Alimentary Lipemia-induced Redistribution of Cholesteryl Ester between Lipoproteins

Studies In Normolipidemic, Combined Hyperlipidemic, and Hypercholesterolemic Men

Robin P.F. Dullaart, Johanna E.M. Groener, Hans van Wijk, Willem J. Sluiter, and D. Willem Erkelens

Alimentary lipemia stimulates the transfer of cholesteryl ester between lipoproteins in vitro and may alter lipoprotein cholesteryl ester distribution in vivo. The effect of a single, large oral fat load on lipoprotein cholesteryl ester redistribution and the activity of cholesteryl ester transfer protein was investigated in six normolipidemic men (Group A), six combined hyperlipidemic men (Group B), and six hypercholesterolemic men (Group C). Fasting triglyceride-rich lipoprotein cholesteryl ester was high in Group B, low in Group A, and intermediate in Group C (A < C < B, p < 0.05). After an oral fat load, total plasma cholesteryl ester was unchanged in all groups. In Group A, cholesteryl ester increased in smaller triglyceride-rich lipoproteins and remained so at 24 hours. Conversely, low density and high density lipoprotein cholesteryl ester decreased and returned to fasting values at 24 hours. In Group B, cholesteryl ester increased in large triglyceride-rich lipoproteins. Low density and high density lipoprotein cholesteryl ester (expressed as percentage of plasma cholesteryl ester) decreased. By contrast, in Group C, triglyceride-rich lipoprotein and low density lipoprotein cholesteryl ester remained unaltered, and only high density lipoprotein cholesteryl ester decreased. The activity of cholesteryl ester transfer protein increased in all groups and returned to fasting values at 24 hours. No differences in response were observed among the three groups. It is concluded that an oral fat load can induce a shift in lipoprotein cholesteryl ester distribution from high and low density lipoproteins to triglyceride-rich lipoproteins without affecting total plasma cholesteryl ester. The induction of triglyceride mass and the activity of cholesteryl ester transfer protein appear to be important factors in these changes. The alterations were most pronounced in normolipidemia and combined hyperlipidemia. Minor changes occurred in hypercholesterolemia.


Interest in the effects on lipoprotein metabolism of a meal or a fat challenge has increased. The importance of the postprandial phase in lipoprotein metabolism is seen, not only as the period when the mechanisms for the formation and catabolism of lipoproteins are activated, but also as the time when potentially atherogenic lipoproteins are produced. It has been suggested that, after hydrolysis of triglyceride, cholesteryl ester in remnant particles are produced. Cholesteryl ester in chylomicrons and chyomicron remnants can be derived from the intestine or from other lipoproteins present in plasma by the action of lipid transfer protein(s). The triglyceride response to a fat load varies remarkably between normolipidemic subjects and in different types of hyperlipidemia. Postprandial lipemia will cause perturbations in the composition and concentrations of lipoproteins of all major density classes. Several metabolic pathways are involved. Among these pathways are the lipoprotein lipase, the hepatic lipase, and the lipid transfer reaction. The transfer of cholesteryl ester and triglyceride between lipoproteins depends on the activity of lipid transfer protein(s) and on the concentration and composition of the lipoproteins involved. In vitro studies have shown that mass transfer of cholesteryl ester occurs from lipoproteins with a higher density toward triglyceride-rich lipoproteins (TRL). Recent in vitro experiments have indicated that lipid transfer processes may be stimulated during alimentary lipemia. The stimulation has been ascribed to alterations in the activity of the lipid transfer protein(s), to an increase in TRL mass, and to differences in the composition of lipoproteins in the postprandial state. Thus, the possibility that the lipid transfer reaction in vivo is strongly affected by alimentary lipemia is of interest. The question arises whether this is reflected in...
Table 1. Clinical Characteristics and Fasting Lipids in 18 Subjects

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<th>Group/subject</th>
<th>Age (yrs)</th>
<th>Weight (kg)</th>
<th>Body mass index (kg/m²)</th>
<th>Plasma cholesterol (mmol/l)</th>
<th>Plasma triglyceride (mmol/l)</th>
<th>HDL cholesterol (mmol/l)</th>
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<td>22.40*</td>
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<td>Range</td>
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<td>0.90–2.97</td>
<td>0.58–0.86</td>
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Group A was normolipidemic men, Group B was combined hyperlipidemic men, and Group C was hypercholesterolemic men.

