days in syringes moistened with a solution of heparin, and plasma samples were collected and processed as described in detail elsewhere (1, 7). Most of each sample of plasma was subjected to ultracentrifugation at densities of 1.019 and 1.063 in order to obtain the following three classes of lipoproteins: very low density (VLDL; d $<$ 1.019), low density (LDL; d 1.019–1.063), and high density (HDL; d $>$ 1.063) lipoproteins. The total lipid separate cholesteryl esters and free cholesterol. These samples were then analyzed in duplicate for cholesterol in each plasma and lipoprotein sample was extracted and was chromatographed on a silicic acid column to mass (8), and were assayed for ^{14}C with a Packard liquid scintillation counter. During radioassay, samples were monitored for quenching by means of an external automatic standard; quenching was not observed. The counting efficiency was identical for all samples from a given subject. The data provided the values for the specific radioactivity of the free cholesterol and for the average specific radioactivity of the esterified cholesterol, in each plasma or lipoprotein sample.

The specific radioactivity of each individual cholesteryl ester was then determined for each sample by separately measuring the distribution of cholesterol mass and of radioactivity among four different cholesteryl ester groups, namely the saturated, mono-, di-, and tetra-unsaturated esters. The distribution of cholesterol mass was calculated as previously described (1) from the composition of the cholesteryl esters as determined by gas–liquid chromatography of the cholesteryl ester fatty acids (as their methyl esters). The distribution of radioactivity among the different cholesteryl esters was determined by thin-layer chromatography on Silica Gel G impregnated with $AgNO_3$, as described before (1, 9). By then comparing the distribution of cholesterol mass with that of ^{14}C, the specific radioactivity of the different (saturated, mono-, di-, and tetra-unsaturated) cholesteryl esters in each sample could be readily calculated (1).

TABLE 1 SUBJECTS STUDIED; EFFECTS OF THERAPY

Subject	Age	Rx*	Plasma Cholesterol†	Plasma Triglyceride†	n‡
			mg/100 ml		
LP	68	0	295 ± 6	48 ± 2	13
LP	68	CHOL	212 ± 7	78 ± 5	9
JP	57	0	313 ± 4	182 ± 6	8
JP	57	CHOL	233 ± 12	169 ± 23	9
RL	62	0	277 ± 6	471 ± 34	5
RL	62	ATR	242 ± 5	230 ± 14	6
AM	52	0	257 ± 9	313 ± 78	4
AM	52	ATR	219 ± 11	141 ± 29	4

* 0, none; CHOL, cholestyramine, 12 g/day; ATR, clofibrate, 2 g/day.

† Mean ± SEM for samples taken during a period of several weeks either without therapy, or during therapy and including the period of the present study. Most of the samples were obtained at weekly intervals.

‡ n, number of samples.

RESULTS

The specific radioactivity of the free and esterified cholesterol in each plasma lipoprotein, for each subject, at four time intervals after the intravenous injection of

[1] Goodman, DeW. S., and R. P. Noble. Unpublished studies.

cholesterol-^{14}C, is shown in Figs. 1–4. Labeled free cholesterol rapidly equilibrated with the free cholesterol in each of the three lipoproteins. Thus, for subjects LP, RL, and AM the free cholesterol specific radioactivities in the three lipoproteins derived from a given plasma sample were nearly identical in all samples. In subject JP (Fig. 2) the specific radioactivity of the HDL free

