

# Effects of fat ingestion on high density lipoprotein profiles in human sera

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**Abstract** Serum concentrations of triacylglycerol, apolipoprotein A-I, apolipoprotein A-II, HDL<sub>2</sub>, and HDL<sub>3</sub> were determined in sera of nine normolipidemic adult males, just before and 3, 5, and 8 hr after ingestion of 250 ml of cream (100 g of triacylglycerol). In all individuals a rapid hypertriglyceridemic response was observed. Triacylglycerol concentrations increased from 624 ± 124 mg/liter of serum to 1435 ± 350 mg/liter of serum 3 hr after cream ingestion. In most individuals the hypertriglyceridemic response was followed by a decline in serum triacylglycerol concentration to below basic levels. As a result of cream ingestion, small but statistically highly significant increases in serum cholesterol and apolipoprotein A-I concentrations were observed that persisted till the end of the observation period. In most individuals a small rise in the apolipoprotein A-II concentration in serum was also present. Marked changes were observed in serum HDL as illustrated in the HDL absorption at 280 nm and cholesterol profiles obtained by single-spin rate-zonal density gradient ultracentrifugation of the sera. Due to a prominent increase in phospholipids (up to about 18%) and a smaller increase in protein (up to about 6%), flotation rates and concentrations of HDL<sub>2</sub> as well as HDL<sub>3</sub> increased. These changes in HDL subclass flotation characteristics and chemical composition are best explained by uptake of surface material from chylomicrons by existing HDL<sub>2</sub> and HDL<sub>3</sub> particles. The data do not support a previously proposed concept in which HDL<sub>3</sub> is converted into HDL<sub>2</sub> by uptake of surface remnants formed during catabolism of triglyceride-rich lipoproteins.—Groot, P. H. E. and L. M. Scheek. Effects of fat ingestion on high density lipoprotein profiles in human sera. *J. Lipid Res.* 1984. **25**: 684–692.

**Supplementary key words** HDL<sub>2</sub> • HDL<sub>3</sub> • rate-zonal ultracentrifugation

The metabolism of serum HDL is complex and not fully understood. This complexity is due to the heterogeneous appearance of serum HDL and caused by the fact that serum HDL is not secreted as such but is the product of complex interactions of precursors of HDL, synthesized in liver and gut, serum factors and enzymes, and other serum lipoproteins.

In most sera, two subfractions of HDL can be distinguished: HDL<sub>2</sub> and HDL<sub>3</sub>. This subdivision is based originally on schlieren profiles of HDL obtained in flotation studies in the analytical ultracentrifuge (1). More recently

a bimodal distribution in plasma HDL was demonstrated by rate-zonal ultracentrifugation and the flotation characteristics of the two isolated HDL components agreed well with those defined by analytical ultracentrifugation (2–4). HDL<sub>2</sub> and HDL<sub>3</sub> differ in chemical composition (HDL<sub>2</sub> is enriched in all lipid constituents) and apolipoprotein pattern (the ratio apolipoprotein A-I/A-II is higher in HDL<sub>2</sub>) (2–6). The HDL subclass distribution may even be more complex as suggested by a variety of other fractionation techniques (7–12) but the physiological significance of these subfractions awaits further study.

HDL<sub>2</sub> and HDL<sub>3</sub> may be metabolically interrelated. Patsch et al. (13) have suggested, based on in vitro studies, that surface fragments of triglyceride-rich lipoproteins, generated by lipoprotein lipase, fuse with existing serum HDL<sub>3</sub> particles to produce HDL<sub>2</sub>-like particles. This process, if operating in vivo, could explain the correlation between the rate of lipolysis and serum HDL<sub>2</sub> concentrations, seen in many studies (for references see 13, 14).

The metabolic relation between the degradation of chylomicrons and HDL subclass composition has been investigated in human subjects fed standard fat-containing meals (15–19). Although in all studies an increase in HDL phospholipid and protein was observed in response to fat ingestion, the contribution of the HDL<sub>2</sub> and HDL<sub>3</sub> subclasses to these changes varied considerably, probably due to differences in methodology used for HDL<sub>2</sub> and HDL<sub>3</sub> separation. Recently we have developed a method (4), based on rate-zonal density gradient ultracentrifugal separation of HDL subclasses in human serum, that produces a detailed HDL subclass profile. This method is very suitable for metabolic studies as many serum samples can be analyzed in parallel. Using this method we addressed the question whether chylomicron degradation in man results in an interconversion of HDL<sub>3</sub> into HDL<sub>2</sub>.

Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; LCAT, lecithin:cholesterol acyltransferase.

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In the present study we describe the effects of an oral fat load on HDL subclass profiles and our data indicate that HDL<sub>3</sub> as well as HDL<sub>2</sub> are involved in the processing of surface surplus generated by chylomicron degradation while a direct transformation of HDL<sub>3</sub> to HDL<sub>2</sub> could not be demonstrated.

## METHODS

### Subjects

The volunteers participating in this study were healthy male members of our department and medical students, aged 21–40 years. Body weights were 65–82 kg. Blood was taken at 9 AM after an overnight fast. Next the subjects consumed 250 ml of cream (fat content 40% w/v) and blood samples were taken after 3, 5, and 8 hr. Blood was allowed to clot for at least 3 hr at 0°C and sera were prepared by low speed centrifugation. EDTA solution (pH 7.4) was added to a final concentration of 2.5 mM.

