Influence of diet on the composition of plasma cholesterol esters in man

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ABSTRACT The effect on the plasma cholesterol esters of
diets rich in either carbohydrate, chocolate, or safflower oil was
studied sequentially in two men. The changes in the choles-
terol esters of the major plasma lipoproteins were studied by
measuring (a) the distribution of fatty acids in the esters and
(b) the distribution of radioactivity among the esters after
the administration of cholesterol-4-^{14}C labeled lipoproteins.

Similar changes were found in the cholesterol esters of the
two major lipoproteins; these changes became apparent within
24 hr after changing diets. Monounsaturated esters pre-
dominated with carbohydrate-rich diets. When the chocolate-
rich diet was substituted, the proportion of saturated and
monounsaturated esters fell and that of cholesteryl linoleate
rose. This indicated the utilization of preexisting linoleate in
preference to the more saturated fatty acids which abounded
in the diet. The substitution of safflower oil led to further in-
crements of cholesteryl linoleate. The possible reasons under-
lying the preferential incorporation of cholesteryl linoleate in
man are discussed.

KEY WORDS   cholesterol esters · plasma lipoproteins ·
dietary influence · carbohydrate · chocolate · safflower
oil · compositional changes · man

T HE LIPIDS of lymph and plasma lipoproteins tend to
resemble the dietary lipids to a variable extent. The
degree of resemblance varies among the different lipids
and among the lipids of the different lipoproteins. Thus
the triglyceride fatty acids of the chylomicrons in the
lymph and in the plasma are very similar to the ingested
fatty acids at the height of absorption (1–5), although
dilution with endogenous fatty acids may be considerable
at other times (3, 4). On the other hand, in man the
lipids of the other lymph lipoproteins are affected to a
lesser degree by a single feeding of a specific fat (4) and
lipids other than triglyceride, namely phospholipids and
cholesterol esters, are only very slightly altered (4, 5).

Kayden, Karmen, and Dumont (4) have also reported
the compositional changes of plasma cholesterol esters
after single meals of corn oil or coconut oil. They have
shown that 8–10 hr later the cholesterol esters of chylo-
microns contain a small proportion of the fed fatty acids
but that the cholesterol esters of the remaining lipopro-
teins remained substantially unaltered (4). Although the
composition of the plasma cholesterol esters may not be
appreciably influenced by a single meal of fat, there is
ample evidence that the prolonged consumption of a fat
such as corn oil will lead to a significant increase in the
proportion of cholesteryl linoleate (6). Furthermore,
diets rich in carbohydrate, which lead to the synthesis of
monounsaturated and saturated fatty acids (7–9), in-
crease the proportions of cholesteryl oleate and cho-
lesteryl palmitate (7, 10, 11).

We have studied in man the changes in the plasma
cholesterol esters for three separate dietary periods during
which either carbohydrate, chocolate, or safflower oil
was the major source of calories. We have made frequent
measurements, especially when one diet was substituted
for another, in order to determine the time course of the
compositional changes and also the contribution of endog-
enous fatty acids to these changes. The studies were
carried out in two men in one of whom the plasma was
separated into three major classes of lipoproteins since
the turnover of cholesterol esters is dissimilar in the
different lipoproteins (11, 12). The changes in the cho-
lesterol esters have been followed by measuring the pro-
portions of the different fatty acids in the esters and the
distribution of radioactivity among the different esters
after the injection of lipoproteins labeled with cho-
lesterol-4-^{14}C.

