# Carbohydrate composition of protein and lipid components in sialic acid-rich and -poor low density lipoproteins from subjects with and without coronary artery disease

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**Abstract** Low density lipoprotein (LDL) from patients with coronary heart disease (CHD) caused 78–286% increase in accumulation of cholesterol in human aortic subendothelial cells compared to 2–17% caused by LDL from normal subjects. Ricin-Sepharose affinity chromatography was used to separate LDL into two subfractions, one sialic acid-rich (SAR) and the other sialic acid-poor (SAP). SAP-LDL from CHD patients caused 156–307% increase in accumulation of cellular cholesterol, whereas SAR-LDL from these patients caused only 14–21% increase. SAP-LDL from normal healthy subjects caused 50–86% increased accumulation, whereas their SAR-LDL induced only 2–12% increase. Carbohydrate analysis of SAP-LDL protein isolated from four CHD patients revealed mean values of 59, 25, 61, and 11 nmoles of N-acetyl glucosamine, galactose, mannose, and sialic acid per mg protein, respectively. Mean values for SAR-LDL protein from these patients were 59, 31, 77, and 24 nmol/mg protein, respectively. Analysis of SAP-LDL protein from four normal healthy subjects indicated respective mean values of 58, 29, 72, and 22 nmol/mg, whereas SAR-LDL protein from normals contained 59, 29, 72, and 29 nmol/mg. The carbohydrate content of LDL lipids represents about 25% of the total carbohydrate present in the lipoprotein. The mean values for SAP-LDL lipids from four CHD patients were about 2, 2, 18, 18, and 2 nmol/mg protein for N-acetyl galactosamine, N-acetyl glucosamine, galactose, glucose, and sialic acid, respectively. The mean values for SAR-LDL lipids from these patients were 3, 4, 34, 41, and 5 nmol/mg, respectively. Analysis of SAP-LDL lipids from four normal healthy subjects indicated respective mean values of 4, 6, 30, 31, and 3 nmol/mg, whereas SAR-LDL lipids from these subjects contained 6, 9, 41, 46, and 7 nmol/mg. These results suggest that the different biological properties of SAR-LDL and SAP-LDL are related to their different carbohydrate compositions.—**Tertov, V. V., A. N. Orekhov, I. A. Sobenin, J. D. Morrisett, A. M. Gotto, Jr., and J. G. Guevara, Jr.** Carbohydrate composition of protein and lipid components in sialic acid-rich and -poor low density lipoproteins from subjects with and without coronary artery disease. *J. Lipid Res.* 1993. **34:** 365–375.

**Supplementary key words** coronary atherosclerosis • low density lipoproteins • carbohydrate • sialic acid

Recently we have reported that the total low density lipoprotein (LDL) fraction isolated from the plasma of patients with coronary heart disease (CHD) stimulates lipid accumulation in human aortic cells cultured from uninvolved intima (1, 2). Comparison of the chemical composition of LDL obtained from the plasma of CHD patients and healthy subjects revealed that these LDL preparations differed only in their carbohydrate composition (3–5). The sialic acid content of LDL from the CHD patients was 2- to 3-fold lower than that of LDL from healthy subjects. A strong negative correlation was established between the capacity of LDL to stimulate intracellular lipid accumulation and its sialic acid content. Moreover, LDL from healthy subjects, when desialylated by neuraminidase treatment, induced the accumulation of intracellular cholesterol. Taken together, these findings suggest that desialylation may increase the atherogenicity of LDL. We have developed a method for the isolation of sialic acid-poor LDL from the total LDL fraction (6). It is based on the capacity of galactose residues of N-bound bi-antenary oligosaccharide chains exposed by desialylation to bind to Ricin-agglutinin ($RCA_{120}$) immobilized on agarose.

This study was undertaken to determine the carbohydrate content of apoB-100 and the lipid fraction of sialic acid-rich and -poor LDL isolated from the plasma of healthy subjects and CHD patients. For carbohydrate

Abbreviations: LDL, low density lipoprotein; CHD, coronary heart disease; SAR, sialic acid-rich; SAP, sialic acid-poor; PBS, phosphate-buffered saline; TFA, trifluoroacetic acid.

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analysis, the method of anion exchange chromatography followed by pulsed amperometric detection was used.

## MATERIALS AND METHODS

### Materials

Monosaccharides used as standards and trifluoroacetic acid were purchased from Sigma Chemical Co. (St. Louis, MO). Agglutinin $RCA_{120}$ insolubilized on cross-linked 4% agarose (2–4 mg protein per ml packed gel) was also obtained from Sigma. Carbonate and tar-free sodium hydroxide was obtained from Fisher Scientific (Rockville, MD). Fetal calf serum, Medium 199, fungizone, penicillin/streptomycin, and glutamine were obtained from GIBCO Europe (Paisley, U.K.). Kits for total cholesterol determinations were purchased from Boehringer Mannheim (Mannheim, Germany).

### Donors

LDL was isolated from individual plasma samples, as well as from pooled plasma of healthy subjects and patients with coronary heart disease (CHD). The latter patients had angiographically documented stenosis of coronary arteries. The characteristics of these CHD patients and healthy donors have been described in detail elsewhere (1, 2, 7). Characteristics such as age and gender in the groups of healthy donors and patients were similar. None of the CHD patients or healthy donors had diabetes mellitus or hypertension. Their plasma cholesterol levels were less than 210 mg/dl.

### Preparation of lipoproteins

Low density lipoprotein was isolated from plasma (0.1% EDTA) by sequential ultracentrifugation according to Lindgren (8). Lipoprotein oxidation and proteolysis were minimized by making the plasma 20 $\mu$M in butylated hydroxytoluene, 1 mM in phenylmethanesulfonyl fluoride, and 10 mM in $\epsilon$-aminocaproic acid. After recentrifugation, LDL preparations were dialyzed against phosphate-buffered saline (PBS) containing 0.1% EDTA, applied to a 5-ml immunoaffinity column containing polyclonal anti-apo[a]-agarose to remove Lp[a] (9), and eluted with 50 ml of PBS. Nonadsorbed lipoproteins were loaded on a 10-ml column of anti-apoB-100-agarose, and washed subsequently with 200 ml of PBS, then with 100 ml of PBS containing 0.5 M NaCl. Finally, adsorbed LDL was eluted with 100 ml 1 N acetic acid. The eluate was immediately adjusted to pH 7.2 with 3.5 M Tris. The eluate density was adjusted to 1.063 g/ml by adding NaBr, and LDL were re-isolated by ultracentrifugation.

