Stimulation of Glycolipid Synthesis and Exchange by Human Serum High Density Lipoprotein-3 in Human Fibroblasts and Leukocytes*

Bill C. P. Kwok, Glyn Dawson, and Mary C. Ritter

From the Departments of Biochemistry, Pediatrics, and Medicine, Pritzker School of Medicine, University of Chicago, Chicago, Illinois 60637

Upon exposure to either human skin fibroblasts or human circulating leukocytes, the composition of human serum high density lipoprotein-3 (HDL₃) was modified by the apparent loss of apolipoprotein A-I and II and a 2- to 4-fold increase in glycosphingolipid content. Exposure of HDL₃ to leukocytes produced an increase in the content of lactosylceramide, which is the major glycolipid in leukocytes, whereas exposure of HDL₃ to human skin fibroblasts produced predominantly an increase in trihexosylceramide, which is the major glycolipid in fibroblasts. Other protein components of HDL₃ (such as apolipoprotein A-I) were unaffected and there were no major changes in either neutral lipid or phospholipid composition. The increase in glycosphingolipid content of both cells and reisolated HDL₃ particles was HDL₃ concentration-dependent up to a concentration of 1 mg/ml and appeared to be the result of a stimulation of cellular glycolipid synthesis by HDL₃ and subsequent transfer to HDL₃ in the medium. A similar stimulation could not be produced by either low density lipoprotein or lipoprotein-deficient serum. The coaddition of HDL₃ and lipoprotein-deficient serum reduced the loss of apolipoprotein A-I and II and the change in HDL₃ glycolipid content, but not the increase in cellular glycolipid content, suggesting that modification of the apolipoprotein A-II peptide may enhance the ability of HDL₃ to acquire new glycolipid from cells.

Glycosphingolipids in blood are found in association with both cellular elements (erythrocytes, leukocytes, platelets, etc.) and lipoproteins of both the low density and high density type (1, 2). The majority of lipoprotein glycolipid (70%) is an integral component of the LDL particle, but in patients with an inherited deficiency of circulating LDL (abetalipoproteinemia and hypobetalipoproteinemia), the HDL particles are sufficiently enriched in glycolipid that the plasma glycolipid levels appear normal (2). Both analytical (1, 2) and metabolic (3) studies indicate that there is little exchange of glycolipid between erythrocytes and lipoprotein, with the possible exception of monohexosylceramides (3, 4). Thus, the relative amounts of individual glycolipid species in the lipoprotein fraction are quite different (1, 3), and the plasma glycolipid elevations seen in patients with Gaucher's disease (glucosylceramide storage) and Fabry's disease (trihexosylceramide storage) do not alter erythrocytic glycolipid composition (4). However, initial observations in this laboratory showed that HDL₃, but not LDL, was capable of stimulating glycolipid synthesis in cultured human skin fibroblasts, where trihexosylceramide is the major glycolipid (7). Our data suggested that glycolipid exchange between HDL₃ and actively dividing cells was a physiologically significant process.

The glycolipid composition of leukocytes is distinct from that of erythrocytes, lymphocytes, and platelets (1, 3, 8, 9) and human skin fibroblasts (10) in that 80% is lactosylceramide (8). Since human serum HDL₃ has been shown to undergo structural modification following exposure to human leukocytes (11), we have attempted to determine if there are concomitant changes in glycolipid composition.

This study reports a comparison of the behavior of the two cell types in response to culture in the presence of HDL₃ and other human serum components. A preliminary report of these findings has been published in abstract form (7).

MATERIALS AND METHODS

Lipoproteins and Lipoprotein-deficient Serum—Human serum LDL, HDL₃, and HDL₄ were isolated from serum of normal, healthy, fasted male A donors by ultracentrifugal flotation as previously described (12). Lipoprotein-deficient serum was prepared by removing any residual lipoprotein at density 1.25 g/ml (12). Typical LPDS preparations contained between 50 and 60 mg of protein/ml. Both HDL₃ and LPDS were dialyzed extensively against 0.15 M NaCl and 0.01% EDTA, pH 7.0, followed by heat treatment at 56°C for 30 min to inactivate lecithin-cholesterol acyltransferase. Following sterilization by passage through Millipore filters (0.22 µm), both HDL₃ and LPDS were stored at 4°C and -20°C, respectively, until required. The LPDS was shown to be free from both esterified and nonesterified cholesterol and contained less than 200 µg/ml of phospholipid.