### Lipoprotein separation

HDL subclass profiles were obtained after rate-zonal density gradient ultracentrifugation of aliquots of serum (1.7 ml, in triplicate), raised to  $d$  1.40 g/ml with solid NaBr, using the Beckman SW 40 rotor. Details of the procedure have been described before (4). Gradients were eluted using a tube piercer (4) and the absorbance at 280 nm was measured in an ISCO 0.5-cm flow-through cell (Cat. no. 68-0080-002) connected with an ISCO UA-5 optical unit (both purchased from ISCO Inc, Lincoln, NE). In order to minimize mixing during the gradient elution, the elution system was set up in such a way that a downward flow could be maintained. From each gradient 38 fractions of 0.28 ml were collected. The baseline drift in the optical system was minimal and recorder tracings of HDL profiles in different sera could be compared directly (comp. Fig. 3). Triplicate tracings of the same serum were always exactly identical and one set of fractions was used to determine a HDL-cholesterol profile (comp. Fig. 2). Based on these data, gradient fractions were combined to compose HDL<sub>2</sub> and HDL<sub>3</sub> pool fractions. Next, the HDL<sub>2</sub> and HDL<sub>3</sub> pool fractions were characterized chemically and immunochemically.

### Chemical and immunological methods

Triacylglycerol concentrations in sera were determined according to Laurell (20). Triacylglycerol concentrations in the HDL<sub>2</sub> and HDL<sub>3</sub> subfractions were determined using a more sensitive method. Lipids in aliquots (0.8 ml) of HDL were extracted according to Bligh and Dyer (21). The chloroform phase of this extraction was transferred to a glass tube and evaporated. The lipid residue was solubilized in 2.5 ml of a mixture of peroxide-free di-

isopropylether and ethanol (95/5; v/v) and 0.3 g of SiO<sub>2</sub> was added to remove phospholipids. Aliquots of the organic phase (2 ml) were removed and transferred to a tube fitted with a glass stopper and 0.2 ml of an alcoholic KOH solution (9.5 ml ethanol mixed with 0.5 ml of 6 M KOH) was added. The tubes were closed and triacylglycerol was saponified during 20 min at 60°C. The tubes were cooled, 0.5 ml of 0.6 M HCl was added, and the contents of the tubes were mixed carefully and left for 15 min. The organic phase was removed and glycerol was determined in the water phase using a fluorometric enzymatic procedure described by Laurell and Tibbling (22), adapted for small samples. Trioleoylglycerol standards were taken throughout the whole procedure.

Phospholipids, cholesterol, unesterified cholesterol, and protein were determined as described earlier (4).

Apolipoproteins A-I and A-II were determined by radial-immunodiffusion as described before (4).

### Statistical methods

Results are expressed as mean  $\pm$  SD. Group means were compared by a two-tailed paired Student's *t* test.

## RESULTS

### Effects of cream ingestion on serum lipids and apolipoprotein concentrations

The effects of cream ingestion on serum concentrations of triacylglycerol, cholesterol, and apolipoproteins A-I and A-II are shown in **Table 1**. Triacylglycerol concentrations increased rapidly in all subjects studied and the highest levels were found 3 hr after fat ingestion. In most subjects the hypertriglyceridemic response was followed by a rapid clearance. In **Fig. 1** serum triacylglycerol concentrations at  $t = 3, 5,$  and 8 hr are expressed as percentage of the initial levels. It is interesting to note that serum triacylglycerol concentration 8 hr after cream ingestion dropped to a level below that found in the fasting state, in seven out of the nine subjects studied. The fasting levels of triacylglycerol in the sera of the two subjects showing the large and extended hypertriglyceridemic response were not very different from those found in the total group; they were 478 and 618 mg/liter of serum.

Cream ingestion also resulted in a slight increase in serum levels of cholesterol (6%) and apolipoprotein A-I (7%) that persisted till the end of the observation period and were found to be statistically highly significant. Serum levels of apolipoprotein A-II behaved less consistently and were found to increase in only six of the eight subjects tested. Comparable responses of serum apolipoprotein A-I and A-II concentrations to an oral fat load in men have been reported by others previously (19, 23).

TABLE 1. Serum concentrations of triacylglycerol, cholesterol, and apolipoproteins A-I and A-II in normolipidemic subjects before and after cream ingestion

| Serum Concentration of<br>(n) <sup>a</sup> | Hours after Cream Ingestion      |                           |                         |                         |
|--------------------------------------------|----------------------------------|---------------------------|-------------------------|-------------------------|
|                                            | 0                                | 3                         | 5                       | 8                       |
|                                            | <i>mg/liter serum, mean ± SD</i> |                           |                         |                         |
| Triacylglycerol (9)                        | 624 ± 142                        | 1435 ± 350 <sup>b,c</sup> | 1078 ± 448 <sup>d</sup> | 693 ± 437               |
| Cholesterol (9)                            | 1910 ± 406                       | 1980 ± 437 <sup>e</sup>   | 1977 ± 359 <sup>f</sup> | 2025 ± 354 <sup>e</sup> |
| Apolipoprotein A-I (9)                     | 1631 ± 276                       | 1750 ± 353 <sup>g</sup>   | 1715 ± 305 <sup>f</sup> | 1745 ± 231 <sup>e</sup> |
| Apolipoprotein A-II (8)                    | 529 ± 81                         | 562 ± 90                  | 555 ± 90                | 548 ± 68                |

<sup>a</sup> Number of experiments.

<sup>b</sup> Statistical significance compared to t = 0 hr, using Student's two-tailed paired *t* test.