For separation of sialic acid-rich and -poor LDL, 20 mg LDL from either healthy subjects or CHD patients was loaded on a 10-ml $RCA_{120}$-agarose column. The column was washed with 200 ml PBS; under these conditions sialic acid-rich LDL emerged unbound. Sialic acid-poor LDL (bound fraction) was eluted from the $RCA_{120}$-agarose column with PBS containing 50 mM galactose. The column was stripped with 100 ml PBS containing 0.5 M NaCl, then re-equilibrated with PBS before reuse. Unbound and bound fractions were separately adjusted to d 1.063 g/ml with solid NaBr, then concentrated by ultracentrifugation. Total sialic acid content (protein- and lipid-bound) was determined by the colorimetric method of Svennerholm (10).

Lipids from LDL preparations were extracted three times with ethanol–diethyl ether 3:1 (v/v), three times with chloroform–methanol 1:2 (v/v), and one time with methanol. Extracts were combined and evaporated to dryness under dry nitrogen stream. Delipidated LDL preparations were washed three times with distilled water (18 megaohm resistivity) to remove salt.

### Hydrolysis of glycoconjugates

For neutral monosaccharide analysis, lipid and protein samples (1–4 mg protein of initial LDL) were hydrolyzed in 2 N trifluoroacetic acid (TFA) at 100°C. For sialic acid determination, samples were heated in 0.05 N TFA at 80°C. After TFA evaporation under dry nitrogen, hydrolysates were dissolved in high purity water and aliquots were used for sugar determination.

### Monosaccharide determination

Carbohydrate content was determined by anion exchange chromatography using the pulsed amperometric detection method of Johnson and Polta (11) and the modification of Hardy, Townsend, and Lee (12). The system used for monosaccharide analysis consisted of a Dionex BioLC Gradient Pump Module with a Model PAD 2 detector (Dionex Co., Houston, TX). The Dionex Eluent Degas Module was used to degas and pressurize the eluting buffers with helium. Monosaccharides were separated on a Dionex Carbopack AS-6 pellicular anion exchange resin column, equipped with an AG-6 guard column and operated at a flow rate of 1 ml/min. Monosaccharide standard solutions were made daily or stored at −30°C until immediately before use. The neutral monosaccharide separation was carried out at an isocratic NaOH concentration (22 mM) for 15 min followed by a 10-min column washing with 200 mM NaOH then 15-min re-equilibration with starting sodium hydroxide solution. Sialic acid analysis was performed with a solution of 8 mM NaOH and 80 mM $CH_3COONa$ for 7 min followed by column regeneration with 200 mM NaOH and re-equilibration. The separated monosaccharides were detected with a gold working electrode using 300 mM NaOH post-column effluent. The following pulse potentials and durations were used for carbohydrate analysis: $E_1$ = 0.10 V ($t_1$ = 360 ms); $E_2$ = 0.60 V ($t_2$ = 120 ms);

$E_3$ = −0.76 V ($t_3$ = 420 ms). The resulting chromatographic data were integrated and plotted using a Shimadzu C-R3A Chromopac integrator (Shimadzu, Kyoto, Japan).

### Cell culture

Subendothelial cells were isolated from grossly normal intima by dispersion of human aortic tissue with 0.15% collagenase (13). Cells were suspended in growth medium containing Medium 199, 10% fetal calf serum, 2 mM L-glutamine, and antibiotics. Cells were seeded into 24-well tissue culture plates at a density of 2–4 × $10^4$ cells per $cm^2$ of growth area. The cells were cultured at 37°C in a humidified $CO_2$ incubator (95% air/5% $CO_2$). The primary cultures contained a mixed cell population made up primarily (95%) of typical and modified smooth muscle cells as defined by their ultrastructural and immunofluorescent features. The medium was changed daily. After 7 days in primary culture, cells were incubated for 6 h in medium containing LDL (100 μg protein/ml) and 10% lipoprotein-deficient serum. Serum was prepared from the blood of healthy donors by ultracentrifugation at d 1.250 g/ml (8). Culture medium and lipoprotein preparations were filtered (0.22 μm pore size) immediately before adding to cell cultures. After incubation, cells were rinsed, cellular lipids were extracted with n-hexane–isopropanol 3:2 (v/v) according to Hara and Radin (14) and total cholesterol content was determined by an enzymatic method described earlier (6). Cellular protein content was determined by the method of Lowry et al. (15).

### Statistical analysis

The significance of differences between group mean values was evaluated by multiple *t*-test and one-way analysis of variance using the BMDP statistical program package (16).

## RESULTS

### Carbohydrate analysis

**Fig. 1A** shows the chromatographic separation of a standard mixture of monosaccharides on a Dionex system. Individual peaks of monosaccharides were completely resolved. In serial experiments, the difference in retention times for a single sugar was not greater than 1%. In the range of 25–1000 pmol, there was a linear relationship between peak area and carbohydrate concentration for the eight sugars measured.

In order to analyze neutral saccharides, the protein and lipid fractions were hydrolyzed with 2 N TFA at 100°C. This treatment does not degrade fucose, galactose, glucose, mannose, glucosamine, or galactosamine at hydrolysis times up to 6 h (**Fig. 2**). As deoxyglucose, a monosaccharide not occurring in mammalian glycoconjugates, is stable under the hydrolysis conditions used and is well resolved from other carbohydrates, it was used as an internal standard. N-acetyl galactosamine and N-acetyl glucosamine hydrolyzed to the corresponding amino su-