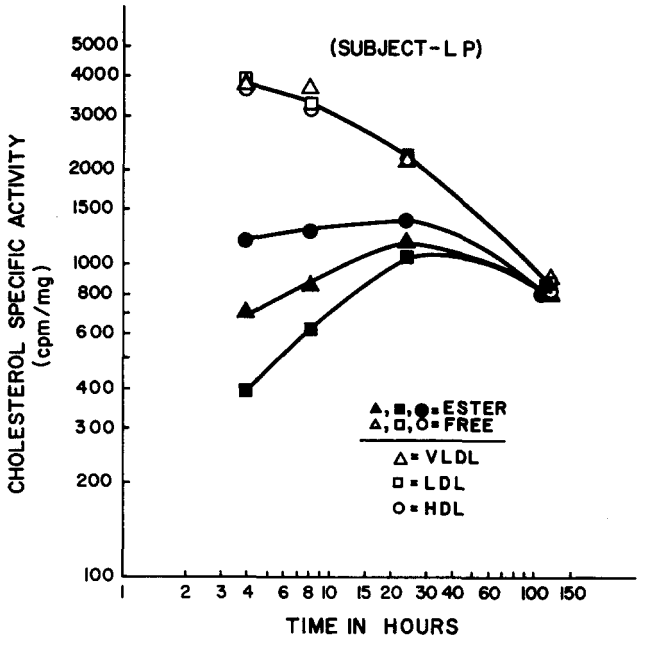

FIG. 1. The specific radioactivity of free and esterified cholesterol in the three plasma lipoprotein fractions of subject LP (during cholestyramine therapy) at various times after intravenous cholesterol-^{14}C.

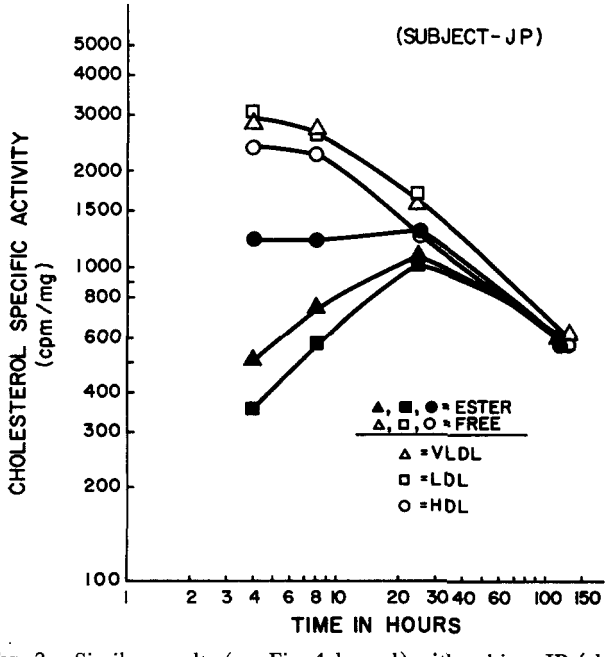

FIG. 2. Similar results (see Fig. 1 legend) with subject JP (cholestyramine therapy).

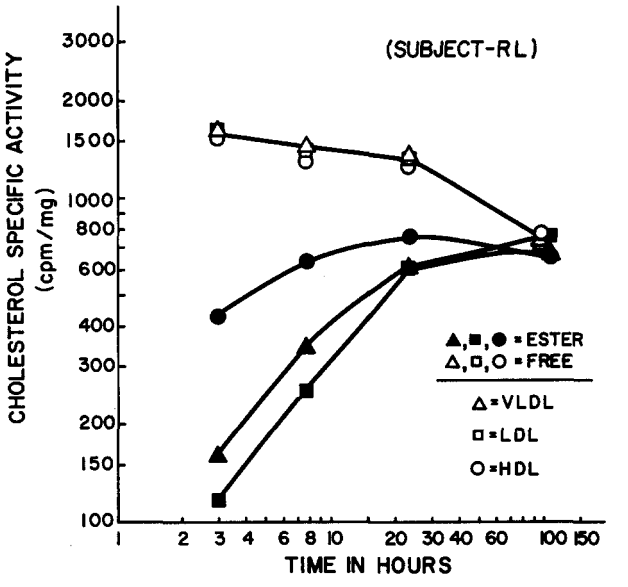

FIG. 3. Similar results (see Fig. 1 legend) with subject RL (clofibrate therapy).