<sup>c</sup> *P* < 0.001.

<sup>d</sup> *P* < 0.02.

<sup>e</sup> *P* < 0.01.

<sup>f</sup> *P* < 0.05.

<sup>g</sup> *P* < 0.005.

### Effects of cream ingestion on serum HDL subclass profiles

The effects of cream ingestion on HDL subclass distribution were analyzed by rate-zonal density gradient ultracentrifugation. The absorption at 280 nm recorder tracings of HDL subclass separations in sera of six subjects

are shown in Fig. 2. (Tracings of the other subjects were obtained using the flow-through cell and recording set-up as described in ref. 4 and are not shown.) Fasting serum levels (t = 0 hr) of HDL<sub>3</sub> (left peak in the tracings) and HDL<sub>2</sub> (right peak) varied substantially. In all subjects studied the absorption at 280 nm in the HDL<sub>3</sub> region of the gradient increased after cream ingestion while the absorption maxima were shifted in the direction of the top of the gradient. Similar effects were observed in the HDL<sub>2</sub> region of the gradient. These data suggest that as a result of cream ingestion the flotation rates of both the HDL<sub>2</sub> and HDL<sub>3</sub> subclass in human serum are increased. The supposition is substantiated by cholesterol measurements performed in all the fractions collected in the HDL profiling procedure. The data from nine subjects are compiled in Fig. 3. Differences between postprandial and fasting HDL cholesterol concentrations were calculated for each individual in each fraction and were evaluated statistically. These data are included in Fig. 3 and illustrate the fact that the redistribution of HDL cholesterol as a result of cream ingestion was consistently found in all subjects. Comparable effects on absorption at 280 nm and cholesterol profiles were obtained in three normolipidemic females from whom data, obtained in sera in the fasting state and 5 hr after cream ingestion, were available, indicating that the postprandial changes in HDL are at least qualitatively similar in both sexes.<sup>2</sup>

The underlying mechanism of these changes in flotation rate of HDL was investigated by chemical and immunological analysis of the total HDL<sub>2</sub> and HDL<sub>3</sub> fractions. These fractions were obtained as indicated in the legend of Fig. 3. Data of the chemical analyses and of measurements of apolipoprotein A-I and A-II in HDL<sub>2</sub> and HDL<sub>3</sub> are given in Table 2 and Table 3, respectively.

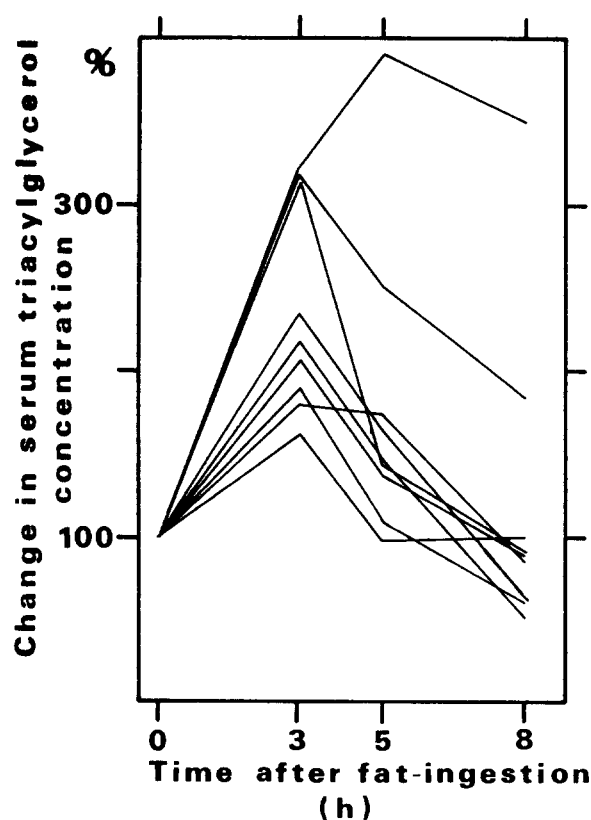
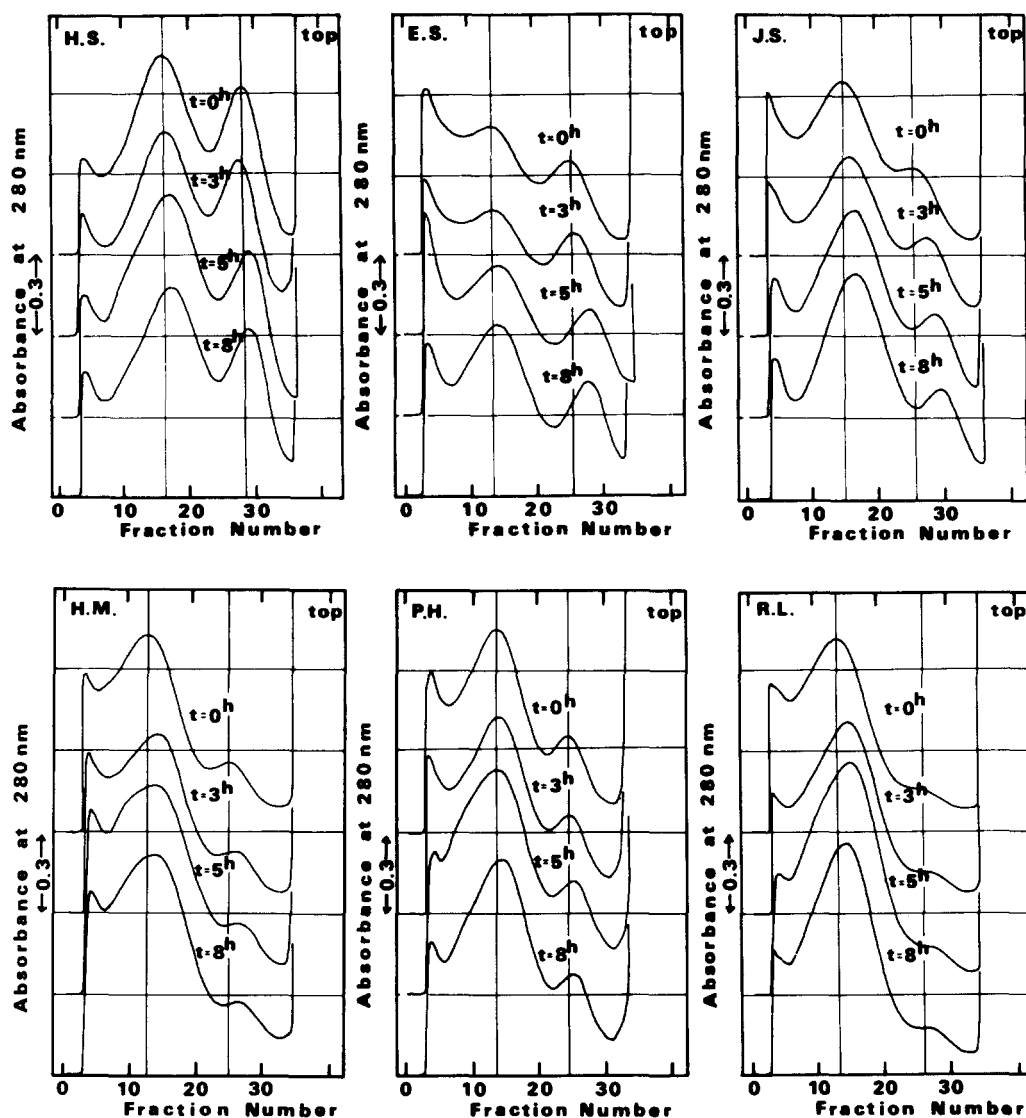