Abbreviations: IHD = ischemic heart disease, PVD = peripheral vascular disease, premature IHD = first-degree relative with IHD before the age of 50, FCH = familial combined hyperlipidemia, FH = familial hypercholesterolemia.

*Group A < Groups B and C, p < 0.05; †Group B > Groups A and C, p < 0.05.

Altersations in the distribution of cholesteryl ester among lipoproteins. The purpose of the present study was to determine the effect of a single, large oral fat load on the distribution of cholesteryl ester among lipoproteins and on the activity of cholesteryl ester transfer protein. Normolipidemic, combined hyperlipidemic, and hypercholesterolemic men were studied.

Methods

Subjects

Three groups of subjects were studied. Group A consisted of six normolipidemic men, Group B consisted of six male patients with primary combined hyperlipidemia (previously type IV), and Group C consisted of six male patients with hypercholesterolemia (previously type IIa). Their clinical characteristics are given in Table 1. All subjects consented to the procedure after explanation of the purpose of the study. No men in Group A used medications. In Groups B and C, all lipid-lowering drugs were withheld for 2 weeks before the study. Patients with dysbetalipoproteinemia, diabetes mellitus, renal disease, or impaired thyroid function were excluded. Each subject consumed his habitual diet before the study day. After a 12-hour fast, each subject consumed a single, large oral fat load consisting of 6 g unstirred cream/kg body weight (2.1 g triglyceride and 7.2 mg cholesterol/kg body weight). Two fasting blood samples were collected before the fat load. Thereafter, 12 samples were obtained at regular intervals up to 8 hours after the fat load in Groups A and C; 13 samples were taken up to 13 hours after the fat load in Group B to collect data at both rising and declining triglyceride concentrations. Two post-experiment (PE) samples were taken after 24 hours. During the course of the experiments, the subjects drank only water.

Lipoproteins

Venous blood samples (20 ml) were drawn from an intravenous catheter, which was kept patent with saline solution (154 mmol NaCl/l). The samples were collected into tubes containing disodium ethylenediaminetetraacetic acid (EDTA) (1 mg/ml) and were kept on ice. Plasma was separated immediately at 1500 g for 15 minutes at
4°C. Chylomicrons were harvested immediately by ultracentrifugation for 35 minutes at 4°C at 100 000 g. Subsequently, the remaining d<1.006 g/ml fraction containing endogenous very low density lipoproteins (VLDL), smaller chylomicrons, and chylomicron remnants was isolated by preparative ultracentrifugation\(^22\) for 20 hours at 4°C and at 105 000 g in a 40.3 rotor in a Beckman L8-80 ultracentrifuge. Density was adjusted with KBr. Lipoprotein fractions were prepared by tube slicing and finally dialyzed against NaCl (154 mM), EDTA (1 mM) and NaN\(_3\) (1.5 mM), pH 7.4.

High density lipoproteins (HDL) were separated from lipoproteins (LDL) in the d>1.006 g/ml fraction by precipitation of LDL with sodium phosphotungstate and MgCl\(_2\).\(^3^2\)

**Lipid Assays**

Total cholesterol, free cholesterol, and triglyceride were assayed by enzymatic procedures (Boehringer Mannheim Diagnostica, Mannheim, FRG, catalogue nos. 23754, 310328, and 70192, respectively). Cholesteryl ester was calculated as the difference between total cholesterol and free cholesterol. Samples were assayed in duplicate. The coefficients of variation were 0.8%, 2.6%, and 1.1% in the total cholesterol, free cholesterol, and triglyceride assays, respectively.

The TRL lipids were calculated as the differences between plasma and the d>1.006 g/ml fraction, and LDL lipids as the differences between the d>1.006 g/ml fraction and the HDL-containing supernatant.