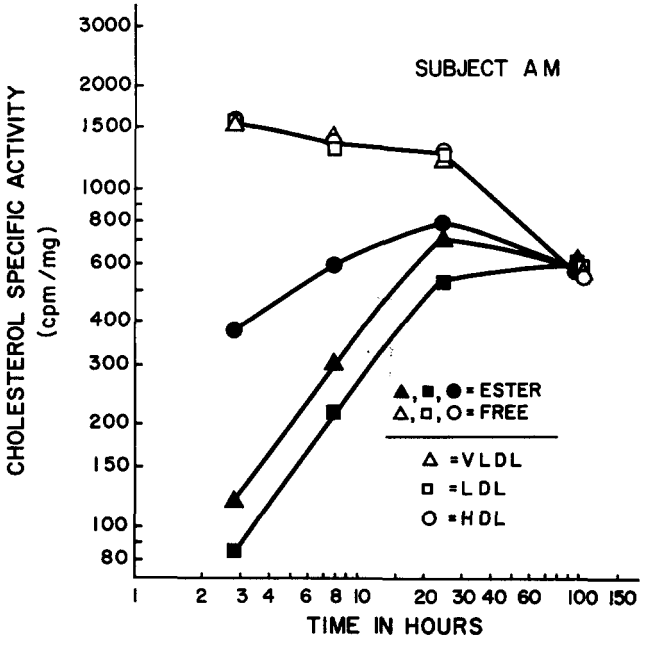

Fig. 4. Similar results (see Fig. 1 legend) with subject AM (clofibrate therapy).

cholesterol was slightly less than that of the LDL and VLDL free cholesterol during the first 24 hr. Equilibration was complete between the free and esterified cholesterol of all three lipoproteins in all subjects by 4–5 days. During the first 24 hr, however, marked differences were seen in the specific radioactivities of the esterified cholesterol of the different lipoproteins. In all subjects, radioactivity appeared much more rapidly in the esterified cholesterol of the HDL than in the ester cholesterol of the other lipoproteins. Radioactivity appeared least rapidly in

TABLE 2 CHOLESTERYL ESTER FATTY ACID COMPOSITION OF WHOLE PLASMA AND PLASMA LIPOPROTEINS

Fatty Acid	% of Total Fatty Acid Methyl Esters*			
	Whole Plasma	VLDL	LDL	HDL
I. Subject LP:				
14:0†	0.3	0.5	0.3	0.4
16:0	10.4 ± 0.2	10.9 ± 0.2	10.1 ± 0.2	11.4 ± 0.5
16:1	2.4 ± 0.1	2.9 ± 0.2	2.4 ± 0.1	2.5 ± 0.1
18:0	0.7	1.5	0.7	0.8
18:1	18.0 ± 0.2	19.6 ± 0.5	17.8 ± 0.2	18.7 ± 0.4
18:2	57.1 ± 0.4	54.2 ± 0.4	57.7 ± 0.6	56.3 ± 1.1
20:0	1.1	1.0	1.1	1.1
20:4	9.9 ± 1.0	7.0 ± 0.6	9.8 ± 0.6	8.9 ± 0.3
II. Subject JP:				
14:0	0.6	0.5	0.8	0.6
16:0	12.3 ± 1.1	10.8 ± 0.2	12.1 ± 0.3	11.7 ± 0.2
16:1	4.3 ± 0.1	4.3 ± 0.1	4.4 ± 0.1	4.6 ± 0.1
18:0	1.0	1.0	0.9	0.9
18:1	18.0 ± 0.4	17.9 ± 0.3	16.9 ± 0.4	16.7 ± 0.3
18:2	51.0 ± 1.8	51.4 ± 0.6	51.4 ± 0.4	51.2 ± 0.4
20:0	1.3	1.4	1.3	1.4
20:4	10.7 ± 0.9	9.9 ± 0.6	11.6 ± 0.2	12.2 ± 0.6
III. Subject RL:				
14:0	0.5	0.4	0.7	0.5
16:0	10.9 ± 0.1	10.1 ± 0.1	11.3 ± 0.2	10.5 ± 0.1
16:1	6.1 ± 0.4	5.8 ± 0.5	6.2 ± 0.5	6.0 ± 0.4
18:0	1.1	1.2	1.1	1.6
18:1	23.9 ± 0.8	24.9 ± 1.0	23.5 ± 1.0	22.3 ± 0.6
18:2	45.2 ± 0.9	44.7 ± 0.5	44.3 ± 0.6	45.1 ± 0.4
18:3	1.6	2.1	2.0	1.8
20:3	1.1	1.3	1.1	1.1
20:4	8.0 ± 0.2	7.1 ± 0.2	7.8 ± 0.3	8.3 ± 0.3
IV. Subject AM:				
14:0	0.5	0.3	0.5	0.5
16:0	9.3 ± 0.1	7.4 ± 0.3	9.6 ± 0.2	9.6 ± 0.4
16:1	10.1 ± 0.1	8.4 ± 0.4	10.2 ± 0.3	9.4 ± 0.6
18:0	0.8	3.7 ± 0.6	0.7	1.1
18:1	28.8 ± 0.3	27.1 ± 0.8	28.7 ± 0.2	27.9 ± 0.3
18:2	39.7 ± 0.7	36.9 ± 0.8	39.1 ± 0.7	39.0 ± 1.1
18:3	1.8	1.6	1.6	2.0
20:3	0.9	2.2	1.1	1.4
20:4	6.2 ± 0.5	5.5 ± 0.7	6.7 ± 0.3	7.0 ± 0.3