Human Skin Fibroblast Studies—Normal human skin fibroblasts from stocks maintained in this laboratory were plated at a density of 2 × 10⁴ cells/60-mm Falcon plastic dish and maintained in 3 ml of DME medium supplemented with 10% fetal calf serum and 10% calf serum at 37°C for 7 days as described previously (10). Prior to labeling or adding lipoprotein, the monolayers were washed three times with phosphate-buffered saline, once with DME medium containing 5% (by volume) human LPDS, and then cultured in 5% (by volume) human LPDS-supplemented DME medium for 16 h ("priming"). Incubation studies were carried out for 24 h in 3 ml of fresh 5% LPDS-supplemented DME medium together with appropriate amounts of HDL₃ or LDL and isotopic precursor (10 µCi of [U-¹³C]Gal or 3 µCi...
of (\textsuperscript{14}C)Gal). All incubations were carried out at 37°C unless stated otherwise. Phospholipids were labeled by incubating cells with \textsuperscript{14}C]-acetate, 3 \muCi/ml for 18 h. In some instances, cells were double-labeled with 10 \muCi of [\textsuperscript{3}H]Leu and 3 \muCi of methyl \textsuperscript{14}C]-thymidine. The level of trichloroacetic acid-precipitable \textsuperscript{3}H and \textsuperscript{14}C radioactivity was used as a measure of \textit{de novo} protein and DNA synthesis. HDL\textsubscript{a} was reisolated from the pooled DME medium from 16 to 20 large (100 mm) Falcon plates (total volume ~200 ml) by dialyzing for 48 h at 4°C against 0.15 \textit{M} NaCl containing 0.01% EDTA and 0.02% NaN\textsubscript{3}, pH 7.0, adjusting the dialysate to a density of 1.21 and carrying out ultracentrifugation as described previously (11, 12). When unfraccionated medium was to be analyzed for radioactive glycolipids, dialysis was carried out for 48 h against 5 mm ammonium bicarbonate buffer, pH 8.5, and the dialysate lyophilized prior to extraction.

**Human Leukocyte Studies**—Isolated mixed human leukocytes (~80% granulocytes, 20% mononuclear cells, but no platelets) from the blood of normal, healthy, fasting A\textsuperscript{+} male donors (11) were cultured in 25-ml Nalgene polycarbonate flasks at 37°C or 4°C for 18 h in RPMI-1640 medium (supplemented with 0.02% NaHCO\textsubscript{3}, 25 mm 4-2-hydroxyethyl-1-piperazineethanesulfonic acid, 100 units of penicillin, 100 \mug of vitamin E/ml) at a density of 2 to 3 X 10\textsuperscript{5} cells (~1 to 2 mg of cell protein) in an atmosphere of 5% CO\textsubscript{2}. Human serum HDL\textsubscript{a}, HDL\textsubscript{b}, LDL, and 3% (by volume) LPDS, and isotopic precursors, \textsuperscript{14}C]-Gal and \textsuperscript{3}H]-Leu (3 pCi/ml) were added to the medium and incubated for 18 h. HDL\textsubscript{a} was reisolated as described for human skin fibroblasts.

**Lipid Analysis of Lipoproteins and Cells**—Harvested cells were disrupted by sonication in 0.5 ml of phosphate-buffered saline for 30 s at setting 5 on a model 185W sonifier (Heat Systems-Ultrasonics, Inc., Plainview, NY). Total cell dispersions, native lipoproteins, reisolated and dialyzed lipoprotein, and lyophilized, radioactive culture medium were extracted for lipids by the Folch procedure (13) and subjected to sequential silicic acid column chromatography and Silica Gel G thin layer chromatography (2, 10). Phospholipid, unesterified cholesterol, cholesterol esters, and triglycerides were determined by procedures described previously (11). Individual glycolipids were either scraped directly from the thin layer chromatography plate into vials for radioactivity determination by liquid scintillation counting (0.5 ml of water plus 10 ml of ACS mixture (Amersham/Searle) or quantitated by gas-liquid chromatography of their derived trimethylsilyl methylglycosides using mannitol as internal standard (1, 2, 10, 14). Gal and Glc were used to calibrate the detector response prior to each series of determinations. All radioactive incorporation data and quantitative analyses are expressed in terms of the protein content as established by the Lowry procedure (15) using bovine serum albumin (Miles Laboratories) (Fraction V) as a standard, unless otherwise specified.

**Polyacrylamide Gel Electrophoresis**—Electrophoresis to resolve the apo-A-I and apo-A-II components of HDL\textsubscript{a} was carried out on 10\% polyacrylamide gels in 8 m urea (16) and in 0.1\% sodium dodecyl sulfate polyacrylamide gels (Fig. 1a) as described previously (18). Quantitation of apo-A-I and apo-A-II on Coomassie blue-stained gels was carried out by a densitometric method as described previously (11).