Fig. 1. Serum triacylglycerol concentrations before and after cream ingestion. For each subject, serum triacylglycerol concentrations are expressed as a percentage of the basal level.

<sup>2</sup> Groot, P. H. E. Unpublished observation.



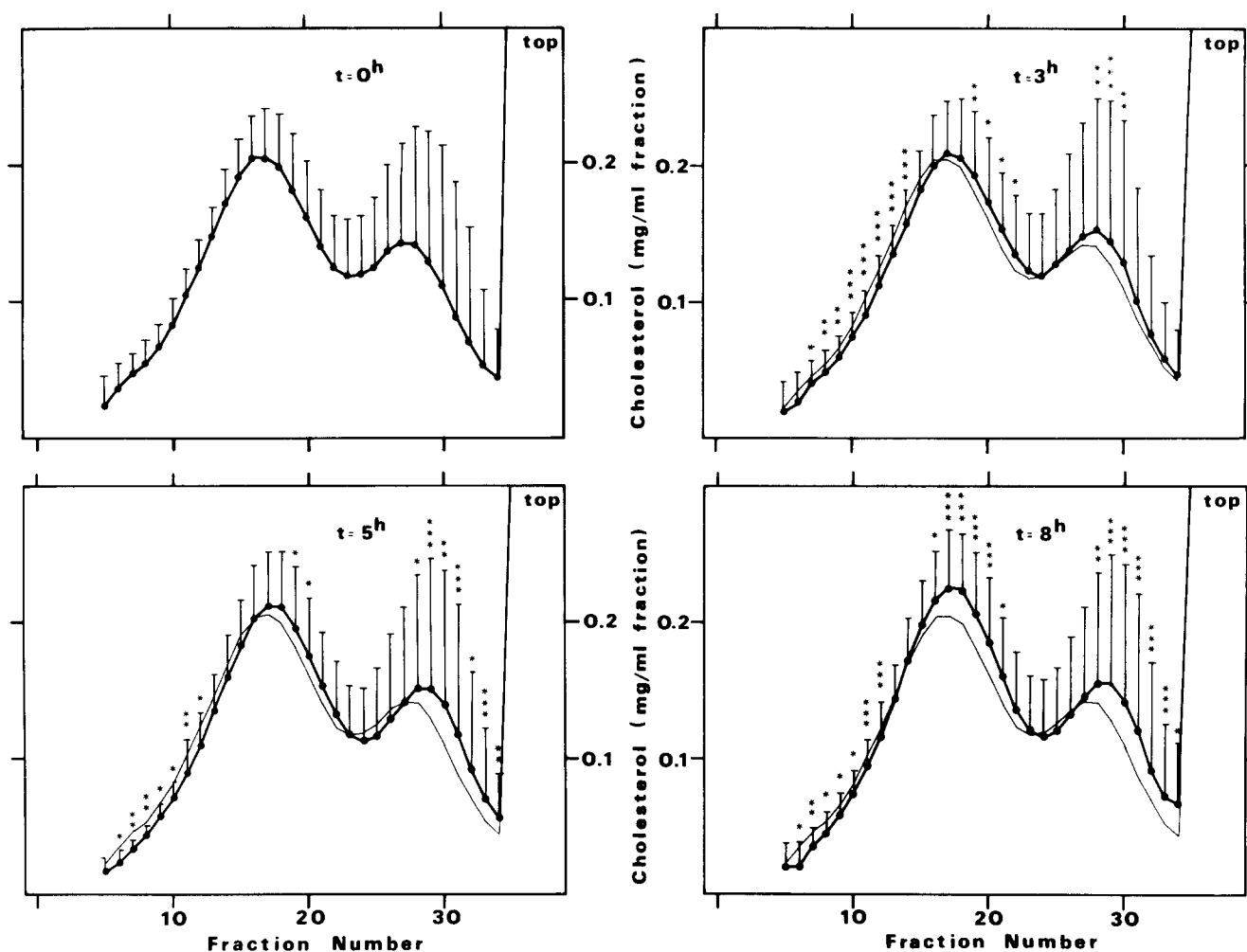
**Fig. 2.** HDL subclass profiles in sera of normolipidemic males before and after cream ingestion. HDL subclass profiles were determined by rate-zonal ultracentrifugation of sera obtained just before ( $t = 0$  hr) and after ( $t = 3, 5, \text{ and } 8$  hr) ingestion of cream. The absorption at 280 nm recordings of the HDL subclass separation were obtained as described in Methods and 38 fractions (0.28 ml each) were collected from each tube. The left peak in these profiles represents HDL<sub>3</sub>, the right peak HDL<sub>2</sub>. VLDL plus LDL are recovered in fractions 36 to 38.