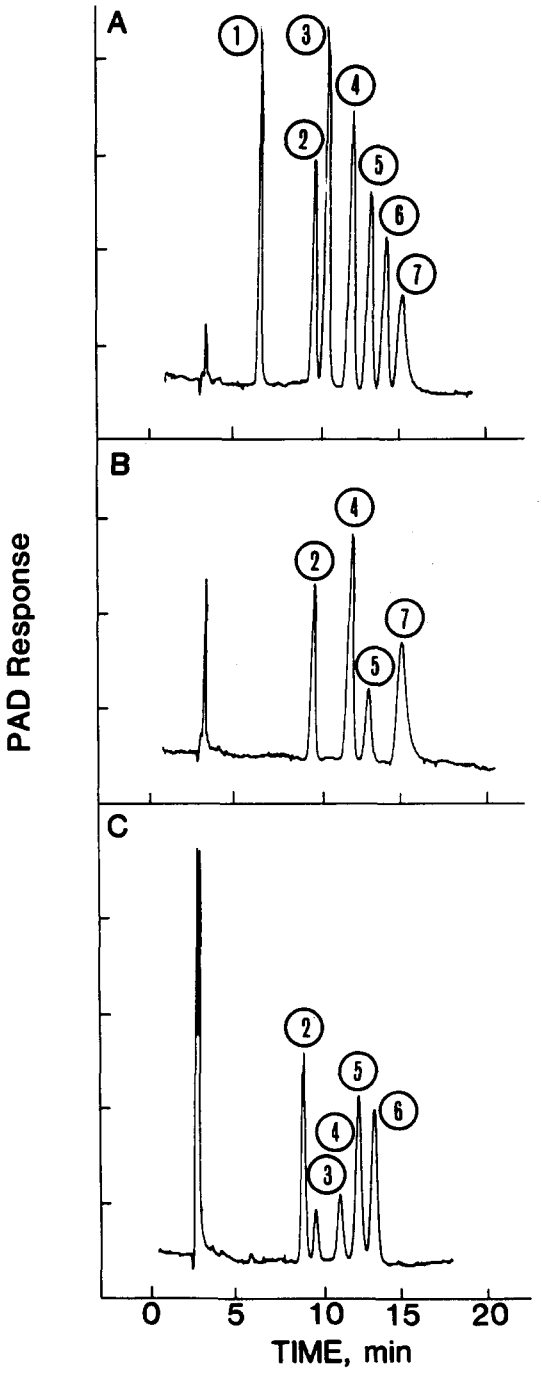


**Fig. 1.** Profile of monosaccharide anion exchange chromatography. A: Mixture of monosaccharide standards (100 pmol). B: Apolipoprotein B-100 monosaccharides. C: Monosaccharides of lipids extracted from LDL. Conditions of chromatography and pulse amperometric determination are described in Materials and Methods; 1, fucose; 2, deoxyglucose; 3, N-galactosamine; 4, glucosamine; 5, galactose; 6, glucose; 7, mannose.

gars after 1 h of hydrolysis (Fig. 2). Thus, this approach allows the determination of N-acetyl galactosamine and N-acetyl glucosamine content by measuring the amounts of galactosamine and glucosamine in the hydrolysate.

The hydrolysate of the protein fraction contains three neutral sugars: glucosamine, galactose, mannose (Fig. 1). After hydrolysis of the lipid fraction with 2 N TFA at 100°C, galactosamine and glucosamine, as well as galactose and glucose, were demonstrated to be present (Fig. 1C). Under these conditions, released monosaccharides increased up to 3 h, after which their amounts remained unchanged up to 5 h (**Fig. 3**).

Upon hydrolysis of glycoconjugates with 2 N TFA, sialic acid was completely degraded (data not shown). Therefore, the sialic acid content was determined after hydrolysis with 0.05 N TFA at 80°C. The amount of sialic acid liberated from the protein-bound glycoconjugates reached a maximum value by 20 min of hydrolysis and then remained unchanged for the next 40 min of incubation (**Fig. 4**). A similar situation was observed with lipid-bound sialic acid (Fig. 4). A strong positive correlation ($r = 0.89$, n = 20, $P < 0.05$) was established between the sialic acid content of lipoproteins determined by this method and by the colorimetric method of Svennerholm (10).

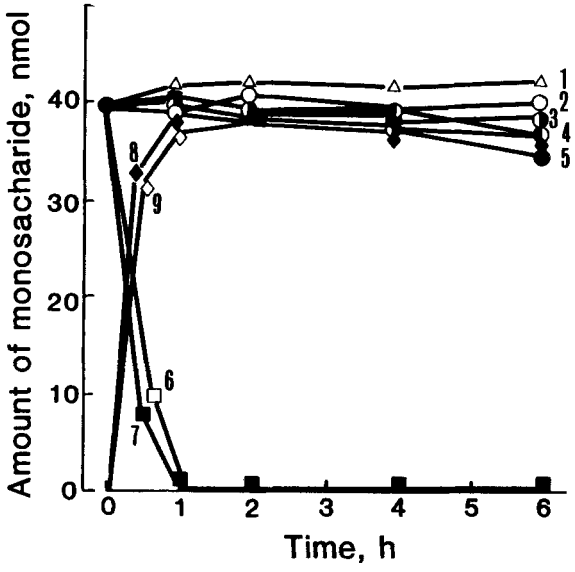


**Fig. 2.** Stability of individual neutral and cationic monosaccharides during trifluoroacetic acid hydrolysis. Forty nmol of deoxyglucose (1), fucose (2), galactose (3), mannose (4), glucose (5), N-acetyl-glucosamine (6), and N-acetyl-galactosamine (7), were incubated in 2 M TFA at 100°C for indicated times and analyzed as described in Materials and Methods. N-galactosamine and N-glucosamine were used as external standards for determination of N-galactosamine (8) and N-glucosamine (9) that appeared during hydrolysis of the corresponding acetylated amino sugars.

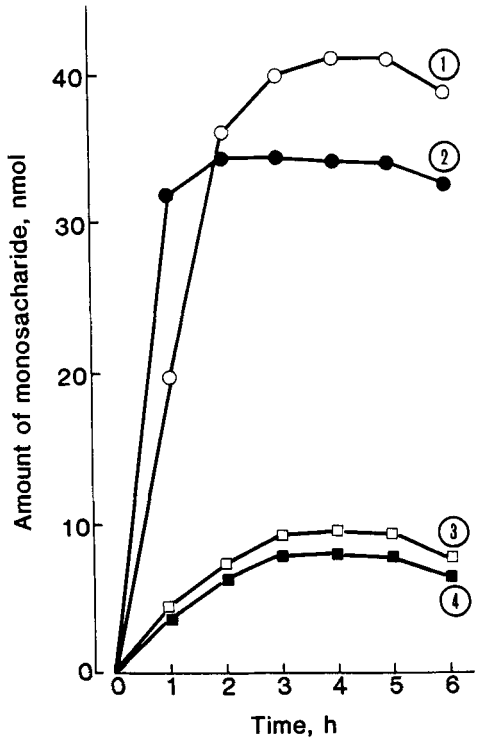


**Fig. 3.** Amounts of neutral monosaccharides in hydrolysates of lipid extracts of LDL obtained from a healthy subject. Lipid samples (1 mg LDL protein) were hydrolyzed in 2 M TFA at 100°C for indicated times; 1, glucose; 2, galactose; 3, N-glucosamine; 4, N-galactosamine.

### Effect of LDL preparations on cholesterol accumulation in cultured cells of uninvolved human aortic intima

The monosaccharide content was determined in LDL preparations of 8 healthy subjects and 8 patients with coronary atherosclerosis, and in LDL isolated from pooled plasma of 10 healthy subjects and from 10 patients. Data presented in **Table 1** show how the cholesterol content of cells cultured from uninvolved human aortic intima was altered under the action of these LDL preparations. The total LDL prepared from either individual or pooled plasma of healthy subjects caused relatively minor accumulation of intracellular cholesterol content (2–17%). In contrast, total LDL from CHD patients induced 78–286% increase in cholesterol content of cultured cells (Table 1).