**RESULTS**

**Modification of HDL\textsubscript{a} Apoprotein**—Exposure of human skin fibroblasts to human serum HDL\textsubscript{a} for 24 h under the conditions described resulted in a maximum 50\% reduction in the amount of recoverable apo-A-II peptide present in the reisolated HDL\textsubscript{a} particle as measured by densitometric scanning of sodium dodecyl sulfate polyacrylamide gels (Fig. 1a). Apo-A-I content was essentially unaffected and the results are, therefore, qualitatively similar to the 100\% loss of apo-A-II observed when human leukocytes were exposed to HDL\textsubscript{a} (0.2 mg/ml) for 18 h (11). These results are in agreement with those obtained by polyacrylamide gel electrophoresis in 8 m urea. The apparent decrease in HDL\textsubscript{a} apo-A-II content following exposure to human skin fibroblasts only reached a maximum of 50\% after a 24-h incubation in the absence of LPDS and was partially blocked by the presence of 5\% (by volume) LPDS (Fig. 1b). Incubation for a further 48 h did not increase the loss of apo-A-II. No changes in peptide composition were observed when the incubations were carried out at 4°C. The disappearance of apo-A-II was completely prevented by increasing the concentration of LPDS to 20\% (by volume) as shown in Fig. 1c. The loss of apo-A-II following exposure to human skin fibroblasts was cell density-dependent, reaching the 50\% maximum after 48 h in cultures where the cell density exceeded 2 X 10\textsuperscript{5}/60-mm Falcon dish (Fig. 1d).

Reisolated HDL\textsubscript{a} had essentially the same flotation density properties as native HDL\textsubscript{a} and the disappearance of apo-A-II peptide following exposure to human skin fibroblasts did not result in the loss of more than 5\% of the protein from the HDL\textsubscript{a} particle (Table I). Preliminary studies and previous work on leukocytes (11) indicate that peptides produced by proteolytic cleavage of apo-A-II remain associated with the HDL\textsubscript{a} particle. The failure to detect the presence of apo-A-II fragments by electrophoresis following exposure to human skin fibroblasts may be due to the staining properties or the size of the degraded peptides.

**Modification of HDL\textsubscript{a} Lipid by Exposure to Cultured Cells**—There was no overall change in HDL\textsubscript{a} lipid composition following exposure to human skin fibroblasts (Table I). However, with both cell types at 37°C, we observed an increase in the amount of glycolipid in the reisolated HDL\textsubscript{a} specific for each cell type, namely a 4-fold increase in lacto-
slyceramide following leucocyte incubation (Table II) and a 2-fold increase in trihexosylceramide following incubation of human skin fibroblasts with HDL3 at a concentration of 0.2 mg/ml (Table I). The increase in LacCer was not observed when HDL3 was exposed to leucocytes at 4°C (Table II). Human skin fibroblasts produced HDL3 concentration-dependent increases in the amount of [3H]glycolipid acquired by exogenous HDL3, up to a HDL3 concentration of 1 mg/ml (Fig. 2b), which is approximately the concentration of HDL3 in normal human male blood (2). This dose-dependent increase in HDL3-associated [3H]glycolipid was followed by culturing human skin fibroblasts in the presence of constant 5% (by volume) LPDS, increasing concentrations of HDL3, and an isotopic precursor of glycolipids ([14C]Gal). Similar results were obtained with leucocytes regarding an HDL3 concentration-dependent increase in [3H]glycolipid in reisolated HDL3 (data not shown). Increasing the LPDS concentration from 5 to 15% reduced the amount of [3H]GbOse,Cer transferred from cultured skin fibroblast cells to HDL3 in the medium and essentially no increased transfer was seen when cells were grown either in serum-free medium (no HDL3) or in medium supplemented with 10% fetal calf serum and 10% calf serum (Fig. 3a), which is the optimum growth conditions for these cells. The ability of 3% LPDS to inhibit the transfer of newly synthesized [14C]galactolipid to HDL3 in the medium (in this case, a 2-fold increase in [14C]LacCer content) was seen more clearly with human leucocytes (Table III). These cells contain virtually no GbOse,Cer and this was reflected in the low level of [14C]GbOse,Cer in both cells and medium.

**Table I**

**Lipid analysis of HDL3 before and after exposure to human skin fibroblasts**

HDL3 (0.2 mg/ml) was incubated at 37°C in the presence or absence of 5% LPDS with human skin fibroblasts (105 cells/10 ml/100-mm Petri dish) under conditions which resulted in 50% loss of apo-A-I. Following incubation, the HDL3 was reisolated from pooled medium (200 ml) and analyzed for protein, neutral lipids, phospholipids, and glycolipids as described in the text.

| Composition        | HDL3 | HDL3 + LPDS |
|--------------------|------|-------------|
|                    | Before | After | After |
|                   | µmol/g HDL3 protein | µmol/g HDL3 protein | µmol/g HDL3 protein |
| Glycolipid         |       |       |       |
| LacCer            | 0.95 ± 0.1 | ND*  | 1.35 ± 0.1 |
| GbOse,Cer        | 0.40 ± 0.1 | ND*  | 0.70 ± 0.1 |
| Phospholipid       |       |       |       |
| Total              | 26.0   | 26.7   | 26.1   |
| Phosphatidylethanolamine | 1.4   | 1.8    | 1.7    |
| Phosphatidylcholine | 20.7   | 21.3   | 20.0   |
| Lyso phosphatidylcholine | 1.0   | 1.0    | 1.2    |
| Sphingomyelin      | 2.9    | 2.6    | 3.0    |
| Neutral lipid      |       |       |       |
| Free cholesterol   | 4.2    | 4.3    | 4.7    |
| Cholesterol        | 14.3   | 13.3   | 13.9   |
| Triglycerides      | 2.3    | 4.1    | 4.3    |
| Protein            | 53.2   | 51.6   | 50.9   |

* Not determined. No glycolipid analyses were performed because fibroblasts require LPDS.