EXPERIMENTAL METHODS

Two healthy young men were studied in a hospital under
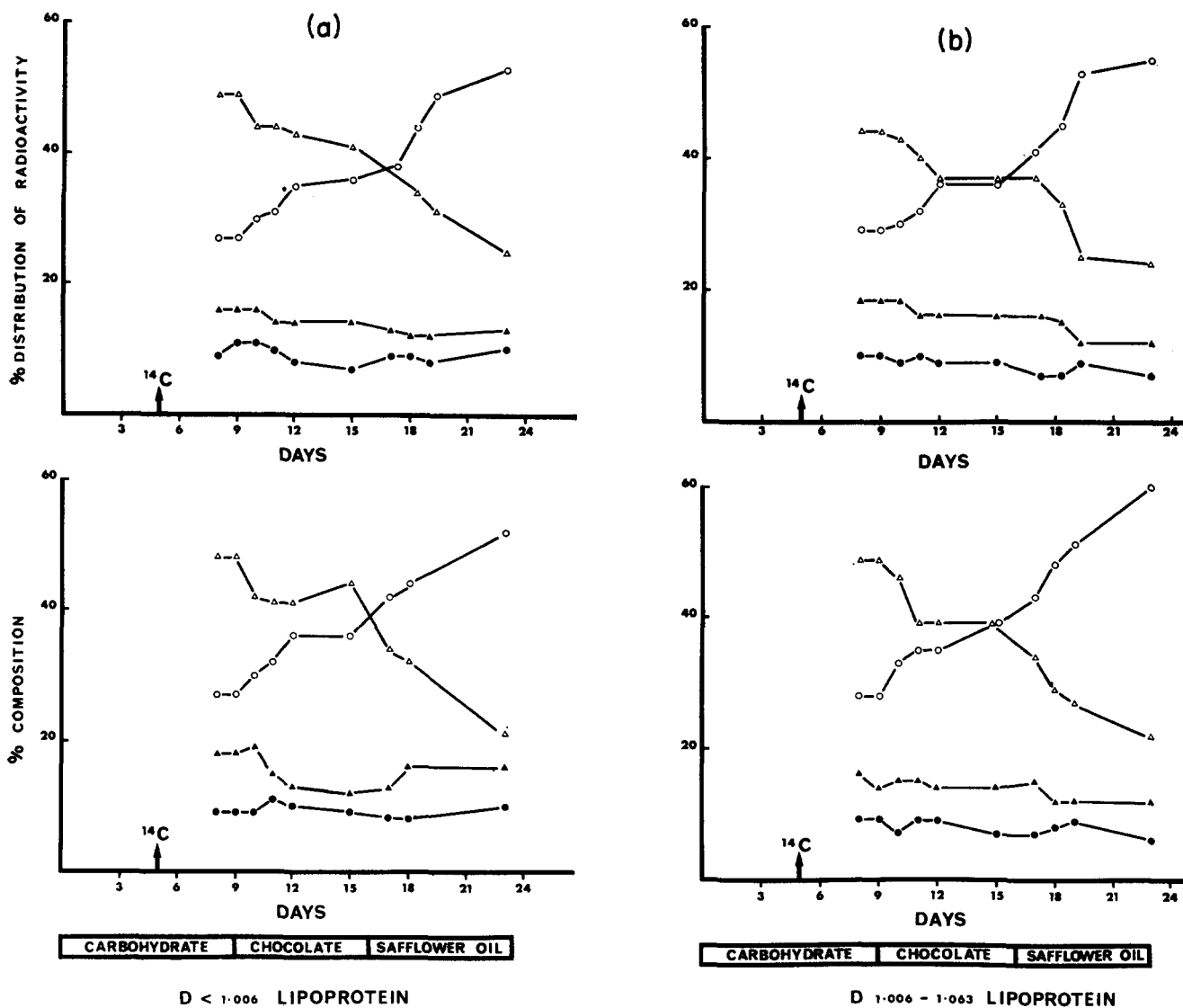metabolic ward conditions. The caloric intake required

FIG. 1. Distribution of radioactivity and fatty acid composition in cholesterol esters of (a) d < 1.006 lipoproteins, subject 1; (b) d 1.006 unsaturated esters, ○ linoleate, ● arachidonate.

to maintain normal weight was estimated beforehand and subsequent variations in body weight did not exceed 1 kg during any dietary period. The subjects were studied over a period of 3–4 weeks during which they consumed a diet rich in carbohydrate for the first 10 days, a diet rich in chocolate for a further 6 days, and a diet rich in safflower oil for the final 4–7 days. Details of the diets are given in Table 1 and the duration of each diet is presented in Fig. 1 a–d. The food was divided into four portions each day, the last meal being eaten at 6 p.m. Fifty microcuries of cholesterol-4-^{14}C (Radiochemical Centre, Amersham, U.K.) was dissolved in 0.5 ml of ethanol. The solution was diluted with 2 ml of 0.9% NaCl and then incubated with 20 ml of the subject's own plasma at 37°C for 1 hr (11, 13); the resultant solution was then injected intravenously on the 5th day. Full equilibration

between plasma free and esterified cholesterol and between the individual cholesterol esters can be expected to have been reached 3 days later when the first measurements were made (11–13).