We have also examined the effect of SAR-LDL and SAP-LDL, separated by affinity chromatography over $RCA_{120}$-agarose, on intracellular lipid accumulation. SAR-LDL of healthy subjects and CHD patients caused respective increases in intracellular cholesterol content of 2–12% and 7–21%, whereas SAP-LDL stimulated respective increases of 50–124% and 156–307% intracellular ac-

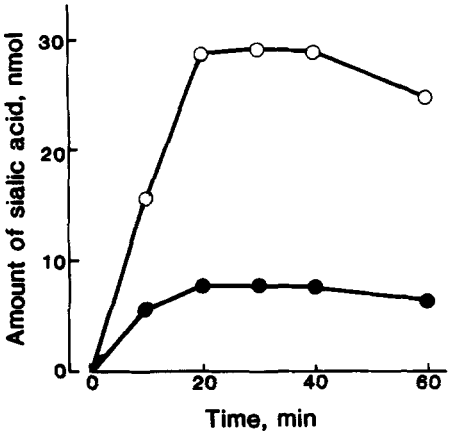


**Fig. 4.** Sialic acid content in hydrolysates of protein (○) and lipids (●) from LDL of healthy subjects. Protein and lipid preparations were hydrolyzed in 0.05 M TFA at 80°C for the indicated times and sialic acid content was determined by pulse amperometric detection as described in Materials and Methods.

cumulation of cholesterol. Thus, it was only the SAP-LDL fraction that exhibited a significant atherogenic potential as judged by lipid accumulation in cultured human aortic cells.

### Carbohydrate content of protein-bound LDL glycoconjugates

Glycoconjugates of apoB-100 from individual total LDL preparations contained glucosamine, galactose, mannose, and sialic acid (**Table 2**). The mean contents of glucosamine, galactose, and mannose in the protein-bound glycoconjugates of total LDL of CHD patients (56.8, 27.1, 73.3 nmol/mg) and of healthy subjects (59.0, 28.6, 73.8 nmol/mg) were similar. In contrast, the mean content of protein-bound sialic acid of CHD patients' LDL was 17.7 nmol/mg, 1.6-fold lower than that of healthy subjects' LDL (29.0 nmol/mg) (Table 2). The sialic acid content of pooled LDL samples was similar if not identical to the mean value for eight individual preparations.

In healthy subjects, the mean sialic acid content of SAP-LDL apolipoprotein (21.9 nmol/mg) was 23% lower than that of SAR-LDL (29.1 nmol/mg) (**Table 3**). In CHD patients, the sialic acid content of apoprotein in SAP-LDL (11.3 nmol/mg) was 54% lower than that of SAR-LDL (24.0 nmol/mg) (Table 3). Hence, the mean sialic acid content in the protein-bound glycoconjugates of SAP-LDL is considerably lower than that of SAR-LDL, regardless of whether the lipoprotein originates from a healthy subject or patient with CHD.

In healthy subjects, the mean contents of the protein-bound glucosamine, galactose, and mannose in SAR-LDL (58.8, 28.7, 71.8 nmol/mg) and SAP-LDL (58.2, 28.9, 72.0 nmol/mg) were essentially the same (Table 3). The apolipoprotein of SAR-LDL and SAP-LDL from CHD patients had the same glucosamine content (59.4 vs. 59.0 nmol/mg), values which were similar to those of the corresponding LDL from healthy subjects (58.8 vs. 58.2 nmol/mg). The mean galactose content of CHD patients' SAR-LDL and SAP-LDL differed modestly (31.2 vs. 24.7 nmol/mg), while the mannose content differed substantially (77.1 vs. 61.1 nmol/mg) (Table 3).

### Carbohydrate content of lipid-bound LDL conjugates

The carbohydrate composition of the lipid moiety differed significantly from that of protein-bound saccharide chains by the absence of mannose and the presence of galactosamine and glucose (Fig. 1, **Table 4**). Some preparations contained traces of fucose. For example, in healthy subjects the mean contents of lipid-bound glucosamine and galactose (8.5 and 42.5 nmol/mg) (Table 4) were quite different from that found in the protein moiety (59.0 and 28.6 nmol/mg) (Table 2). In the LDL lipids, the mean sialic acid level (7.7 nmol/mg) was ~3.5-fold lower than in the apoprotein (29.0 nmol/mg).

TABLE 1. Effect of LDL on cholesterol level in cells cultured from uninvolved human aortic intima

| Number | LDL Preparation | Total Cholesterol Accumulation | |
|---|---|---|---|
| | | Healthy Subjects | CHD Patients |
| | | *% above control* | |
| 1 | Total | 6 ± 6 | 126 ± 15[a] |
| 2 | Total | 12 ± 5 | 107 ± 14[a] |
| 3 | Total | 14 ± 7 | 78 ± 9[a] |
| 4 | Total | 2 ± 7 | 85 ± 6[a] |
| 5 | Total | 7 ± 8 | 102 ± 4[a] |
| | SAR-LDL | 7 ± 5 | 14 ± 6 |
| | SAP-LDL | 50 ± 6[a] | 198 ± 14[a] |
| 6 | Total | 8 ± 4 | 108 ± 7[a] |
| | SAR-LDL | 2 ± 5 | 17 ± 11 |
| | SAP-LDL | 86 ± 4[a] | 156 ± 14[a] |
| 7 | Total | 10 ± 9 | 215 ± 21[a] |
| | SAR-LDL | 12 ± 6 | 21 ± 12 |
| | SAP-LDL | 68 ± 10[a] | 307 ± 32[a] |
| 8 | Total | 13 ± 10 | 286 ± 20[a] |
| | SAR-LDL | 9 ± 6 | 20 ± 16 |
| | SAP-LDL | 73 ± 5[a] | 215 ± 21[a] |
| Pool | Total | 17 ± 5 | 124 ± 10[a] |
| | SAR-LDL | 8 ± 6 | 7 ± 5 |
| | SAP-LDL | 124 ± 21[a] | 215 ± 21[a] |

Arterial cells were incubated for 24 h in Medium 199 containing 10% lipoprotein-deficient serum (LDS) and freshly filtered (0.22 μm pore diameter) lipoprotein preparations at a concentration of 100 μg protein/ml. Control cells were incubated in Medium 199 containing 10% LDS. The data are the means of four determinations ± SEM.

[a] Significant difference from the control, $P < 0.05$.