**Table II**

**Glycosphingolipid analysis of HDL3 before and after exposure to leucocytes at 37°C and 4°C**

HDL3 (0.2 mg/ml) was incubated for 18 h at 37°C or 4°C in the presence or absence of 3% (by volume) LPDS with leucocytes (105 cells/ml in a total volume of 2.5 ml). After incubation, the medium from 40 flasks was pooled, and the HDL3 was reisolated and analyzed for glycosphingolipids as described in the text.

| Incubation condition | GlyCer* | LacCer | GbOse,Cer |
|----------------------|---------|--------|-----------|
|                      | µmol/g HDL3 protein* | µmol/g HDL3 protein* | µmol/g HDL3 protein* |
| Before incubation    | 1.3 ± 0.2 | 1.2 ± 0.5 | 0.4 ± 0.1 |
| After incubation at 37°C | 1.3 ± 0.2 | 5.0 ± 1.2 | 0.3 ± 0.1 |
| HDL3 ± LPDS          | 1.4 ± 0.1 | 2.1 ± 0.1 | 0.3 ± 0.1 |
| After incubation at 4°C | 0.9 ± 0.1 | 1.1 ± 0.2 | 0.2 ± 0.2 |
| HDL3 ± LPDS          | 1.1 ± 0.1 | 0.9 ± 0.1 | 0.3 ± 0.1 |

* Glucosylceramide.

**Table III**

**Stimulation of glycolipid synthesis in human leucocytes by human serum HDL3 and HDL3**

Isolated human leucocytes (105 cells/ml in total volume of 2.5 ml/flask) were incubated with 10 µCi of [14C]Gal at 37°C for 18 h in the presence of HDL3 (0.2 mg/ml) or HDL3 (0.2 mg/ml), in the presence or absence of 3% LPDS (by volume) or LDL (0.4 mg/ml). Cellular [14C]glycolipids and [14C]glycolipids in the medium were analyzed as described in the text.

| Medium supplement | Cells | Medium |
|-------------------|------|-------|
|                   | LacCer | GbOse,Cer | LacCer | GbOse,Cer |
|                   | cpm/mg cell protein* | cpm/mg cell protein* | cpm/mg cell protein* | cpm/mg cell protein* |
| None              | 2105 ± 172 | 145 ± 36 | 1270 ± 37 | 510 ± 50 |
| LPDS              | 2300 ± 100 | 100 ± 15 | 1000 ± 25 | 410 ± 45 |
| HDL3              | 3200 ± 125 | 200 ± 19 | 2420 ± 75 | 580 ± 60 |
| HDL3 + LPDS       | 2800 ± 300 | 275 ± 40 | 1260 ± 50 | 475 ± 50 |
| HDL3              | 3100 ± 150 |            |          |          |
| HDL3 + LPDS       | 2400 ± 200 |            |          |          |
| LDL               | 2570 ± 150 | 244 ± 15 | 1300 ± 45 | 419 ± 40 |

* Values represent the mean ± standard deviation of three separate determinations.
(Table III). No transfer of cellular glycolipid to LDL in the medium was observed under these conditions (Table III). Further, since leukocytes do not divide or increase their surface area, the increase in HDL₃ glycolipid is a specific phenomenon unrelated to an increase in total cell membrane.

Stimulation of Glycolipid Synthesis in Fibroblasts by HDL₃—The presence of HDL₃ in the culture medium was found to stimulate both the synthesis of cellular glycolipid, as well as its egress from the cell to form an HDL₃-glycolipid complex. In normal human skin fibroblasts grown under four different culture conditions, we observed both a stimulation of [³H]Gal incorporation into GbOse₃Cer (Fig. 3b) and an absolute increase in the amount of cell-associated glycolipid/mg of cell protein. Exposure of human skin fibroblasts (in 5% LPDS) to HDL₃ increased the GbOse₃Cer level from 4.0 ± 0.2 to 5.6 ± 0.4 nmol/mg of protein in one cell strain and from 3.6 ± 0.2 to 5.6 ± 0.3 nmol/mg of protein in a second cell strain.