**Cholesteryl Ester Transfer Activity**

The activity of cholesteryl ester transfer protein, designated cholesteryl ester transfer activity (CETA), was measured as the exchange of cholesteryl ester between \(^3\)H-cholesteryl ester-labeled HDL and LDL obtained from pooled plasma, as described earlier.\(^3^3\) The plasma samples were first delipidated using diisopropyl ether/butanol (60:40, vol/vol) at OX.\(^3^4\) Incubations to measure CETA were carried out at 37°C for 16 hours. Under these circumstances, \(^3\)H-cholesteryl ester exchange was linear with time and added protein. Control incubations were carried out at 0°C and at 37°C without the addition of delipidated plasma to correct for cholesteryl ester exchange without an exogenous source of CETA. The radioactivities from these control incubations (less than 5% in LDL at 0°C and 5±2% in the assays without delipidated plasma) were subtracted. CETA was calculated according to the method of Barter and Jones.\(^3^6\) The plasma delipidations and the assays were performed in duplicate. CETA determinations from each subject were carried out in one run. The within-assay coefficient of variation of the CETA determinations was 10%. Changes in CETA were expressed relative to the mean value of the two fasting samples from each subject, which activity was set at 100%.

**Statistical Analysis and Calculations**

Changes in plasma and lipoprotein cholesteryl ester concentration and in CETA were assessed by calculating the mean of measurements in the two fasting samples; the mean of three measurements (peak samples) around the absolute triglyceride peak of each subject (one directly before, one at peak, and one directly after the peak); the mean of three measurements at 1, 2, or 3 hours after the absolute triglyceride peak (post-peak [PP] samples); and the mean of two measurements obtained 24 hours after the fat load (PE samples). In addition, changes in cholesteryl ester distribution between lipoproteins, expressed as percentages of total plasma, were assessed by comparison of the fasting measurements to those obtained at the end of the study day (i.e., 8 hours after an oral fat load in Groups A and C and 13 hours after an oral fat load in Group B). The sign test on rank differences of median values was used for comparison of cholesteryl ester concentrations in six combined hyperlipidemic subjects (Group B).

**Results**

**Changes in Triglyceride Concentration after Oral Fat Ingestion**

Fasting plasma triglyceride was higher in the combined hyperlipidemic subjects (Group B) than in the normolipidemic (Group A) or in the hypercholesterolemic (Group C) subjects (p<0.05) (Table 1). After an oral fat load, the maximal increase in triglyceride levels in Group B was...
greater \((p<0.05)\) and was measured later than in Groups A and C \((p<0.05)\). After 24 hours, plasma triglyceride concentrations had returned to the fasting levels in Groups A, B, and C. The plasma triglyceride response curves of Groups A and C were comparable. The median values for the three groups are shown in Figure 1.

The TRL triglyceride responses for the three groups (Figures 2A, 2B, and 2C) are comparable with those described for total plasma.

The differences in response among Groups A, B, and C were due to a greater increase in triglyceride in large TRL in Group B \((B>A \text{ and } C, p<0.05)\). There were no differences in triglyceride responses in smaller TRL among the three groups (data not shown).

**Cholesteryl Ester Distribution between Lipoproteins**

Data on cholesteryl ester concentrations in plasma and various lipoprotein fractions of the three groups of subjects before and after an oral fat load are given in Table 2. Fasting total plasma cholesteryl ester concentration was high in Group C, intermediate in Group B, and low in Group A. Fasting TRL cholesteryl ester was low in Group A, while in Group C, TRL contained about 10% of plasma cholesteryl ester. In Group B, TRL contained about 40% of plasma cholesteryl ester. The LDL cholesteryl ester concentration was higher in Group C than in Groups A and B \((p<0.05)\). HDL cholesteryl ester concentrations were not significantly different in the three groups, but tended to be lower in Group B.

No significant changes in total plasma cholesteryl ester were found in any group during the postprandial phase. However, significant changes in the distribution of cholesteryl ester among the lipoproteins were observed (Table 2).