TABLE 2. Carbohydrate content of total LDL glycoprotein isolated from healthy subjects and CHD patients

| Subject | Carbohydrate Content | | | |
|---|---|---|---|---|
| | N-Glu | Gal | Man | S.A. |
| | *nmol/mg protein* | | | |
| Healthy subjects | | | | |
| 1 | 55.9 ± 0.7 | 26.2 ± 0.5 | 70.9 ± 0.2 | 26.2 ± 0.6 |
| 2 | 52.3 ± 1.8 | 23.1 ± 2.1 | 70.1 ± 0.5 | 27.8 ± 0.7 |
| 3 | 60.8 ± 1.1 | 30.5 ± 2.0 | 66.9 ± 1.2 | 31.2 ± 1.0 |
| 4 | 67.4 ± 1.0 | 29.8 ± 0.7 | 85.6 ± 1.1 | 32.8 ± 1.5 |
| 5 | 65.2 ± 1.8 | 31.4 ± 0.6 | 74.3 ± 1.6 | 31.6 ± 0.5 |
| 6 | 58.5 ± 0.8 | 28.1 ± 0.9 | 78.1 ± 1.1 | 25.9 ± 0.3 |
| 7 | 53.0 ± 0.8 | 27.8 ± 0.8 | 66.6 ± 3.5 | 25.9 ± 0.7 |
| 8 | 59.3 ± 2.5 | 31.8 ± 0.9 | 77.9 ± 1.8 | 30.2 ± 0.4 |
| Mean ± SD | 59.0 ± 5.0 | 28.6 ± 2.7 | 73.8 ± 6.1 | 29.0 ± 2.6 |
| ± SEM | ± 1.9 | ± 1.0 | ± 2.3 | ± 1.0 |
| Pool | 60.6 ± 1.1 | 28.3 ± 1.5 | 78.8 ± 1.8 | 26.5 ± 0.5 |
| CHD patients | | | | |
| 1 | 54.8 ± 1.3 | 27.2 ± 1.0 | 67.1 ± 1.0 | 17.2 ± 0.7 |
| 2 | 55.7 ± 1.5 | 24.6 ± 0.7 | 63.6 ± 1.5 | 13.5 ± 0.6 |
| 3 | 60.4 ± 1.5 | 28.7 ± 0.5 | 82.9 ± 2.2 | 20.7 ± 0.9 |
| 4 | 63.1 ± 1.5 | 34.6 ± 0.5 | 87.5 ± 1.2 | 21.1 ± 0.6 |
| 5 | 59.0 ± 0.9 | 29.6 ± 1.1 | 75.3 ± 1.5 | 20.8 ± 0.9 |
| 6 | 52.9 ± 1.4 | 27.4 ± 1.2 | 83.2 ± 1.8 | 20.2 ± 0.9 |
| 7 | 52.6 ± 2.1 | 22.9 ± 1.4 | 65.6 ± 0.9 | 16.1 ± 1.0 |
| 8 | 56.3 ± 0.8 | 22.0 ± 2.1 | 61.2 ± 1.6 | 12.3 ± 0.9 |
| Mean ± SD | 56.8 ± 3.5 | 27.1 ± 3.8 | 73.3 ± 9.6 | 17.7 ± 3.3[a] |
| ± SEM | ± 1.3 | 2.3 | ± 3.6 | ± 1.2 |
| Pool | 61.5 ± 0.5 | 27.7 ± 1.0 | 77.2 ± 1.0 | 17.5 ± 0.5[a] |

Data are the mean of three determinations. N-Glu, N-acetyl glucosamine; Gal, galactose; Man, mannose; S.A., sialic acid.

[a]Significant difference from healthy subjects, $P < 0.05$.

The content of all neutral sugars (galactosamine, glucosamine, galactose, and glucose) in lipids of LDL from patients with coronary atherosclerosis were 1.5- to 2-fold lower than that in LDL from healthy subjects (Table 4). The mean sialic acid level in the lipid fraction of CHD patients' LDL (4.0 nmol/mg) was also lower than that of healthy subjects' LDL (7.7 nmol/mg). The levels of all lipid-bound monosaccharides in SAP-LDL of healthy subjects were 47–75% of those in SAR-LDL (**Table 5**). In CHD patients, lipid-bound sugars in SAP-LDL were about 34–52% of those in SAR-LDL.

## DISCUSSION

The carbohydrate content of glycoconjugates in various total human LDL preparations has been examined by several researchers. Apolipoprotein carbohydrate content ranged widely from 137 to 499 nmol/mg protein (17–29) (**Table 6**). In the present study, the carbohydrate content of various apoLDL preparations extended from 175 to 191 nmol/mg protein. The widely ranging content of individual sugars in LDL is probably a reflection of different dietary regimens, postprandial conditions, and general states of health or disease (19). Specific conditions might favor the formation of a particular glycosylated form of LDL. In the present study, the population of healthy subjects had apoLDL that, on average, was 11 nmol/mg richer in sialic acid than the apoLDL from CHD patients (Table 2). The carbohydrate composition of an individual's total apoLDL is determined by the proportion of differently glycosylated apoLDL subpopulations. Ten such species have been separated by isoelectric focusing of Nonidet P-40 delipidated apoLDL (30). In the present study we have used lectin affinity chromatography to separate two subpopulations of LDL that differ primarily in their content of sialic acid; in CHD patients these two subpopulations also differ in their content of galactose and mannose (Table 3). The glucosamine, galactose, mannose, and sialic acid were present at a molar ratio of about 2:1:2.5:1, which is consistent with the results reported by others (21–22, 24).

Protein-bound LDL glycoconjugates are represented by two forms of chains: bi-antenary sialylated chains and

TABLE 3. Carbohydrate content of glycoprotein in sialic acid-rich (SAR) and sialic acid-poor (SAP) LDL subfractions