* Mean ± SEM were not calculated for fatty acids comprising less than 2.5% of the total. For each subject, all minor components (less than 0.5% of the total fatty acids) have been omitted from this table. Four samples of whole plasma and of each plasma lipoprotein were analyzed for each subject.

† Number of carbon atoms: number of double bonds.

the esterified cholesterol of the LDL. The differences between the three lipoproteins were most marked in the early hours of the study. These findings are similar to those previously obtained with normal untreated subjects (1).

The cholesteryl ester fatty acid composition of whole plasma and of each class of plasma lipoprotein, for each subject, is shown in Table 2. For each subject, the cholesteryl ester composition of the three lipoproteins was almost identical. Similar findings were previously reported (7) for normal subjects. It thus seems likely that neither drug selectively alters the cholesteryl ester composition of one particular lipoprotein. The cholesteryl ester fatty acid pattern of the subjects (RL and AM) being treated with clofibrate showed an unusually high proportion of monoenoic (16:1 and 18:1) fatty acids. This finding was undoubtedly an effect of clofibrate therapy, since clofibrate is known to increase the relative amount of 16:1 and 18:1 and to decrease the relative amount of 18:2 in plasma cholesteryl esters of patients under treatment with this drug (4, 5).[1] Although we did not determine the cholesteryl ester composition of these patients prior to drug therapy, we have conducted more detailed studies in other subjects, to be reported elsewhere, on the effects of both cholestyramine and clofibrate on the composition