In Group A, there was a significant increase in cholesteryl ester in TRL in the samples obtained after the individual triglyceride peaks (PP, which persisted after 24 hours PE) (Table 2). As can be seen in Figure 2A, the median TRL triglyceride peak was at 5 hours, while the median TRL cholesteryl ester peak was at 6 hours. Beyond the peak, TRL triglyceride declined faster than did TRL cholesteryl ester. The increase in TRL cholesteryl ester was accompanied by a significant decrease in LDL
Table 2. Cholesteryl Ester Concentrations in Plasma and Lipoproteins

<table>
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<th>Group</th>
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<th>TGRP</th>
<th>LDL</th>
<th>HDL</th>
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<td>2.11</td>
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</tr>
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<td>0.01–0.44</td>
<td>1.55–2.95</td>
<td>0.47–0.76</td>
</tr>
</tbody>
</table>

Group B

| Fasting | 4.67*  | 1.59† | 1.95‡ | 0.42 |
|         | 3.35–5.55 | 1.24–4.03 | 0.93–3.40 | 0.23–1.07 |
| Triglyceride peak | 4.62 | 2.57 | 1.85 | 0.38 |
|         | 4.73–8.08 | 1.57–4.20 | 0.88–3.58 | 0.16–0.64 |
| Post-peak | 4.67 | 2.51* | 1.77 | 0.38 |
|         | 4.34–7.90 | 2.19–4.19 | 0.66–3.30 | 0.18–0.74 |
| Post-experimental | 5.02 | 2.44 | 1.89 | 0.42 |
|         | 3.61–8.83 | 1.61–3.07 | 1.11–3.60 | 0.17–1.07 |

Group C

| Fasting | 6.01*  | 0.60† | 4.83‡ | 0.54 |
|         | 4.59–10.85 | 0.22–0.81 | 3.99–9.50 | 0.43–0.63 |
| Triglyceride peak | 6.15 | 0.74 | 4.91 | 0.48* |
|         | 4.85–10.37 | 0.39–1.29 | 3.83–8.61 | 0.36–0.59 |
| Post-peak | 6.07 | 0.53 | 5.01 | 0.48* |
|         | 4.82–10.37 | 0.34–1.29 | 3.92–8.61 | 0.37–0.58 |
| Post-experimental | 6.15 | 0.45 | 5.08 | 0.55 |
|         | 5.05–10.52 | 0.40–0.72 | 4.27–9.51 | 0.37–0.61 |

Data are expressed in mmol/l and given in medians and ranges.
Abbreviations: LDL=low density lipoprotein, HDL=high density lipoprotein.
*Change within a group, p<0.05; †values in Group A<B<C, p<0.05; ‡values in Group A<C<B, p<0.05; ††values in Group A and B<C, p<0.05.

and HDL cholesteryl ester (Table 2). After 24 hours, LDL and HDL cholesteryl ester had returned to fasting levels. The changes of cholesteryl ester in TRL, LDL, and HDL (expressed as percentage of plasma cholesteryl ester in Group A) are shown in Figure 3A. It can be seen that TRL cholesteryl ester, as percentage, increased from 0.5% (0–7) (median, range) to 10% (1–18), p<0.01, analyzed from 0 to 8 hours, whereas HDL and LDL cholesteryl ester decreased from 24% (19–26) to 18% (13–23), p<0.01, and from 83% (63–90) to 69% (64–88), p<0.05, respectively.

In Group B, a significant increase in TRL cholesteryl ester was observed in the PP samples (Table 2). Also in this group, the median TRL triglyceride peak was observed earlier (at 7 hours) than the cholesteryl ester peak (at 9 hours) (Figure 2B). The decline in TRL triglyceride was faster than the decline in TRL cholesteryl ester.

In absolute terms, the amount of cholesteryl ester in LDL and HDL did not change significantly (Table 2). However, as is illustrated in Figure 3B, HDL and LDL cholesteryl ester as percentage of plasma declined after an oral fat load from 10% (6–11) to 8% (4–11), p<0.05, analyzed from 0 to 8 hours. LDL and TRL cholesteryl ester did not change significantly from 81% (78–88) to 82% (79–88), and from 10% (5–12) to 11% (5–14), respectively.