It can be seen from Fig. 3b that the stimulation in cellular GbOse₃Cer synthesis by HDL₃ (0.2 mg/ml) was additive to that produced by the addition of 5% LPDS, 15% LPDS, or 10% fetal calf serum, plus 10% calf serum. The stimulation of glycolipid synthesis occurred independently of cell density and was HDL₃ concentration-dependent up to 1 mg/ml for 24 h (Fig. 2a). This stimulation was also observed for fibroblasts incubated with HDL₃ for 48 h.

Stimulation of Glycolipid Synthesis in Leukocytes by HDL₃—HDL₃ increased both the incorporation of [¹⁴C]Gal into leukocyte glycolipid (predominantly LacCer) (Table III) and the absolute amount of cell-associated glycolipid (Table IV). The coaddition of LPDS did not reduce the stimulation of LacCer synthesis but completely inhibited the transfer of lactosylceramide from cells to extracellular HDL₃. HDL₃ had a similar effect to HDL₃ and the coaddition of LPDS completely suppressed the stimulation. LDL was less effective than either HDL species in promoting cellular glycolipid synthesis and did not stimulate any egress of glycolipid from the cell (Table III). As observed with human skin fibroblasts, the stimulation of cellular glycolipid synthesis by HDL₃ was HDL₃ concentration-dependent over the range 0 to 1.0 mg/ml as shown in Fig. 4a. It can also be seen that the slight stimulation by LDL at low concentrations (Table III) became a marked inhibition at higher LDL concentrations (Fig. 4a). In addition, increasing the concentration of HDL₃ or LDL in the culture medium inhibited the synthesis of phospholipids as typified by phosphatidylethanolamine (Fig. 4b). In contrast, sphingomyelin synthesis was slightly stimulated in a manner comparable to that of the glycosphingolipid lactosylceramide. The observed HDL₃ stimulation of glycolipid synthesis in human leukocytes was even more striking when compared to the fact that addition of HDL₃ or LDL suppressed protein synthesis (as measured by [³H]Leu incorporation into trichloroacetic acid-precipitable material) to a level only 50 to 60% of that observed in cells incubated for 18 h in buffer alone.

The coaddition of LPDS did not reverse this 40% suppression of overall cellular protein synthesis.

Quantitative analysis of treated leukocytes from several different patients showed that HDL₃ produced a consistent 2- to 3-fold elevation which was essentially unaffected by the coaddition of LPDS, but blocked by lowering the temperature to 4°C (Table IV). The addition of LPDS alone produced no stimulation of LacCer synthesis. Of interest was the fact that

---

**Fig. 3. Stimulation of GbOse₃Cer synthesis in cultured skin fibroblasts by HDL₃.** Human skin fibroblasts (2 × 10⁶ cells) were primed as described in the legend of Fig. 1a and then cultured in the presence of [³H]Gal (10 μCi/3 ml of medium/24 h) under four different conditions: optimum growth conditions (supplemented with 10% calf serum and 10% fetal calf serum), low growth conditions (serum-free medium), and in serum-free medium supplemented with either 5% (by volume) LPDS or 15% (by volume) LPDS in the presence (c) or absence (b) of HDL₃ (0.2 mg/ml). a, [³H]GbOse₃Cer isolated from dialyzed, lyophilized media as described in the text. b, [³H]-GbOse₃Cer isolated from washed, harvested cells as described in the text.

---

**Table IV**

| Treatment        | Patient 1 | Patient 2 |
|------------------|-----------|-----------|
|                  | GlcCer⁶   | LacCer⁷   | GlcCer⁸   | LacCer⁹   |
| None (freshly iso| 1.3 ± 0.2 | 3.8 ± 0.6 | 1.5 ± 0.2 | 11.5 ± 1.0|
| leucocytes)      |           |           |           |            |
| After incubation  |           |           |           |            |
| at 37°C          |           |           |           |            |
| None             | 1.5 ± 0.3 | 4.6 ± 0.8 | 1.9 ± 0.3 | 10.3 ± 1.5|
| LPDS             | 1.2 ± 0.2 | 4.8 ± 0.8 |           |            |
| HDL₃             | 1.9 ± 0.2 | 9.4 ± 0.7 | 1.8 ± 0.2 | 22.9 ± 0.6|
| HDL₃ + LPDS      | 1.1 ± 0.3 | 9.6 ± 0.6 | 1.7 ± 0.4 | 19.9 ± 1.0|
| After incubation  |           |           |           |            |
| at 4°C           |           |           |           |            |
| None             | 1.8 ± 0.2 | 4.0 ± 0.1 |           |            |
| LPDS             | 1.3 ± 0.3 | 4.1 ± 0.3 |           |            |
| HDL₃             | 1.4 ± 0.2 | 4.2 ± 0.3 |           |            |
| HDL₃ + LPDS      | 1.6 ± 0.3 | 4.2 ± 0.3 |           |            |

⁶ Glucosylceramide.
⁷ Values represent the mean ± standard deviation of three determinations.
patients could be divided into two populations based on high and low LacCer content; the results in Table IV are the mean from two patients of each type. The immunological classification of these mixed leukocyte populations is unknown.