Blood was collected into iced heparinized tubes at 9 a.m. after a 15 hr fast. The plasma was then separated into three lipoprotein fractions, d < 1.006, d 1.006–1.063 and d > 1.063 in subject 1 and into two fractions, d < 1.006 and d > 1.006, in subject 2 (14).

The subsequent procedures have been described in detail elsewhere (11). Briefly, the lipids were extracted into chloroform–methanol and the esterified cholesterol was separated from free cholesterol on columns of silicic acid. Aliquots of the free and esterified cholesterol were assayed for radioactivity and chemical concentration (15). Contamination of esterified by free cholesterol was
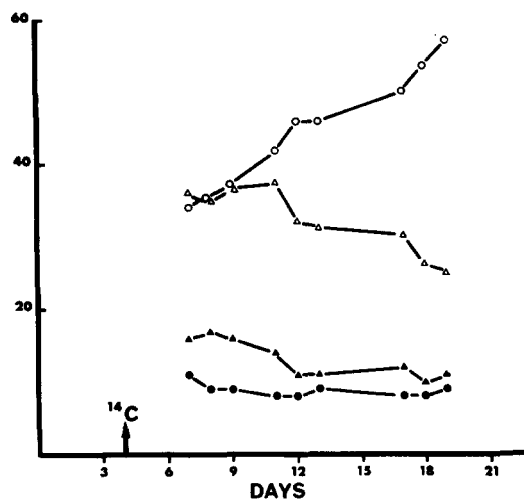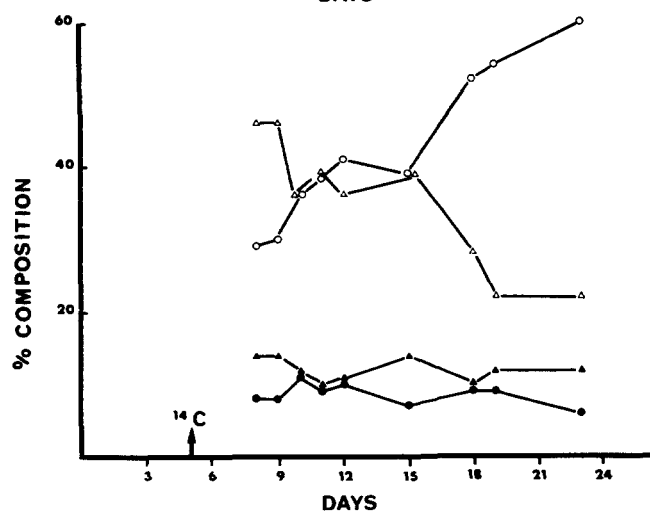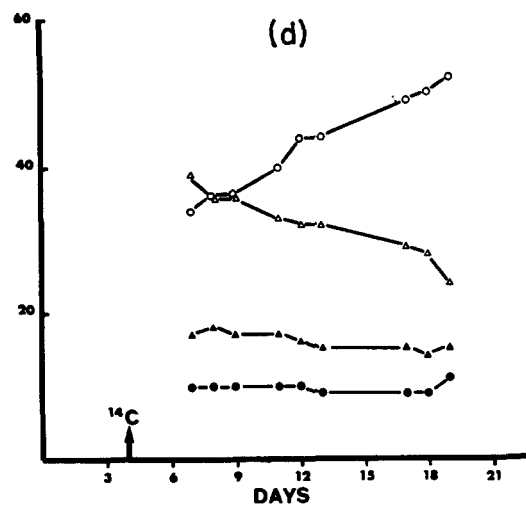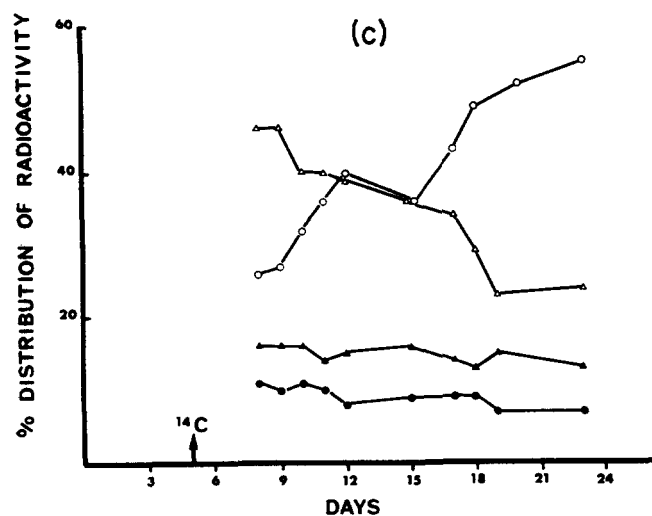
(c)