| Subject | Carbohydrate Content | | | |
|---|---|---|---|---|
| | N-Glu | Gal | Man | S.A. |
| | *nmol/mg LDL protein* | | | |
| Healthy subjects | | | | |
| Subject 5 | | | | |
| SAR-LDL | 66.8 ± 2.3 | 31.7 ± 0.9 | 74.4 ± 4.2 | 30.9 ± 0.9 |
| SAP-LDL | 63.6 ± 3.1 | 30.8 ± 1.2 | 75.0 ± 5.5 | 23.0 ± 1.2[a] |
| Subject 6 | | | | |
| SAR-LDL | 57.8 ± 2.4 | 26.5 ± 0.5 | 74.9 ± 6.3 | 26.1 ± 0.3 |
| SAP-LDL | 60.2 ± 3.3 | 27.5 ± 1.7 | 74.0 ± 7.5 | 22.0 ± 1.1[a] |
| Subject 7 | | | | |
| SAR-LDL | 53.0 ± 1.7 | 27.5 ± 1.7 | 62.6 ± 7.0 | 26.2 ± 0.5 |
| SAP-LDL | 52.5 ± 2.2 | 28.9 ± 1.8 | 65.1 ± 2.3 | 18.9 ± 0.4[a] |
| Subject 8 | | | | |
| SAR-LDL | 57.5 ± 1.4 | 29.2 ± 2.2 | 75.1 ± 2.9 | 33.1 ± 1.3 |
| SAP-LDL | 56.5 ± 2.7 | 28.5 ± 1.9 | 74.0 ± 3.7 | 23.6 ± 1.4[a] |
| Mean ± SD ± SEM | | | | |
| SAR-LDL | 58.8 ± 5.0 ± 2.9 | 28.7 ± 2.0 ± 1.1 | 71.8 ± 5.3 ± 3.1 | 29.1 ± 3.0 ± 1.7 |
| SAP-LDL | 58.2 ± 4.1 ± 2.4 | 28.9 ± 1.2 ± 0.7 | 72.0 ± 4.0 ± 2.3 | 21.9 ± 1.8[a] ± 1.0 |
| Pool | | | | |
| SAR-LDL | 59.9 ± 2.3 | 28.5 ± 0.6 | 75.9 ± 2.3 | 26.8 ± 1.2 |
| SAP-LDL | 62.6 ± 1.5 | 28.0 ± 1.1 | 77.0 ± 5.0 | 22.9 ± 0.4[a] |
| CHD patients | | | | |
| Patient 5 | | | | |
| SAR-LDL | 65.6 ± 2.8 | 33.7 ± 0.6 | 75.5 ± 2.0 | 25.5 ± 1.0 |
| SAP-LDL | 67.2 ± 1.5 | 31.7 ± 0.5 | 74.9 ± 0.9 | 15.2 ± 1.0[a] |
| Patient 6 | | | | |
| SAR-LDL | 60.0 ± 1.6 | 27.0 ± 1.2 | 81.7 ± 2.5 | 26.9 ± 0.8 |
| SAP-LDL | 58.7 ± 2.3 | 25.3 ± 1.4 | 77.2 ± 3.8 | 15.3 ± 0.2 |
| Patient 7 | | | | |
| SAR-LDL | 54.9 ± 2.1 | 29.4 ± 0.5 | 68.0 ± 1.8 | 25.6 ± 0.6 |
| SAP-LDL | 52.1 ± 1.1 | 20.5 ± 0.5[a] | 53.7 ± 1.6[a] | 7.6 ± 0.2[a] |
| Patient 8 | | | | |
| SAR-LDL | 57.3 ± 2.3 | 34.7 ± 1.3 | 83.1 ± 1.5 | 17.9 ± 0.2 |
| SAP-LDL | 58.3 ± 1.5 | 21.4 ± 0.8[a] | 38.8 ± 4.7[a] | 7.1 ± 0.1[a] |
| Mean ± SD ± SEM | | | | |
| SAR-LDL | 59.4 ± 4.0 ± 2.3 | 31.2 ± 3.1 ± 1.8 | 77.1 ± 6.0 ± 3.4 | 24.0 ± 3.5 ± 2.0 |
| SAP-LDL | 59.0 ± 5.4 ± 3.1 | 24.7 ± 4.4 ± 2.5 | 61.1 ± 15.8 ± 9.1 | 11.3 ± 4.0[a] ± 2.3 |
| Pool | | | | |
| SAR-LDL | 63.4 ± 1.5 | 26.5 ± 1.1 | 85.0 ± 2.2 | 26.8 ± 0.8 |
| SAP-LDL | 62.1 ± 2.4 | 25.0 ± 1.3 | 83.6 ± 2.7 | 11.6 ± 0.2[a] |

Fractions of SAR-LDL and SAP-LDL were isolated by affinity chromatography as described in Materials and Methods. N-Glu, N-acetyl glucosamine; Gal, galactose; Man, mannose; S.A., sialic acid.

[a] Significant difference from SAR-LDL, $P < 0.05$.

TABLE 4. Carbohydrate content of glycolipids from unfractionated LDL obtained from healthy subjects and CHD patients

| Subject | N-Gal | Carbohydrate Content | | | |
|---|---|---|---|---|---|
| | | N-Glu | Gal | Glu | S.A. |
| | | *nmol/mg protein* | | | |
| Healthy subjects | | | | | |
| 1 | 5.2 ± 0.1 | 6.0 ± 0.2 | 42.9 ± 1.3 | 44.2 ± 1.0 | 7.4 ± 0.1 |
| 2 | 6.2 ± 0.1 | 7.8 ± 0.1 | 36.7 ± 2.5 | 39.7 ± 2.5 | 9.8 ± 0.5 |
| 3 | 5.7 ± 0.3 | 7.8 ± 0.1 | 43.7 ± 2.2 | 50.2 ± 0.4 | 7.4 ± 0.9 |
| 4 | 9.5 ± 0.3 | 11.5 ± 0.6 | 60.2 ± 1.6 | 61.2 ± 0.8 | 8.4 ± 0.1 |
| 5 | 5.3 ± 0.3 | 8.5 ± 0.6 | 32.6 ± 0.6 | 35.6 ± 1.5 | 6.1 ± 0.1 |
| 6 | 5.9 ± 0.4 | 8.6 ± 0.7 | 44.7 ± 1.5 | 51.2 ± 1.6 | 7.3 ± 0.1 |
| 7 | 5.5 ± 0.2 | 7.6 ± 0.2 | 36.1 ± 0.4 | 39.8 ± 2.8 | 6.8 ± 0.2 |
| 8 | 6.3 ± 0.8 | 10.0 ± 0.4 | 43.2 ± 2.9 | 50.2 ± 3.5 | 8.1 ± 0.2 |
| Mean ± SD | 6.2 ± 1.3 | 8.5 ± 1.6 | 42.5 ± 7.8 | 46.5 ± 7.8 | 7.7 ± 1.0 |
| ± SEM | ± 0.5 | ± 0.6 | ± 3.0 | ± 2.9 | ± 0.4 |
| Pool | 5.9 ± 0.2 | 7.4 ± 0.1 | 40.8 ± 0.3 | 42.6 ± 1.4 | 6.3 ± 0.1 |
| CHD patients | | | | | |
| 1 | 2.9 ± 0.1 | 4.0 ± 0.2 | 25.1 ± 1.0 | 27.8 ± 2.0 | 4.2 ± 0.1 |
| 2 | 2.9 ± 0.1 | 7.2 ± 0.2 | 26.4 ± 0.5 | 26.4 ± 0.4 | 4.9 ± 0.1 |
| 3 | 2.6 ± 0.1 | 5.7 ± 0.3 | 35.5 ± 1.8 | 36.4 ± 2.0 | 4.7 ± 0.2 |
| 4 | 2.5 ± 0.1 | 5.5 ± 0.3 | 35.5 ± 1.8 | 36.4 ± 2.0 | 4.1 ± 0.1 |
| 5 | 2.7 ± 0.1 | 3.0 ± 0.2 | 30.6 ± 0.4 | 38.1 ± 1.1 | 5.8 ± 0.1 |
| 6 | 3.0 ± 0.1 | 4.2 ± 0.1 | 29.4 ± 1.1 | 28.8 ± 1.1 | 4.2 ± 0.2 |
| 7 | 1.8 ± 0.1 | 1.8 ± 0.1 | 22.8 ± 1.1 | 25.1 ± 1.9 | 2.3 ± 0.1 |
| 8 | 2.0 ± 0.1 | 2.1 ± 0.1 | 23.6 ± 1.7 | 26.1 ± 1.7 | 1.9 ± 0.1 |
| Mean ± SD | 2.6 ± 0.4[a] | 4.2 ± 1.8[a] | 28.6 ± 4.7[a] | 30.6 ± 5.0[a] | 4.0 ± 1.2[a] |
| ± SEM | ± 0.1 | ± 0.7 | ± 1.8 | ± 1.9 | ± 0.5 |
| Pool | 3.2 ± 0.1[a] | 3.9 ± 0.2[a] | 28.3 ± 1.0[a] | 32.1 ± 1.5[a] | 2.7 ± 0.1[a] |