In Group C, no significant change in TRL or LDL cholesteryl ester was found (Table 2, Figure 2C). There was a small, but significant, decrease in HDL cholesteryl ester (Table 2). Figure 3C illustrates that HDL cholesteryl ester as percentage of plasma declined after an oral fat load from 10% (6–11) to 8% (4–11), p<0.05, analyzed from 0 to 8 hours. LDL and TRL cholesteryl ester decreased after an oral fat load from 10% (6–11) to 8% (4–11), p<0.05, analyzed from 0 to 8 hours. LDL and TRL cholesteryl ester did not change significantly from 81% (78–88) to 82% (79–88), and from 10% (5–12) to 11% (5–14), respectively.

To further assess the relationship between the triglyceride and the cholesteryl ester response in TRL, the median TRL cholesteryl ester concentrations from each sampling point obtained at rising TRL triglyceride concentrations were compared with the values obtained when triglyceride concentrations were declining. The data from Group A are illustrated in Figure 4A. The median TRL cholesteryl ester levels and the corresponding TRL triglyceride levels from each sampling point are shown.

TRL cholesteryl ester increased with increasing TRL triglyceride concentrations. TRL cholesteryl ester was lower with rising triglyceride from 0 to 5 hours than with declining triglyceride from 6 to 24 hours (p<0.05).

As illustrated in Figure 4B, TRL cholesteryl ester in Group B was also lower with increasing triglyceride from 0 to 7 hours than with declining triglyceride from 8 to 24 hours (p<0.05).
These data suggest that part of the observed increase in TRL cholesteryl ester was not concomitant with intestinal triglyceride synthesis and secretion.

To elucidate whether the increases in TRL cholesteryl ester in Groups A and B were due to alterations in the larger or the smaller TRL, these fractions were analyzed separately. In Group A, the increase in cholesteryl ester was measured in smaller TRL (p < 0.025) but not in chylomicrons. In Group B, the increase in cholesteryl ester was measured in the large TRL (p < 0.025), but not in the smaller TRL (data not shown).

**Cholesteryl Ester Transfer Activity**

Changes in CETA were expressed as percentages of the fasting measurements (100%). Data from all subjects were combined since there were no differences in CETA response among the three groups. CETA increased at the triglyceride peak and PP measurements [median increase of 5.5% (~7 to 418) p < 0.01 and of 6.0% (~17 to 418), p < 0.01, respectively] and was unchanged PE (0.5% [44 to 32]).

**Discussion**

The effect of postprandial lipemia on lipoprotein cholesterol has been extensively studied. This investigation shows that alimentary lipemia-induced changes in cholesteryl ester distribution between the major lipoprotein fractions were substantially different in normolipidemia, combined hyperlipidemia, and hypercholesterolemia. In agreement with previous studies, this investigation showed considerable differences in lipoprotein cholesteryl ester distribution between normo- and hyperlipidemic subjects in the fasting state. There was no significant change in any group in total plasma cholesteryl ester after the fat challenge.

In the normolipidemic group, TRL cholesteryl ester increased in smaller TRL. It was not possible to differentiate between cholesteryl ester in chylomicron remnants and endogenous VLDL. An increase in endogenous VLDL during the postprandial phase, due either to an increased hepatic synthesis or to a decreased removal in the presence of chylomicrons, has been suggested. Comitantly, LDL and HDL cholesteryl ester decreased. Similar alterations in cholesteryl ester distribution in normolipidemic subjects and TRL cholesteryl ester peaking after triglyceride have been recently reported. Earlier reports also showed an increase in TRL cholesterol and a decrease in LDL cholesterol after induction of TRL. In another report, LDL cholesterol was unaltered after ingestion of 100 g soybean oil.
This decrease coincided with a more pronounced triglyceride response than in normolipidemic controls.

In discussing the mechanisms that are responsible for the changes in lipoprotein cholesteryl ester after ingestion of fat, it should be noted that the composition and concentration of lipoproteins is continuously modified as a result of a complicated sequence of events in which several metabolic pathways operate.