Comparison of Glycolipid Synthesis, Protein synthesis, and Cell Division—Under the culture conditions described, we did not observe any incorporation of methyl\[^{14}C\]thymidine into trichloroacetic acid-precipitable material (a measure of DNA synthesis) in human leukocytes so that the observed enhanced glycolipid synthesis resulted in a net increase in the amount of glycolipid/cell. As noted previously, there was in fact a substantial inhibition of overall protein synthesis as judged by \[^{1}H\]Leu incorporation.

Cell division, DNA, and protein synthesis in human skin fibroblasts are heavily dependent on serum protein supplement and can be restored to control levels (10% fetal calf serum and 10% calf serum) by the addition of 15% LPDS. This is in contrast to the effect on glycolipid synthesis where the coaddition of HDL\(_3\) and LDL (0) for 18 h as described in the text. b, \[^{1}C\]phosphatidylethanolamine synthesis by freshly isolated cultures (2.5 X 10\(^5\) cells/2.5 ml of medium and 10 \(\mu\)Ci of \[^{1}H\]Gal) in the presence of increasing concentrations of HDL\(_3\) (C) and LDL (0) for 18 h as described in the text.

**DISCUSSION**

High density lipoprotein-3 (HDL\(_3\)) represents a well characterized and relatively homogeneous subpopulation of human serum HDL (19). The particle is ~50% protein (apo-A-I and apo-A-II in the weight ratio 2:1) and 50% lipid, whereas the other major HDL particle, HDL\(_1\), is ~40% protein (apo-A-I and apo-A-II in the weight ratio 9:1) and 60% lipid (12). HDL\(_3\) is thought to be involved in the regulation of cholesterol metabolism by promoting sterol egress from cells (20, 21) through a mechanism involving lecithin-cholesterol acyltransferase (EC 2.3.1.43) (22) and there have been some reports that it specifically bind to certain types of cells in culture (23). However, its role appears to be minor compared to the receptor-mediated control of cholesterol metabolism by LDL (24).

When HDL\(_3\) was exposed to human leukocytes at 37°C for 18 h (11), the reisolated HDL\(_3\) was found to retain the ultracentrifugal and spectral properties of native HDL\(_3\), despite an almost complete loss of the A-II peptide (apo-A-II) and a modest increase in lysophosphatidylcholine content. This HDL\(_3\) modification is believed to result from the extracellular proteolytic cleavage of apo-A-II in the intact HDL\(_3\) particle since it is blocked by the addition of proteolytic inhibitors such as diisopropyl fluorophosphate. Our studies indicate that a similar but quantitatively less dramatic modification of apo-A-II can occur in another cultured cell system, the human skin fibroblast, and that it is also time- and cell density-dependent.

When HDL\(_3\) was incubated with fibroblast cultures, no more than 50% of apo-A-II was lost under any conditions and a maximum 80% loss occurred after a 24 h exposure, compared to 100% loss after a 1 h exposure to leukocytes. In addition, 20% LPDS (by volume) is required for complete suppression of apo-A-II loss following exposure to fibroblasts, compared to 3% LPDS in leukocytes, and exposure to fibroblasts produced no discernible change in phospholipid composition. Despite these apparent quantitative differences, apo-A-II modification was accompanied by a transfer of newly synthesized glycolipid from both human leukocytes and skin fibroblasts to the exogenous HDL\(_3\), and with both cell types there was a substantial stimulation of cellular glycolipid synthesis.

Our most striking finding is that at least two types of cell can interact with human serum HDL\(_3\) to increase the amount of glycolipid/particle from an average of 1 molecule of glycolipid/native HDL\(_3\) particle to an average of 2 to 4 molecules of glycolipid/HDL\(_3\) particle. Previous studies of glycolipid lipoprotein interaction (25) have claimed that when human serum is incubated with trihexosylceramide at 37°C, most of the glycolipid (at least 60%) is associated with HDL (isolated as a density 1.063 to 1.21 g/ml fraction by preparative ultracentrifugation). However, more detailed studies in this laboratory (26) indicate that micellar trihexosylceramide floats in the same density range as HDL\(_3\) so that these observations could be challenged. We have resolved this problem by incorporating different glycolipids (galactosylceramide, lactosylceramide, and trihexosylceramide) into phospholipid vesicles and have shown by continuous density gradient separation that glycolipid (~1 mol/mol of HDL\(_3\)) can be acquired by either isolated HDL\(_3\) or HDL\(_3\) in human serum.