--1.063 lipoproteins, subject 1; (c) d > 1.063 lipoproteins, subject 1; (d) d > 1.006 lipoproteins, subject 2.   ▲ saturated esters, △ mono-

<0.5%; of cholesterol ester fatty acids by triglyceride fatty acids <0.03%.

Further aliquots of the cholesterol esters were separated on thin layers of silver nitrate-impregnated silicic acid into individual cholesterol esters (16) for measurement of radioactivity. Reproducibility was at least 90% on triplicate runs; recovery of esters, including linoleate and arachidonate, was at least 90%.

The mass of each individual cholesterol ester was determined from the fatty acid distribution of the total cholesterol esters after the separation of the methylated fatty acids by gas–liquid chromatography. Agreement with the compositions given for NHI Fatty Acid Standards was within 8% for the major and 15% for the minor components.

RESULTS

The changes in the fatty acid composition and in the distribution of radioactivity among the cholesterol esters in the two studies are presented in Fig. 1 a–d.

The figure shows that the distribution of radioactivity among the cholesterol esters and their fatty acid composition were similarly influenced by diet and that the changes which followed the substitution of one diet for another were evident within 24 hr. Although there were some differences in fatty acid composition among the esters of the different lipoproteins (e.g., a higher proportion of monounsaturated esters in the d < 1.006 fraction), a similar over-all pattern prevailed in the three classes of lipoproteins in subject 1 (Fig. 1 a–c) and in the d > 1.006 lipoproteins in subject 2 (Fig. 1d). In subject 2 there was

TABLE 1 Composition of the Diets Consumed by the Two Subjects

Subject	Diet	Calories	Cholesterol Intake	Caloric Distribution			Fatty Acids	
				Carbohydrate	Fat	Protein	Saturated and Monounsaturated	Poly-unsaturated
		per day	*mg/day*	%			*% of calories*	
1	Carbohydrate	2600	800	70	15	15	12	3
	Chocolate	2600	800	15	70	15	65	5
	Safflower oil	2600	800	15	70	15	30	40
2	Carbohydrate	2200	800	70	15	15	12	3
	Chocolate	2200	800	15	70	15	65	5
	Safflower oil	2200	800	15	70	15	30	40

insufficient radioactivity in the d < 1.006 lipoproteins for accurate measurement throughout the study but the compositional changes were similar to those found in the d > 1.006 lipoproteins.

In both subjects the monounsaturated esters were predominant during the carbohydrate-rich diet. The change to the chocolate-rich diet was associated with a fall in the proportion of monounsaturated esters and an increase in cholesteryl linoleate. There was also a probably significant fall in the proportion of saturated esters but not of cholesteryl arachidonate. A further increase in the proportion of cholesteryl linoleate and a further decrease in monounsaturated esters followed the consumption of safflower oil.