The average concentration in serum of all the constituents of HDL<sub>2</sub> as well as HDL<sub>3</sub> increased in the hours following cream ingestion, but the response in serum HDL was delayed in comparison to the hypertriglyceridemic response and was more persistent (Tables 2 and 3 versus Table 1 and Fig. 1). Marked changes were seen in the HDL<sub>2</sub> and HDL<sub>3</sub> phospholipid concentrations which increased in all subjects tested (the average increase at 8 hr was 18%). Smaller but statistically significant rises in HDL<sub>2</sub> as well as HDL<sub>3</sub> protein concentrations were also evident and apolipoprotein measurements revealed that the increments were mainly due to increases in apolipoprotein A-I concentration. Furthermore, statistically

significant rises were seen in the HDL<sub>2</sub> unesterified cholesterol concentration at 5 and 8 hr and in the concentrations of triacylglycerol associated with HDL<sub>3</sub> at 3 and 5 hr after cream ingestion. Data of these minor lipid constituents were only available for six subjects (same subjects as in Fig. 2).

Data on the chemical composition of the HDL<sub>2</sub> and HDL<sub>3</sub> subclasses are summarized in Table 4 and Table 5, respectively. In the hours following cream ingestion, a slight decrease in protein and an increase in phospholipid content in both HDL subclasses was observed resulting in statistically significant decreases in the protein/phospholipid weight ratios.





**Fig. 3.** HDL cholesterol profiles in sera of normolipidemic males before and after cream ingestion. HDL subclass profiles of nine normolipidemic males were obtained as indicated in Fig. 2 and cholesterol concentrations were measured in all the individual fractions. Shown are the mean cholesterol concentrations  $\pm$  SD calculated for each fraction. For reasons of comparison the basal profile (upper left) is also plotted in figures showing the profiles 3 hr (upper right), 5 hr (lower left), and 8 hr (lower right) after cream ingestion. Differences in cholesterol concentration between  $t = 3, 5,$  or  $8$  hr and  $t = 0$  hr were calculated for each subject in each fraction and elaborated statistically. Probability ( $P$ ) values are indicated in the figure (\* $P < 0.05$ ; \*\* $P < 0.02$ ; \*\*\* $P < 0.01$ ). Chemical analysis of HDL<sub>2</sub> and HDL<sub>3</sub> were performed in two pooled fractions composed after measurement of the cholesterol profile. Separation between the HDL<sub>2</sub> and HDL<sub>3</sub> regions was executed based on the nadir in the cholesterol profile. This point differed slightly from individual to individual and was also affected by the nutritional status. Fractions were pooled in the following way (mean fraction number  $\pm$  SD,  $n = 9$ ). Sera at  $t = 0$  hr HDL<sub>2</sub> 23.9  $\pm$  1.4–33.3  $\pm$  0.9; HDL<sub>3</sub>, 7–22.9  $\pm$  1.4. Sera at  $t = 3$  hr: HDL<sub>2</sub>, 24.1  $\pm$  1.3–33.3  $\pm$  0.9; HDL<sub>3</sub>, 7–23.1  $\pm$  1.3. Sera at  $t = 5$  hr: HDL<sub>2</sub>, 24.3  $\pm$  1–33.4  $\pm$  0.9; HDL<sub>3</sub>, 7–23.1  $\pm$  1.0. Sera at  $t = 8$  hr: HDL<sub>2</sub>, 24.3  $\pm$  1–33.4  $\pm$  0.9; HDL<sub>3</sub>, 7–23.3  $\pm$  1.0. The chemical analyses are given in Tables 2 and 3.

## DISCUSSION

The results of a number of studies have linked the catabolism of triacylglycerol-rich lipoproteins to changes in serum HDL (13, 14, 24–29) but details of the nature of this interaction are not fully understood. The present study was undertaken to investigate the concentration and composition of the HDL<sub>2</sub> and HDL<sub>3</sub> subclasses in human serum during a period of enhanced chylomicron degradation induced by ingestion of a large dose of cream. As found in a number of other studies, we observed a rapid increase in serum triacylglycerol concentration after fat ingestion (15–19) followed by a decline to below fasting

levels (16), probably due to diurnal or meal-induced changes in the activity of the lipoprotein lipase system (compare ref. 30).