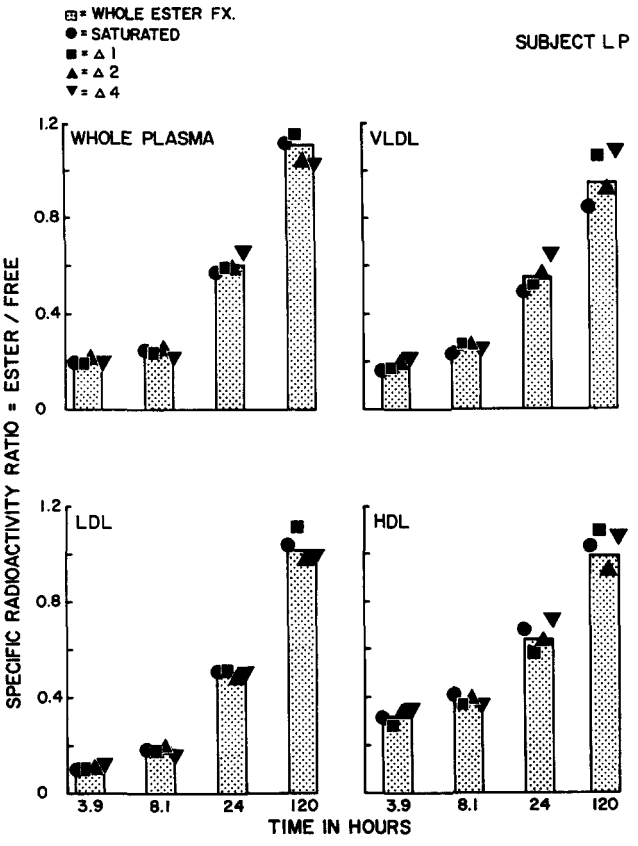

FIG. 5. The relative specific radioactivity of the cholesteryl esters in plasma and in the three plasma lipoprotein fractions of subject LP (cholestyramine therapy) after intravenous cholesterol-^{14}C.

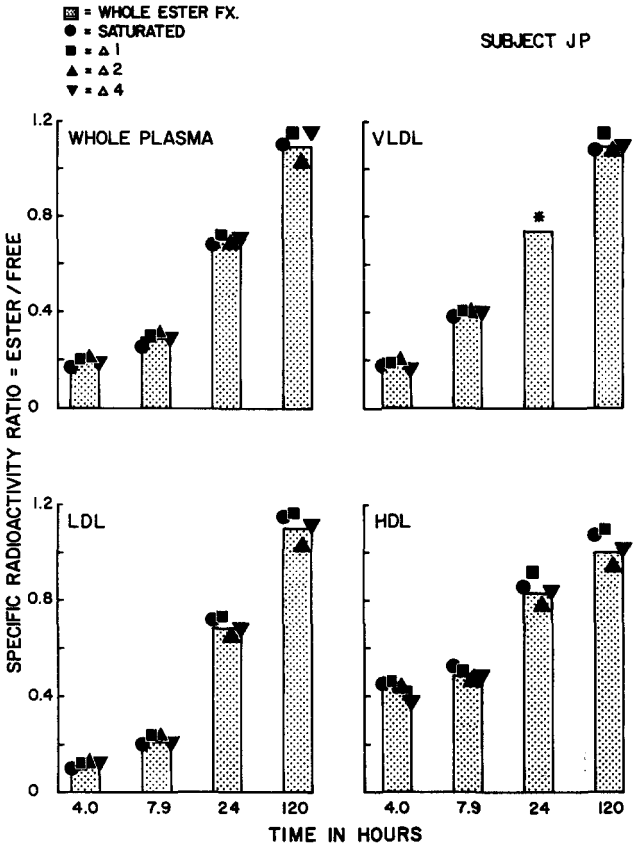

FIG. 6. Similar results (see Fig. 5 legend) with subject JP (cholestyramine therapy). The 24 hr VLDL sample (marked *) was not analyzed for individual esters because of sample loss.

of several plasma lipid classes. These studies have shown the above effect of clofibrate in every subject, and they have shown that cholestyramine has very little effect on the composition of the plasma cholesteryl esters.

Figs. 5–8 show, for each subject, the relative specific radioactivity of each individual cholesteryl ester in whole plasma and in each lipoprotein, at each time interval after giving cholesterol-^{14}C. In these figures, each vertical column shows the results obtained with one sample of plasma or lipoprotein. In each column the height of the stippled bar represents the ratio of the average specific radioactivity of the cholesteryl esters as a whole, compared with the specific activity of the free cholesterol in the same sample. The relative specific radioactivities of the four individual cholesteryl ester groups in each sample are shown as points in the same vertical column. For each subject, both with whole plasma and with each lipoprotein, at each time interval the relative specific radioactivities of the different cholesteryl esters were all virtually identical. This indicates that in each subject the different individual cholesteryl esters were turning over at the same fractional rate, both in whole plasma and within each lipoprotein. These results are similar to those obtained previously (1) with normal subjects.