The cellular modification of apo-A-II, and perhaps other structural alterations, may facilitate the physiological uptake and incorporation of glycolipid into the HDL\(_3\) particle and explain why we see more glycolipid transfer to HDL\(_3\) when HDL\(_3\) is incubated with cells rather than with vesicles (26). If this is the case, then the increase in the glycolipid content of the HDL in serum from patients with abetalipoproteinemia and hypobetalipoproteinemia could occur either during assembly of the HDL\(_3\) particle or following cell-mediated modification of apo-A-II. The role of apo-A-II in the stimulation of glycolipid synthesis is not entirely clear, but it is perhaps noteworthy to point out that the stimulation was greatest in leukocytes (where the proteolytic cleavage of apo-A-II could be observed most readily) and that HDL (which contains little apo-A-II) and LDL had little stimulatory effect.

In the two cell systems studied, we find that HDL\(_3\) promotes both the synthesis of cellular glycolipid (the type of glycolipid being synthesized is actually cell-specific) and the egress of glycolipid to HDL\(_3\). In human leukocytes, the stimulation of glycolipid synthesis can be shown to be independent of general membrane synthesis since it occurred under conditions where there was no increase in cell number, no inhibition of lysosomal hydrolase activity, no measurable DNA synthesis, and, in fact, an overall 60% inhibition of \(\text{de} \text{ novo}\) protein synthesis.
Glycolipid synthesis was not blocked by the coaddition of LPDS, whereas the transfer of newly synthesized glycolipid (mainly lactosylceramide) from cell to HDL\textsubscript{3} was blocked by LPDS. Synthesis was blocked by the coaddition of puromycin\textsuperscript{2} which supports the concept that de novo synthesis of glycolipid is a transmembrane transferase. However, it must be pointed out that the leukocyte preparations are mixtures of cells and subpopulations might be responding quite differently.

In human skin fibroblasts, the stimulation of glycolipid synthesis by HDL\textsubscript{3} could be seen to be superimposed upon that attributable to cell proliferation even up to concentrations as high as 15\% (by volume) of LPDS. However, as also seen with LacCer in human leukocytes, increasing LPDS concentrations tended to inhibit the transfer of glycolipid (predominantly trihexosylceramide) from fibroblasts to HDL\textsubscript{3}. Thus, although it was easier to separate the stimulation of glycolipid synthesis from the stimulation of cell proliferation in cultured skin fibroblasts, the results in the two cell systems were essentially the same.

Cells which line the aortic walls are exposed to HDL\textsubscript{3} concentrations in the region of 1 mg/ml together with varying amounts of HDL\textsubscript{3}, LDL, and very low density lipoprotein. In our studies on fibroblasts, we have shown that the ability to stimulate glycolipid synthesis remains unsaturated at concentrations up to 1 mg/ml.

The ability of HDL\textsubscript{3} to specifically stimulate glycolipid synthesis in a concentration-dependent manner appeared to be independent of cell density and could also be observed under our optimum culture conditions (10\% fetal calf serum plus 10\% calf serum). Thus, it appears unlikely that culture of cells in LPDS is artificially suppressing glycolipid synthesis. Chatterjee et al. (27) made a similar observation that incubation of normal fibroblasts in LPDS for 24 h had no effect on glycolipid or phospholipid content. In contrast, they reported that culture of cells from a patient with homozgous familial hypercholesterolemia in LPDS for 24 h reduced the glycolipid content from three times normal to near normal levels. We have been unable to repeat this observation in three different homozgous familial hypercholesterolemia fibroblast strains and the mechanism of this reduction remains unclear.

Kruth et al. (28) have concluded that low density, rapidly dividing fibroblast cultures have many more LDL receptors than high density (essentially quiescent) cultures, so the HDL\textsubscript{3} receptor (if it exists) is regulated quite differently. The evidence for HDL\textsubscript{3} binding sites on cell surfaces is somewhat incomplete (23), but Nakazai et al. (29) have presented evidence that HDL\textsubscript{3} is bound, taken up, and then degraded within lysosomes in isolated rat liver parenchymal cells. However, other studies on fibroblasts indicate that HDL\textsubscript{3} is a weak competitor for the LDL binding site (23), and that most of the apparent uptake and binding could be accounted for by normal membrane pinocytosis (23, 30). We have found that saturation binding of \[^{125}\text{I}]\text{HDL}\textsubscript{3} occurs at a concentration of 0.05 mg/ml\textsuperscript{2} so that all the studies reported have been carried out at concentrations considerably in excess of the amount needed to saturate any putative "receptors."

The biological role of surface carbohydrate in lipoproteins remains problematic. HDL\textsubscript{3} contains very little peptide-associated carbohydrate, in contrast to LDL\textsuperscript{31}, so that most of the carbohydrate resides in the 1 to 2 molecules of glycolipid/HDL\textsubscript{3} particle (2). A large number of specific recognition roles have been proposed for cell surface glycolipids, ranging from receptors to adhesion or transport modulators (32-34) and lipoprotein glycolipid could fulfill one or more of these roles. It has also been shown that cell proliferation can be inhibited by increasing the amount of glycolipid in the plasma membrane of cells (35, 36). Thus, it is possible that HDL\textsubscript{3}, because of its ability to stimulate the synthesis of cell membrane glycolipid, could have a role in preventing the early stages of atherosclerotic plaque formation by inhibiting smooth muscle proliferation. It is also tempting to speculate that the acquisition of glycolipid by HDL\textsubscript{3} could facilitate the displacement of either surface lipids or peptides from the particle and trigger (membrane-associated) events which might be essential prerequisites for the complicated process of in vivo HDL\textsubscript{3} processing and degradation (37).