Confirmatory to previous reports (15–19, 31) we have demonstrated that, as a result of fat ingestion, the concentrations of phospholipids and apolipoproteins associated with serum HDL are increased, but the use of a recently developed technique of rate-zonal density gradient ultracentrifugation (4) enabled us to study these changes in serum HDL in great detail. Using this method, we observed, based on absorption at 280 nm and cholesterol profiles, that the flotation rates of both HDL<sub>2</sub> and HDL<sub>3</sub> gradually increase due to a prominent increase

TABLE 2. Concentration of HDL<sub>2</sub> constituents in serum before and after cream ingestion

| Time after Cream Ingestion | Serum Concentration of HDL <sub>2</sub> Constituents |                        |                        |                        |                              |                     |                        |                         |
|----------------------------|------------------------------------------------------|------------------------|------------------------|------------------------|------------------------------|---------------------|------------------------|-------------------------|
|                            | Protein (9) <sup>a</sup>                             | Phospholipids (9)      | Total Cholesterol (9)  | Cholesteryl Esters (6) | Unesterified Cholesterol (6) | Triacylglycerol (6) | Apolipoprotein A-I (9) | Apolipoprotein A-II (6) |
| <i>hr</i>                  | <i>mg/liter, mean ± SD</i>                           |                        |                        |                        |                              |                     |                        |                         |
| 0                          | 380 ± 247                                            | 322 ± 225              | 199 ± 137              | 258 ± 176              | 63 ± 44                      | 27 ± 18             | 271 ± 200              | 42 ± 14                 |
| 3                          | 394 ± 242 <sup>b</sup>                               | 353 ± 216 <sup>c</sup> | 207 ± 137 <sup>b</sup> | 262 ± 175              | 67 ± 46                      | 35 ± 11             | 290 ± 195 <sup>b</sup> | 48 ± 16                 |
| 5                          | 405 ± 236 <sup>d</sup>                               | 368 ± 221 <sup>e</sup> | 211 ± 131 <sup>b</sup> | 261 ± 171              | 70 ± 43 <sup>b</sup>         | 36 ± 15             | 286 ± 184              | 48 ± 13                 |
| 8                          | 403 ± 247 <sup>c</sup>                               | 381 ± 231 <sup>e</sup> | 217 ± 135 <sup>b</sup> | 265 ± 177              | 72 ± 45 <sup>f</sup>         | 32 ± 18             | 292 ± 200 <sup>b</sup> | 46 ± 15                 |

<sup>a</sup> Number of experiments.

<sup>b</sup> Statistical significance compared to *t* = 0 hr, using Student's two-tailed paired *t* test; *P* < 0.05.

<sup>c</sup> *P* < 0.02.

<sup>d</sup> *P* < 0.01.

<sup>e</sup> *P* < 0.001.

<sup>f</sup> *P* < 0.005.

in phospholipids and a smaller increase in apolipoprotein concentrations. Increased concentrations of some minor lipid constituents of HDL may also have contributed to these findings. It is well known that the synthesis of apolipoprotein A-I by human small intestine is increased during fat absorption (32, 33). Apolipoprotein A-I is a major constituent of nascent chylomicrons (25, 34) and it has been shown that its concentration in serum is increased in response to fat ingestion (19, 23) as was found in the present study. Effects on apolipoprotein A-II are less well documented. Apolipoprotein A-II is present on human mesenteric lymph and thoracic duct chylomicrons (25, 33, 34) and an incorporation of radioactive amino acids into apolipoprotein A-II by human intestinal biopsies has been demonstrated (35). However, direct evidence for an increased synthesis in human gut during chylomicron assembly is not available. Tall et al. (19) observed a slight increase in serum apolipoprotein A-II after fat ingestion, while we were unable to demonstrate that the increase is of statistical significance. Confirming a previous report

(19), we found that the rise in serum levels of apolipoprotein A-I persisted in a period when serum triacylglycerol concentrations declined to (below) basal levels. It can be calculated from our data that the rise in serum apolipoprotein A-I is mainly localized in the HDL fraction.

Results of a number of studies in man and rat in vivo, and of in vitro studies using isolated lipoproteins and a lipolytic system, have revealed that during chylomicron or VLDL catabolism surface constituents (mainly phospholipid, apoC, and apoA-I) are transferred to HDL (13, 15–19, 24–27, 31). A possible mechanism for these processes was presented by Tall and Small (28). It was proposed that as the triacylglycerol core shrinks by the action of lipoprotein lipase, spike-formed bilayers of phospholipid are formed in the surface film of chylomicrons (and VLDL) stabilized by apolipoproteins (apoA-I, apoC) that could be used for HDL assembly after being released from the chylomicron surface. At present the mechanisms by which surface components are taken up in the HDL fraction are not fully understood. The excess surface bi-