In the present study, it was not possible to differentiate between alimentary lipemia-induced cholesteryl ester in TRL derived from intestinal secretion and that derived from other lipoproteins by the action of lipid transfer protein(s). The increase of cholesteryl ester in TRL with a simultaneous decrease in LDL and HDL that does not affect total plasma cholesteryl ester (as observed in the normolipidemic and combined hyperlipidemic subjects) suggests a contribution of cholesteryl ester transfer from LDL and HDL toward TRL. This is also supported by the observations that cholesteryl ester in TRL increased after triglyceride and that cholesteryl ester in TRL was higher in declining than in rising TRL triglyceride levels.

One alternative explanation would be intestinal secretion of TRL with a high cholesteryl ester content as the postprandial phase proceeds. However, we know of no human studies to support this. Another explanation would be that triglyceride hydrolysis results in TRL relatively enriched in cholesteryl ester. Although this would not absolutely increase TRL cholesteryl ester, it may be that cholesteryl ester–enriched TRL are catabolized relatively slowly. This has been documented in rats. In our hypercholesterolemic subjects, no increase in TRL cholesteryl ester was shown. It may be that the triglyceride-rich lipoprotein pool relative to the cholesteryl ester–rich lipoprotein pool was so small that only minor changes occurred. Furthermore, an accelerated chylomicron clearance in hypercholesterolemic subjects has been reported. Cholesteryl ester transferred to TRL could be so rapidly cleared that it does not result in a measurable increase in cholesteryl ester content. The alimentary lipemia–induced alterations in LDL cholesteryl ester could be partly related to cholesteryl ester transfer from LDL to TRL in vivo. In vitro studies have shown a transfer of cholesteryl ester mass from LDL to VLDL. A third explanation is decreased postprandial synthesis or increased catabolism of LDL. That an altered LDL metabolism may contribute to the decrease in LDL cholesteryl ester appears to be indicated by the observation of a postprandial decrease in apolipoprotein B.

Alimentary lipemia–induced alterations in HDL cholesteryl ester will depend on the quantity of chylomicron surface material transferred to HDL during chylomicron metabolism and on the subsequent esterification of free cholesterol. A second important factor is the rate of cholesteryl ester mass transfer from HDL to lipoproteins of lower densities. The rate of transfer of cholesteryl ester from HDL to VLDL in vitro depends upon their size and composition. In normolipidemic subjects, postabsorptive total HDL cholesterol, HDL₂ cholesteryl, and the decrease in HDL₃ cholesterol are related to the magnitude of the postprandial triglyceride response. The exchange of cholesteryl ester for triglyceride by lipoprotein transfer is a significant process that may contribute to the decrease in HDL cholesteryl ester.
transfer protein(s) results in a triglyceride-enriched and cholesteryl ester–depleted HDL2 particle that can be converted to HDL3 by lipase-mediated triglyceride hydrolysis. This HDL2 to HDL3 conversion has been observed in normolipidemic subjects with a high postprandial triglyceride response. It has been proposed that the decrease in HDL cholesteryl ester will depend on the rise in triglyceride levels. The present results do not indicate that the net decrease in HDL cholesteryl ester is quantitatively related to the magnitude of the triglyceride response when the different groups are compared.

An increase in the activity of cholesteryl ester transfer protein after a single oral fat load has been reported in normolipidemic subjects. No data are available on a possible induction of CETP after a single oral fat load in combined hyperlipidemic and hypercholesterolemic subjects. In this study, a small, but significant, increase of CETP has been measured in all groups. No differences among the three groups were found. The mechanisms responsible for the alimentary lipemia–induced response of CETP remain to be elucidated. In long-term dietary experiments, an increase in CETP has been observed in hyperlipidemic humans and in rabbits, concomitant with an increase in plasma cholesterol.

In view of a stimulated postprandial cholesteryl ester transfer between lipoproteins in vitro, the increase in TRL mass and the increase in CETP may be co-acting factors in the cholesteryl ester redistribution between lipoproteins.

In conclusion, this study shows a marked and prolonged effect of a single large oral fat load on cholesteryl ester redistribution, suggesting that in vivo LDL and HDL are donors for cholesteryl ester, while triglyceride-rich lipoproteins are acceptors. The effects are more pronounced in normolipidemia and combined hyperlipidemia, while only minor alterations occur in hypercholesterolemia.

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