The total cholesterol concentrations at the end of the three dietary periods were 330, 365, and 335 mg/100 ml respectively in subject 1 and 190, 225, and 215 mg/100 ml in subject 2.

DISCUSSION

Since the radioactive cholesterol had been administered 3 days before the first measurements were made, it is virtually certain that complete equilibration between free and esterified cholesterol and among the individual cholesterol esters had been reached (11, 13). We would therefore not be able to detect any changes in turnover rate resulting from changes in the diet. However, we have shown previously that the relationship between the fractional turnover rates of the individual cholesterol esters is not altered by dietary change (11). The design of the previous study (11) differed from that of the present study in that the label was administered when the subjects had been on a given diet for some time and when no further compositional changes were taking place. Since diet did not appear to lead to changes in the fractional turnover rates in the previous study, it seems reasonable to assume that the striking changes in composition and distribution of radioactivity in the present study occurred without major changes in the turnover of any individual ester in the plasma.

The predominance of monounsaturated esters during the carbohydrate-rich diets reflects increased synthesis of these fatty acids and a decreased availability of polyunsaturated fatty acids and is consistent with previous studies (7, 10, 11). The striking rise in the proportion of cholesteryl linoleate and the reciprocal fall in the monounsaturated esters when the chocolate diet was substituted for the carbohydrate diet are of considerable interest since this diet was characterized by a low linoleate and a high monounsaturated and saturated fatty acid content. This suggests that the more saturated fatty acids are less readily incorporated into cholesterol esters when they are derived from the diet than when they are derived from endogenous synthesis. It is possible that there are several pools of precursor fatty acids available for cholesterol esterification and that the dietary fatty acids are not as readily accessible. Since the intake of linoleate was low during this diet, it seems likely that the rise in the proportion of cholesteryl linoleate reflected the utilization of preexisting linoleate. When safflower oil provided the majority of the fat calories, the proportion of cholesterol esterified with linoleate continued to rise. Cholesteryl linoleate was therefore the major cholesterol ester formed whenever fat provided the bulk of the calories.

It is possible that in man the enzymes esterifying cholesterol in sites such as the liver may display a specificity towards linoleate. This would be consistent with our recent (unpublished) finding that whereas the fractional turnover rates of the plasma cholesterol esters are similar, within the liver the fractional turnover rate of linoleate is higher than that of the other esters. The increase in the proportion of cholesteryl linoleate during the two fat diets may therefore reflect (*a*) changes in the precursor fatty acid pools, (*b*) an increase in the turnover of hepatic cholesteryl linoleate, and (*c*) the possible specificity of the enzyme esterifying cholesterol for linoleate. The possibility of minor changes in the turnover of cholesteryl linoleate in the plasma has not been excluded.

The possible mechanisms which might influence the incorporation of cholesterol esters into plasma lipopro-

teins have been reviewed in detail by Goodman (17). Hydrolysis of cholesterol esters may occur during the circulation of plasma lipoproteins through the liver; esterification of free cholesterol molecules may then occur in the liver, in the plasma, or in both sites. A second mechanism might involve the removal and replacement of intact cholesterol esters during the circulation of plasma lipoproteins through the liver. Either of these mechanisms would allow changes in the precursor fatty acid pools or changes in the turnover of individual cholesterol esters to produce the compositional changes observed in this study.

The preferential production of cholesteryl linoleate in man may be analogous to the formation of cholesteryl oleate in the rat, where there is a specificity towards the esterification of cholesterol with oleate both in the intestine (18) and in the liver (19) and the fractional turnover rate of cholesteryl oleate exceeds that of the other esters in the liver and in the plasma (20).

The changes in cholesterol ester composition were apparent within 24 hr and indicate that rapid adjustments in response to dietary change are therefore possible in man as in the rat (21). This early change was apparent in all lipoprotein fractions studied. This finding is not inconsistent with the absence of significant changes in higher density lipoproteins described by Kayden et al. (4). Only a single meal of fat was given in their study and measurements in the plasma were made about 8 hr later, which may have been too soon in the light of the known turnover rates of cholesterol esters (11, 12).

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