TABLE 3. Concentration of HDL<sub>3</sub> constituents in serum before and after cream ingestion

| Time after Cream Ingestion | Serum Concentration of HDL <sub>3</sub> Constituents |                        |                       |                        |                              |                      |                         |                         |
|----------------------------|------------------------------------------------------|------------------------|-----------------------|------------------------|------------------------------|----------------------|-------------------------|-------------------------|
|                            | Protein (9) <sup>a</sup>                             | Phospholipids (9)      | Total Cholesterol (9) | Cholesteryl Esters (6) | Unesterified Cholesterol (6) | Triacylglycerol (6)  | Apolipoprotein A-I (9)  | Apolipoprotein A-II (6) |
| <i>hr</i>                  | <i>mg/liter, mean ± SD</i>                           |                        |                       |                        |                              |                      |                         |                         |
| 0                          | 1422 ± 203                                           | 705 ± 132              | 376 ± 58              | 505 ± 74               | 89 ± 24                      | 52 ± 13              | 1120 ± 224              | 394 ± 74                |
| 3                          | 1454 ± 215                                           | 759 ± 138 <sup>b</sup> | 381 ± 68              | 509 ± 98               | 88 ± 20                      | 58 ± 12 <sup>c</sup> | 1154 ± 226              | 406 ± 65                |
| 5                          | 1509 ± 203 <sup>d</sup>                              | 798 ± 140 <sup>e</sup> | 384 ± 73              | 515 ± 99               | 89 ± 21                      | 67 ± 11 <sup>f</sup> | 1168 ± 214              | 402 ± 86                |
| 8                          | 1512 ± 195 <sup>e</sup>                              | 833 ± 118 <sup>d</sup> | 401 ± 69              | 527 ± 104              | 95 ± 19                      | 44 ± 22              | 1236 ± 216 <sup>e</sup> | 408 ± 68                |

<sup>a</sup> Number of experiments.

<sup>b</sup> Statistical significance compared to *t* = 0 hr, using Student's two-tailed paired *t* test; *P* < 0.05.

<sup>c</sup> *P* < 0.01.

<sup>d</sup> *P* < 0.001.

<sup>e</sup> *P* < 0.005.

<sup>f</sup> *P* < 0.02.

TABLE 4. Chemical composition of HDL<sub>2</sub> before and after cream ingestion

| Time after Cream Ingestion | Composition of HDL <sub>2</sub> in Weight Percentage |                         |                         |                              |                     |                                        |
|----------------------------|------------------------------------------------------|-------------------------|-------------------------|------------------------------|---------------------|----------------------------------------|
|                            | Protein (6) <sup>a</sup>                             | Phospholipids (6)       | Cholesteryl Esters (6)  | Unesterified Cholesterol (6) | Triacylglycerol (6) | Weight Ratio Protein/Phospholipids (9) |
| <i>hr</i>                  | <i>percentage, mean ± SD</i>                         |                         |                         |                              |                     |                                        |
| 0                          | 36.0 ± 2.2                                           | 30.5 ± 1.2              | 24.2 ± 1.6              | 5.8 ± 0.5                    | 3.4 ± 1.3           | 1.19 ± 0.08                            |
| 3                          | 35.5 ± 2.3                                           | 32.0 ± 1.6              | 22.9 ± 0.7              | 5.9 ± 0.3                    | 3.7 ± 1.2           | 1.10 ± 0.10 <sup>b</sup>               |
| 5                          | 35.7 ± 2.9                                           | 32.5 ± 1.8              | 22.2 ± 1.3 <sup>b</sup> | 6.0 ± 0.5                    | 3.6 ± 1.2           | 1.11 ± 0.13                            |
| 8                          | 34.7 ± 2.4                                           | 33.5 ± 1.5 <sup>b</sup> | 22.7 ± 2.2              | 6.3 ± 0.4                    | 3.0 ± 1.1           | 1.06 ± 0.10 <sup>b</sup>               |

<sup>a</sup> Number of experiments.<sup>b</sup> Statistical significance compared to t = 0 hr, using Student's two-tailed paired *t* test; *P* < 0.05.

layer may seal as a phospholipid bilayer vesicle or may form small bilayer discs that are eventually converted into cholesteryl ester-filled spherical HDL particles by the action of the serum LCAT activity (28). Alternatively, surface bilayer may be taken up by existing serum HDL particles. Evidence for the latter process has been presented by Patsch and co-workers (13). In *in vitro* studies using VLDL and HDL<sub>3</sub> mixed with purified lipoprotein lipase, the formation of a larger HDL<sub>2</sub>-like particle was demonstrated as lipolysis proceeds. From these experiments a scheme was proposed, in which HDL<sub>2</sub> is supposed to be the product of fusion of surface fragments of VLDL (or chylomicrons) with HDL<sub>3</sub>. However, our present findings indicate that fat ingestion in man does not result in an acute transformation of HDL<sub>3</sub> into HDL<sub>2</sub>. Our data suggest that surface constituents of chylomicrons associate with HDL<sub>3</sub> as well as HDL<sub>2</sub>, resulting in an increase in the mean flotation rate of both subclasses. Whether surface material is taken up by existing HDL particles or, alternatively, whether a spectrum of surface bilayer-derived HDL particles is superimposed on the basal (fasting) spectra of HDL<sub>2</sub> and HDL<sub>3</sub> particles, cannot be concluded unequivocally from our present data. However, we would suggest, based on our HDL-cholesterol profile data, that the former mechanism is operating. We observed, in the hours following cream ingestion, a de-