DISCUSSION

These studies were designed to investigate whether pharmacologic agents which lower serum lipid levels might selectively affect the turnover of one particular cholesteryl ester, or of the cholesteryl esters in one particular plasma lipoprotein. Cholestyramine and clofibrate were chosen for study because these drugs are known to have very different effects on cholesterol metabolism in the body, and because these are two effective agents currently used for the treatment of hyperlipidemia. Cholestyramine, a bile acid sequestering resin, greatly increases the total body turnover of cholesterol (3), mainly by increasing the rate of production and excretion of bile acids (10). The question, therefore, arose as to whether the accelerated rate of cholesterol catabolism to bile acids induced by cholestyramine might be accompanied by a differential effect on the turnover of one particular portion of the plasma cholesteryl esters. Boyd has, for example, suggested that esterification of cholesterol with linoleic acid might facilitate hydroxylation of the sterol at carbon No. 7, and he has also suggested that esterified cholesterol might serve to some extent as the immediate precursor of bile acids in the liver (11). Although evi-

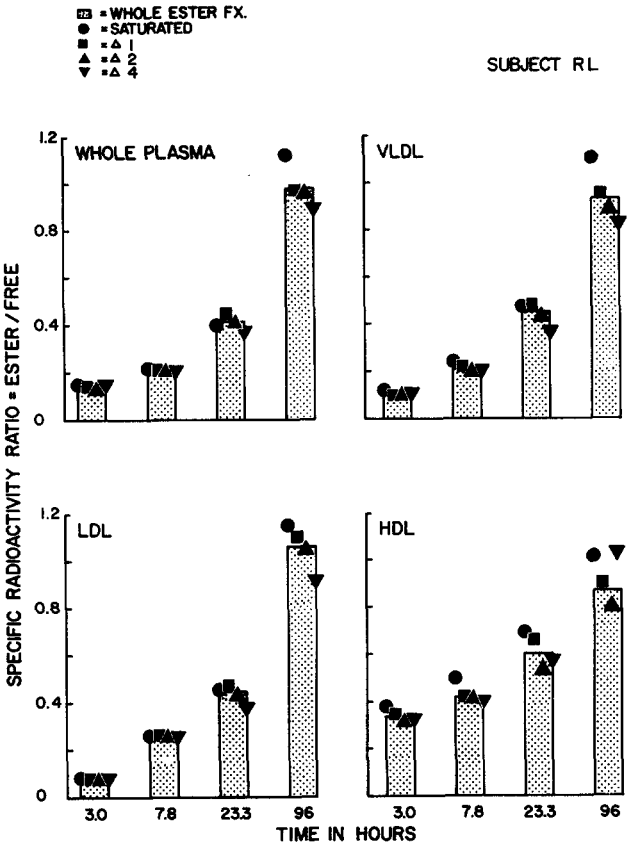

FIG. 7. Similar results (see Fig. 5 legend) with subject RL (clofibrate therapy).