Acknowledgment—We would like to acknowledge the helpful advice and encouragement of Dr. A. M. Scannu, Department of Medicine, University of Chicago.

REFERENCES
1. Vance, D. E., and Sweeley, C. C. (1967) J. Lipid Res. 8, 621-630
2. Dawson, G., Kruski, A. W., and Scannu, A. M. (1976) J. Lipid Res. 17, 125-131
3. Dawson, G., and Sweeley, C. C. (1970) J. Biol. Chem. 245, 410-416
4. Vance, D. E., Krivit, W., and Sweeley, C. C. (1969) J. Lipid Res. 10, 188-192
5. Dawson, G., and Oh, J. Y. (1977) Clin. Chim. Acta 75, 149-153
6. Clarke, J. T. R., Stoltz, J. M., and Malcovey, M. R. (1976) Biochim. Biophys. Acta 431, 317-325
7. Kwok, G. P., Dawson, G., and Ritter, M. (1979) Circulation 60, II-185
8. Hüllebrand, J., Stryckmans, P., and Stolzyn, P. (1971) J. Lipid Res. 12, 361-366
9. Tao, R. V. P., Sweeley, C. C., and Jamieson, G. A. (1973) J. Lipid Res. 14, 19-25
10. Dawson, G., Matalon, R., and Dorfman, A. (1972) J. Biol. Chem. 247, 5944-5950
11. Ritter, M. C., and Scannu, A. M. (1980) J. Biol. Chem. 255, 3763-3769
12. Scannu, A. M. (1969) J. Lipid Res. 7, 295-306
13. Folch, J., Lees, M., and Sloane Stanley, G. H. (1957) J. Biol. Chem. 226, 497-509
14. Dawson, G. (1975) in Lipid Chromatographic Analysis (Marietti, G. V., ed) Vol 2, pp. 663-669, Marcel Dekker, Inc., New York
15. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275
16. Davis, B. J. (1964) Ann. N. Y. Acad. Sci. 121, 404-427
17. Weber, K., and Osborn, M. (1969) J. Biol. Chem. 244, 4406-4412
18. Scannu, A. M., Toth, J., Edelstein, C., Kogg, S., and Stiller, E. (1969) Biochemistry 8, 3309-3316
19. Edelstein, C., Krezy, F. J., Scannu, A. M., and Shen, B. W. (1979) J. Lipid Res. 20, 143-153
20. Stein, Y., Glangeaud, M. C., Fainaru, M., and Stein, O. (1975) Biochim. Biophys. Acta 380, 106-118
21. Stein, O., Vanderhoek, J., and Stein, Y. (1976) Biochim. Biophys. Acta 431, 347-352
22. Glomset, J. A. (1968) J. Lipid Res. 9, 155-167
23. Miller, N. E., Wenstein, D. B., and Steinberg, D. (1977) J. Lipid Res. 18, 438-450
24. Brown, M. S., and Goldstein, J. L. (1976) Science 191, 150-154
25. Clarke, J. T. R., and Stoltz, J. M. (1976) Biochim. Biophys. Acta 441, 165-169
26. Kwok, B., and Dawson, G. (1980) Fed. Proc. 39, 2183
27. Chatterjee, S., Sakerke, C. S., and Krischik, P. O. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 4339-4343
28. Kruth, K. S., Avigan, J., Garbe, W., and Vaughan, M. (1979) J. Cell Biol. 83, 588-594
29. Nakai, T., Otto, P. S., Kenney, D., and Whyane, T. F., Jr. (1976) J. Biol. Chem. 251, 4914-4921
30. van Berkel, T. J. C., Kruijt, J. K., van Gent, T., and van Tol, A. H. (1980) Biochim. Biophys. Res. Commun. 92, 1002-1008
31. Swanznathan, N., and Aladjem, F. (1976) Biochemistry 7, 1516-1522
32. Karlsson, K. A. (1977) in Structure of Biological Membranes
33. Moss, J., Fishman, P. H., Manganiello, V. C., Vaughan, M., and Brady, R. O. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 1034-1037
34. Hansson, H.-A., Holmgren, J., and Svennerholm, L. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 3782-3786
35. Laine, R. A., and Hakomori, S.-I. (1973) Biochem. Biophys. Res. Commun. 54, 1039-1045
36. Keenan, T. W., Schmid, E., Franke, W. W., and Wiegandt, H. (1975) Exp. Cell Res. 92, 259-270
37. Getz, G. S. (1979) Artery 5, 330-345