Data are the mean of three determinations. N-Gal, N-acetyl galactosamine; N-Glu, N-acetyl glucosamine; Gal, galactose; Glu, glucose; S.A., Sialic acid.
[a]Significant difference from healthy subjects, $P < 0.05$.

mannose-rich chains (23, 31, 32). Using the assumption that 13–16 asparagine residues are glycosylated, Taniguchi et al. (32) have calculated that an apoB-100 molecule contains 5–6 high mannose and 8–10 bi-antenary sialylated chains. These calculations and the ratios of various monosaccharides present in high mannose and bi-antenary chains suggest the following stoichiometry for monosaccharides on apoB-100: 42–52 mol glucosamine, 14–18 mol galactose, 60–73 mol mannose, and 12–14 mol of sialic acid per mol apoB-100. Our measurements indicate that apoB from healthy subjects contains about 14 mol of galactose and 15 mol of sialic acid. These measurements are consistent with the calculations of Taniguchi et al. (32). However, our measured levels of glucosamine and mannose were 30 and 38 mol/mol apoB, respectively, about 65% and 58% of the values calculated by Taniguchi et al. Other laboratories have also measured glucosamine and mannose levels lower than those calculated by Taniguchi et al. (23, 24).

In healthy subjects, the mean sialic acid content of SAP-LDL (21.9 nmol/mg) was 25% lower than that of SAR-LDL (29.1 nmol/mg). In patients with coronary atherosclerosis, this difference was much greater (53%). ApoB of SAR-LDL and SAP-LDL from healthy subjects had virtually identical content of neutral sugars (Table 3). In CHD patients the neutral sugar content of SAR-LDL and SAP-LDL prepared from pooled plasma and from two patients (#5 and 6) were similar. However, in the SAP-LDL of two other patients (#7 and 8), the levels of protein-bound mannose and galactose were significantly lower than in their SAR-LDL. These data indicate that in some cases SAP-LDL from patients can have a neutral sugar as well as acidic sugar content lower than that of SAR-LDL.

Carbohydrate constituents of lipid-bound glycoconjugates in human LDL consist of glucosamine, galactosamine, galactose, glucose, and sialic acid. In CHD patients, the lipid fraction of LDL has a lower content of individual monosaccharides than does the corresponding fraction from healthy subjects (Table 4). In healthy subjects, the neutral sugar content of SAP-LDL is 67–75% of that in SAR-LDL. This difference is even larger in CHD patients. Similarly, the acidic sugar content of SAP-LDL is only 34–47% of that in SAR-LDL.

TABLE 5. Carbohydrate content of glycolipids in sialic acid-rich (SAR) and -poor (SAP) subfractions of LDL obtained from healthy subjects and CHD patients