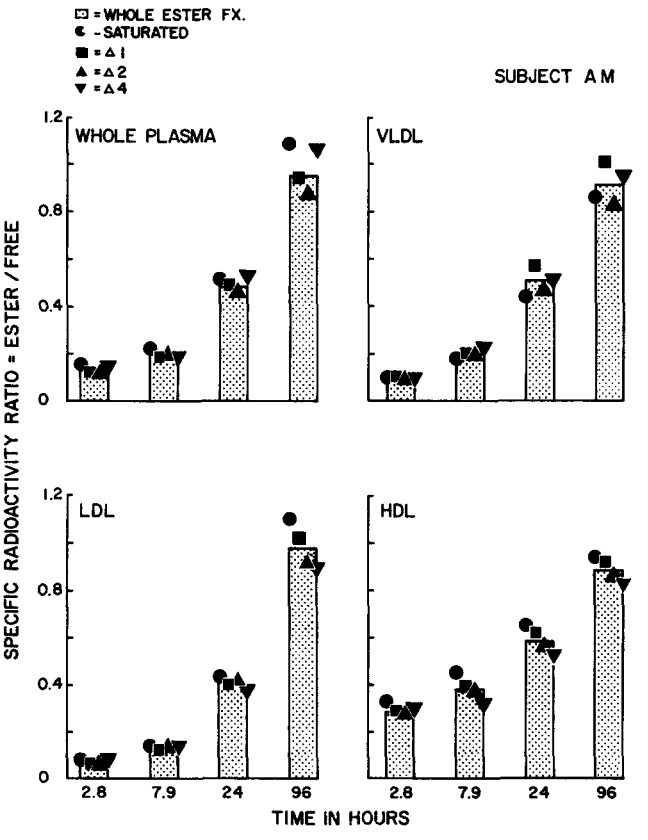

FIG. 8. Similar results (see Fig. 5 legend) with subject AM (clofibrate therapy).

dence supporting this suggestion is not available, we felt that information about the effect of cholestyramine on the turnover of plasma cholesteryl esters would be of value.

In contrast to cholestyramine, which has very little effect on the plasma cholesteryl ester composition, clofibrate is known to alter considerably the composition of plasma cholesteryl esters (4, 5).[1] The mechanism for this effect is not known. The possibility existed, however, that clofibrate alters the cholesteryl ester composition by selectively affecting the fractional turnover rate of certain individual cholesteryl esters.

The possible mechanisms responsible for plasma cholesteryl ester turnover have been discussed in detail previously (1). One possibility is that turnover reflects the continuing hydrolysis of plasma cholesteryl esters (perhaps taking place in the liver), followed by the reesterification of free cholesterol. Other possibilities include the removal and replacement of intact cholesteryl esters during circulation of lipoproteins through the liver and other tissues, and the equilibration by exchange of plasma cholesteryl esters. At the present time, it seems likely that cholesterol esterification in plasma by way of the plasma lecithin-cholesterol acyltransferase reaction (12) plays an important role in the normal formation and turnover of plasma cholesteryl esters. Thus, the initial rate of cholesterol transesterification seen with human plasma in vitro is similar to the turnover rate of cholesteryl esters seen in vivo (13). In general, the pattern of cholesteryl esters formed in vitro is roughly similar to the normal composition of plasma cholesteryl esters. Moreover, it has been shown recently that labeled cholesterol is incorporated in vitro into the cholesteryl esters of different plasma lipoproteins at different rates (HDL > VLDL > LDL) (14, 15), similar to the relative differences observed here for cholesteryl ester turnover in vivo. The evidence suggesting that the acyltransferase reaction serves as a physiologically important source of plasma cholesteryl esters has been summarized by Glomset (12).

The results reported here demonstrate that in all four subjects on drug therapy the fractional turnover rates of the different individual cholesteryl esters were the same in whole plasma, and in each of the three plasma lipoproteins. In addition, in each subject the cholesteryl ester composition of the three plasma lipoprotein classes was almost identical. These results are similar to those previously obtained with normal subjects. The relative turnover rates of the cholesteryl esters in the different plasma lipoproteins were also similar to those observed before in

normal subjects. Thus, in all subjects the fractional turnover rate of cholesteryl esters differed from lipoprotein to lipoprotein; the HDL cholesteryl esters showed the greatest fractional turnover rate, and the LDL cholesteryl esters showed the smallest. These results suggest that neither cholestyramine nor clofibrate has a strongly selective effect on the turnover of one particular cholesteryl ester or on the turnover or composition of the cholesteryl esters in one particular class of lipoprotein.

In view of these findings, the mechanism whereby clofibrate results in the alteration of plasma cholesteryl ester composition remains obscure. One possibility is that clofibrate first induces a change in the composition of the precursor pool of fatty acyl residues which take part in cholesterol esterification. It should also be stressed that these studies were conducted after several weeks of drug therapy, when the patients apparently had reached a new steady state with regard to serum lipid levels and composition. It is possible that clofibrate produced a transient change in the relative turnover rate of one or more particular cholesteryl esters, and that this effect was no longer apparent by the time the steady state was reached. Further studies will be needed in order to define the mechanism of the effect of clofibrate on cholesteryl ester composition.

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