crease in cholesterol concentration in slow floating HDL<sub>2</sub> and HDL<sub>3</sub>, while the concentration increased in the fast floating regions of both subfractions (Fig. 3). We would interpret these findings as evidence in favor of the mechanism in which (phospho)lipids and proteins are taken up by existing HDL<sub>2</sub> and HDL<sub>3</sub> particles. The increase in size resulting from such a process may also contribute to the increase in flotation rate and be superimposed on the effects due to a decrease in protein/lipid ratio. The uptake of surface film from chylomicrons by HDL could explain the increase in unesterified cholesterol concentration seen in HDL<sub>2</sub>. An increase of this constituent was not observed in the HDL<sub>3</sub> fraction and it is tempting to speculate that uptake of unesterified cholesterol by HDL<sub>3</sub> is masked by lecithin:cholesterol acyltransferase activity. A preference of the enzyme for HDL<sub>3</sub> over HDL<sub>2</sub> has been described (36, 37). The triacylglycerol concentration in HDL increased also after cream ingestion, as demonstrated earlier (31), but declined afterwards. During the hyperchylomicronemic phase, the transfer of triacylglycerol from chylomicrons to HDL, catalyzed by serum lipid transfer protein(s), may be stimulated by increased substrate availability (38–40). Whether the triacylglycerol transfer from triacylglycerol-rich lipoproteins to HDL (and LDL) is in some way coupled to an opposite transfer of cholesteryl ester is not fully understood (41,

TABLE 5. Chemical composition of HDL<sub>3</sub> before and after cream ingestion

| Time after Cream Ingestion | Composition of HDL <sub>3</sub> in Weight Percentage |                         |                        |                              |                        |                                        |
|----------------------------|------------------------------------------------------|-------------------------|------------------------|------------------------------|------------------------|----------------------------------------|
|                            | Protein (6) <sup>a</sup>                             | Phospholipids (6)       | Cholesteryl Esters (6) | Unesterified Cholesterol (6) | Triacylglycerol (6)    | Weight Ratio Protein/Phospholipids (9) |
| <i>hr</i>                  | <i>percentage, mean ± SD</i>                         |                         |                        |                              |                        |                                        |
| 0                          | 52.2 ± 2.2                                           | 24.9 ± 2.1              | 17.9 ± 1.1             | 3.1 ± 0.4                    | 1.8 ± 0.4              | 2.05 ± 0.26                            |
| 3                          | 51.4 ± 2.1 <sup>b</sup>                              | 25.9 ± 1.6              | 17.7 ± 0.8             | 3.0 ± 0.3                    | 2.0 ± 0.3 <sup>b</sup> | 1.94 ± 0.20 <sup>b</sup>               |
| 5                          | 51.7 ± 2.5                                           | 26.3 ± 2.0              | 16.9 ± 0.8             | 2.9 ± 0.3                    | 2.3 ± 0.6              | 1.91 ± 0.23 <sup>b</sup>               |
| 8                          | 50.6 ± 2.1                                           | 27.4 ± 1.5 <sup>b</sup> | 17.1 ± 1.1             | 3.1 ± 0.3                    | 1.8 ± 0.7              | 1.83 ± 0.16 <sup>b</sup>               |

<sup>a</sup> Number of experiments.<sup>b</sup> Statistical significance compared to t = 0 hr, using Student's two-tailed paired *t* test; *P* < 0.05.

42) but it is interesting to note that the increase in triacylglycerol in HDL is localized in that fraction, i.e., HDL<sub>3</sub>, where cholesterol esterification by LCAT is expected to occur (43).

In a concurrent study, Tall et al. (19) have analyzed HDL subclass changes in man after fat ingestion using isopycnic density gradient centrifugation. Although the resolution of their technique is inferior to the method used in our study, a similar pattern in HDL mass redistribution was observed, i.e., an increase in mass associated with "light" HDL (in the density range between 1.07 and 1.11 g/ml) and "heavy" HDL (1.11 <  $P$  < 1.17 g/ml) accompanied by shifts of centers of mass to a lower density. Using limiting pore electrophoresis (that resolves HDL in four or five partially separated bands) on fractions obtained by isopycnic centrifugation, it was found that the mass associated with two bands increased (a 10.7 nm-sized "light" HDL and a 9.2–10 nm-sized "heavy" HDL). No indications were found for the formation of new HDL species and our data seem to be in line with that conclusion. Data obtained in a number of other studies (15–18), employing sequential density ultracentrifugation for HDL<sub>2</sub> and HDL<sub>3</sub> separation, are difficult to compare inasmuch as the density of postprandial high density lipoproteins changes and this may affect the quality of HDL<sub>2</sub>/HDL<sub>3</sub> separation.

The question of how plasma HDL<sub>2</sub> is formed has not been resolved in the present study. We think that a direct interconversion of HDL<sub>3</sub> into HDL<sub>2</sub> by the uptake of surface remnants from chylomicrons is unlikely as no indications for the operation of such a process are found in the present studies. However, plasma levels of HDL<sub>2</sub> do correlate with parameters for VLDL and chylomicron catabolism (14, 29, 44) suggesting a metabolic interrelationship. Recently, a central role of plasma LCAT in the formation of HDL<sub>2</sub> has been proposed (45, 46). These studies have shown that during prolonged action of LCAT on HDL<sub>3</sub> in serum, a larger, HDL<sub>2</sub>-like lipoprotein is formed. One may speculate that the availability of phosphatidylcholine and unesterified cholesterol on HDL<sub>3</sub> affects the progress of this process and this availability could be increased by uptake of surface remnants. In individuals who are able to degrade VLDL and chylomicrons rapidly, a better supply of lipid substrates for LCAT may be maintained and those individuals are probably capable of building up high levels of HDL<sub>2</sub> over a prolonged period of time (29). Further studies are needed to verify this hypothesis. ■

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