| Subject | Carbohydrate Content | | | | |
|---|---|---|---|---|---|
| | N-Gal | N-Glu | Gal | Glu | S.A. |
| | *nmol/mg protein* | | | | |
| Healthy subjects | | | | | |
| Subject 5 | | | | | |
| SAR-LDL | 5.5 ± 0.1 | 8.6 ± 0.2 | 34.6 ± 2.3 | 38.2 ± 1.5 | 6.2 ± 0.2 |
| SAP-LDL | 3.5 ± 0.3[a] | 7.4 ± 0.1[a] | 28.4 ± 0.6[a] | 30.8 ± 0.8[a] | 3.9 ± 0.1[a] |
| Subject 6 | | | | | |
| SAR-LDL | 5.9 ± 0.1 | 8.6 ± 0.2 | 45.5 ± 0.4 | 51.4 ± 1.8 | 7.4 ± 0.2 |
| SAP-LDL | 4.7 ± 0.1[a] | 6.8 ± 0.3[a] | 34.8 ± 1.2[a] | 41.1 ± 0.6[a] | 3.0 ± 0.1[a] |
| Subject 7 | | | | | |
| SAR-LDL | 5.6 ± 0.1 | 7.7 ± 0.1 | 34.9 ± 0.8 | 40.9 ± 1.3 | 6.9 ± 0.1 |
| SAP-LDL | 4.1 ± 0.1[a] | 6.7 ± 0.1[a] | 27.3 ± 0.9[a] | 25.0 ± 3.1[a] | 3.7 ± 0.3[a] |
| Subject 8 | | | | | |
| SAR-LDL | 6.6 ± 0.2 | 9.0 ± 0.2 | 48.4 ± 1.4 | 53.8 ± 1.0 | 8.2 ± 0.1 |
| SAP-LDL | 3.8 ± 0.4[a] | 4.3 ± 0.1[a] | 27.9 ± 0.7[a] | 28.4 ± 0.7[a] | 3.1 ± 0.2[a] |
| Mean ± SD ± SEM | | | | | |
| SAR-LDL | 5.9 ± 0.4<br>± 0.2 | 8.5 ± 0.5<br>± 0.3 | 40.8 ± 6.2<br>± 3.6 | 46.1 ± 6.6<br>± 3.8 | 7.2 ± 0.7<br>± 0.6 |
| SAP-LDL | 4.0 ± 0.4<br>± 0.3 | 6.3 ± 1.2<br>± 0.7 | 29.6 ± 3.0<br>± 1.7 | 31.3 ± 6.0<br>± 3.5 | 3.4 ± 0.4[a]<br>± 0.2 |
| Pool | | | | | |
| SAR-LDL | 5.9 ± 0.2 | 7.6 ± 0.1 | 42.6 ± 0.6 | 44.8 ± 2.7 | 6.5 ± 0.2 |
| SAP-LDL | 5.0 ± 0.2[a] | 7.0 ± 0.1[a] | 34.6 ± 1.3[a] | 35.2 ± 1.1[a] | 2.6 ± 0.3[a] |
| CHD patients | | | | | |
| Patient 5 | | | | | |
| SAR-LDL | 3.6 ± 0.1 | 4.0 ± 0.1 | 39.5 ± 0.6 | 50.6 ± 1.4 | 7.4 ± 0.3 |
| SAP-LDL | 1.5 ± 0.1[a] | 1.9 ± 0.1[a] | 21.2 ± 0.1[a] | 18.8 ± 0.9[a] | 3.3 ± 0.2[a] |
| Patient 6 | | | | | |
| SAR-LDL | 4.0 ± 0.1 | 6.4 ± 0.1 | 35.4 ± 0.6 | 41.6 ± 0.2 | 6.8 ± 0.1 |
| SAP-LDL | 2.1 ± 0.1[a] | 2.6 ± 0.2[a] | 19.2 ± 0.7[a] | 18.8 ± 0.7[a] | 2.0 ± 0.1[a] |
| Patient 7 | | | | | |
| SAR-LDL | 2.5 ± 0.1 | 2.5 ± 0.3 | 26.0 ± 0.7 | 36.8 ± 2.2 | 3.4 ± 0.2 |
| SAP-LDL | 1.0 ± 0.1[a] | 1.0 ± 0.1[a] | 15.4 ± 0.4[a] | 18.0 ± 2.2[a] | 1.5 ± 0.1[a] |
| Patient 8 | | | | | |
| SAR-LDL | 2.8 ± 0.1 | 3.0 ± 0.3 | 34.5 ± 1.5 | 35.4 ± 1.4 | 3.6 ± 0.3 |
| SAP-LDL | 1.4 ± 0.1[a] | 1.4 ± 0.1[a] | 14.2 ± 1.0[a] | 17.1 ± 0.4[a] | 0.5 ± 0.1[a] |
| Mean ± SD ± SEM | | | | | |
| SAR-LDL | 3.2 ± 0.6<br>± 0.3 | 4.0 ± 1.5<br>± 0.9 | 33.8 ± 4.9<br>± 2.8 | 41.1 ± 5.9<br>± 3.4 | 5.3 ± 1.8<br>± 1.0 |
| SAP-LDL | 1.5 ± 0.4<br>± 0.2 | 1.7 ± 0.6<br>± 0.3 | 17.5 ± 2.8<br>± 1.6 | 18.2 ± 0.7<br>± 0.4 | 1.8 ± 1.0<br>± 0.6 |
| Pool | | | | | |
| SAR-LDL | 4.5 ± 0.2 | 5.2 ± 0.1 | 35.0 ± 0.7 | 40.6 ± 1.8 | 3.6 ± 0.1 |
| SAP-LDL | 2.1 ± 0.1[a] | 2.4 ± 0.1[a] | 19.9 ± 0.7[a] | 18.7 ± 1.1[a] | 1.0 ± 0.1[a] |

Data are the mean of three determinations. N-Gal, N-acetyl galactosamine; N-Glu, N-acetyl glucosamine; Gal, galactose; Glu, glucose; S.A., sialic acid.

[a]Significant difference from SAR-LDL, $P < 0.05$.

TABLE 6. Carbohydrate content of apolipoprotein from human LDL

| Glucosamine | Galactose | Mannose | Sialic Acid | Total | Reference |
|---|---|---|---|---|---|
| | | *nmol/mg apolipoprotein* | | | |
| 59 | 29 | 74 | 29 | 191 | Table 2 |
| 57 | 27 | 73 | 18 | 175 | Table 2 |
| 37 | 27 | 46 | 27 | 137 | 22 |
| 100 | 50 | 150 | 25 | 325 | 24 |
| 52 | 116 | 276 | 55 | 499 | 23 |
| 50 | 100 | 205 | 19 | 374 | 26 |
| 112 | 150 | 150 | 48 | 460 | 27 |
| 82 | 40 | 68 | 38 | 228 | 19 |

It has been reported recently that there is a lower neutral sugar and sialic acid content in LDL from subjects with a predominance of small, dense LDL particles than in LDL from subjects with larger LDL particles (33). We have prepared LDL fractions containing various proportions of sialic acid and demonstrated that a fraction depleted in neutral sugars and sialic acid (SAP-LDL) is smaller and more dense than a fraction richer in these sugars (SAR-LDL) (results accepted for publication). These findings imply a relationship between carbohydrate content and the size/density of LDL particles.

There are multiple possible reasons for the observed differences in carbohydrate composition of LDL subfractions. These differences could originate from differences in the degree of LDL glycosylation in the Golgi apparatus and/or post-synthetic or post-secretory enzyme degradation of carbohydrate chains. Various glycosidases have been isolated from human blood (34–36). Although these enzymes have acidic pH optima, they may function at suboptimal levels in the healthy state, and at higher levels in disease or stress states. Although free sialic acid is not normally detected in the plasma of healthy individuals (36), desialylation of glycoconjugates may occur in certain pathological conditions. In addition, LDL with reduced carbohydrate content may enter the bloodstream after partial glycolytic degradation in the lysosomes of endothelial cells and other cell types. LDL may also lose significant carbohydrate by dissociation of glycolipids from the LDL surface and subsequent association with other lipoprotein classes. Preliminary data indicating a low phospholipid content of SAP-LDL can be regarded as indirect support for such a possibility. Clearly, further studies will be required to elucidate the mechanism(s) responsible for controlling the carbohydrate content of LDL.

The carbohydrate content of LDL may affect its catabolic route and destiny. One group has reported that uptake and degradation of LDL by cultured human arterial smooth muscle cells is controlled by the sialic content of those particles (28). However, other workers have reported that LDL freed of sialic acid and most of its glucosamine and hexoses by glycosidase digestion bind to fibroblasts with an affinity similar to that of native LDL (29). Low density lipoproteins desialylated by treatment with neuraminidase were more actively metabolized by mouse macrophages (37). In our experiments, neuraminidase treatment of LDL enhanced uptake and increased lipid accumulation in cultured human aortic intimal cells (3, 5). A similar effect was produced by sialic acid-poor LDL isolated from the plasma of patients with coronary atherosclerosis (6). Elucidating the function of changing carbohydrate composition in lipoproteins will be the focus of future studies.

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