Sialic acid content of low density lipoprotein and its relation to lipid concentrations and metabolism of low density lipoprotein and cholesterol

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Abstract **A low sialic acid content in low density lipoprotein (LDL) has been associated with atherogenicity and coronary artery disease (CAD) in many but not all studies. We investigated associations of the sialic acid-to-apolipoprotein B (apoB) ratio of LDL with lipoprotein lipid concentrations, kinetics of LDL, metabolism of cholesterol, and the presence of CAD in 98 subjects (CAD^+, n = 56; CAD^-, n = 42). The sialic acid ratios of total, dense, and very dense LDL were lower in the CAD^+ than CAD^- subjects, especially at high sialic acid ratios. The LDL sialic acid ratio was inversely associated with respective lipid and apoB concentrations and positively with lipid-to-apoB ratios of LDL. The transport rates (TRs) for total and dense LDL apoB were negatively associated with their sialic acid ratios. The sialic acid ratio of dense LDL, but not that of total LDL, was inversely correlated with serum levels of cholesterol precursor sterols, indicators of cholesterol synthesis, and positively with serum levels of plant sterols, indicators of cholesterol absorption. In addition, the TR for dense LDL was positively correlated with cholesterol synthesis. In conclusion, a low LDL sialic acid ratio was associated with CAD, high numbers of small LDL particles, and a high TR for LDL apoB, and in dense LDL also with high synthesis and low absorption of cholesterol.**—Lindbohm, N., H. Gylling, and T. A. Miettinen. **Sialic acid content of low density lipoprotein and its relation to lipid concentrations and metabolism of low density lipoprotein and cholesterol.** ***J. Lipid Res.*** **2000.** 41: **1110–1117.**

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Sialic acids are located in the terminal ends of many carbohydrate chains of glycolipids and glycoproteins, including apolipoprotein B (apoB) (1, 2). Desialylation of low density lipoprotein (LDL) has been shown to increase its binding to arterial proteoglycans (3) and its uptake into cells (4–7). In addition, a strong negative correlation has been reported between the sialic acid content of LDL and the amount of cholesterol accumulated intracellularly (7–10). However, two other studies reported no effect of the sialic acid content of LDL on its binding and degradation in cells (11, 12). LDL of mainly male patients with coronary artery disease (CAD) has been shown to be sialic acid poor compared with LDL from healthy controls in some (7–9, 13) but not all (14–16) studies. Thus, the role of the sialic acid content of LDL in atherogenesis is somewhat controversial, but as a whole, earlier studies suggest that the sialic acid content of LDL might affect its ability to cause atherogenesis.

Only a few studies of a limited number of patients have investigated the effect of the sialic acid content of LDL on its metabolism in vivo. Malmendier et al. (17) showed that desialylation increased the catabolic rate of LDL. However, in Type II diabetic patients the sialic acid-to-apoB ratio of dense LDL was positively related to its fractional catabolic rate (14), while in nondiabetic subjects (18) the ratio was not associated with LDL apoB catabolism; however, the lower the sialic acid ratio, the higher the production rate of dense LDL apoB. Associations of LDL sialic acid content with the metabolism of cholesterol have not been previously investigated.

Variable findings about the importance of LDL sialic acid content provoked us to investigate associations of the sialic acid content of total LDL and its subfractions with the presence of CAD, lipoprotein lipid concentrations, kinetics of LDL, and metabolism of cholesterol in a large group of hypercholesterolemic subjects without and with CAD.

PATIENTS AND METHODS

Patients

Ninety-eight subjects, 53 women and 45 men, were recruited for the study from the outpatient clinic of our hospital. Fifty-six subjects (CAD^+), 30 men and 26 women, were diagnosed with CAD on the basis of a history of myocardial infarction (n = 37), coronary angiography (n = 14), or exercise bicycle test (n = 5),

Abbreviations: BMI, body mass index; CAD, coronary artery disease; FCR, fractional catabolic rate; Lp[a], lipoprotein [a]; LDL, low density lipoprotein; TR, transport rate.

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while the remaining 42 subjects (CAD^-) had no symptoms or manifestations of CAD on clinical interview and examination, or as revealed by electrocardiogram (ECG). None of the subjects had diabetes mellitus or gastrointestinal, liver, or kidney disease. Three women had been receiving thyroxin treatment for hypothyroidism, and they had been euthyroid for several years. The subjects were not taking any lipid-lowering medication at the time of the study. Subjects had been counseled at least 6 months earlier to adhere to a low-fat, low-cholesterol diet, and they had done so. Forty-four subjects were taking beta-blocking agents, 6 subjects were taking thiazide diuretics, and 15 of the 53 women were receiving hormone replacement therapy. Twenty-four subjects were current smokers.

All the subjects gave informed consent to participate in the study. The study protocol was accepted by the ethics committee of our hospital.

Methods

Blood samples were drawn after a 12-h fast. Serum lipoproteins were separated by ultracentrifugation (19). After total LDL had been separated, it was further fractionated into three subfractions: light (d 1.019–1.036 g/ml), dense (d 1.037–1.055 g/ml), and very dense (d 1.056–1.063 g/ml) in a 60 Ti fixed-angle rotor (Beckman Instruments, Fullerton, CA) for 44 h at 58,000 rpm and 10°C, as described in detail previously (20). Total and free cholesterol, triglycerides, phospholipids, apoB, and lipoprotein [a] (Lp[a]) in serum and lipoproteins were analyzed with commercial kits (Boehringer Diagnostica, Mannheim, Germany; Wako Pharmaceuticals, Kyoto, Japan; Orion Diagnostica, Espoo, Finland; and Mercodia AB, Uppsala, Sweden). Sialic acid was analyzed from total LDL and its subfractions by the modified resorcinol method (21, 22), using *N*-acetylneuraminic acid (Sigma, St. Louis, MO) as the standard. In the following, the sialic acid content of LDL is expressed as its ratio to apoB and is referred to as the sialic acid ratio. To examine differences in CAD^+ and CAD^- subjects at different sialic acid levels, both CAD^+ and CAD^- subjects were divided into quartiles by the sialic acid ratios of total and dense LDL, with equal numbers of subjects in each quartile (Q_1–Q_4 from low to high sialic acid ratios).

In 82 subjects, serum noncholesterol sterols, including the cholesterol precursor sterols Δ^8-lathosterol, desmosterol, and lathosterol, the plant sterols campesterol and sitosterol, and choestanol were determined in nonsaponifiable serum extracts by gas–liquid chromatography on a 50-m long SE-30 capillary column (23). Because they are transported in serum by lipoproteins, like cholesterol, the concentrations of the noncholesterol sterols are highly dependent on serum cholesterol levels. Thus, the values are expressed as $10^2 \times$ mmol/mol of serum cholesterol, i.e., as ratios to cholesterol, to eliminate the effect of variation in cholesterol levels.

Of the total 98 subjects, 58 randomly selected subjects volunteered to participate in the lipoprotein kinetic study. First, 50 ml of fasted EDTA plasma was drawn, and total (d 1.019–1.063 g/ml) and dense (d 1.037–1.055 g/ml) LDL were separated by serial density ultracentrifugations. Total LDL apoB was iodinated with ^{131}I, and dense LDL apoB with ^{125}I, by a modification of the iodine monochloride method (24, 25). Approximately 1 mg of a mixture of the autologous labeled total and dense LDL apoB was mixed with 5% human serum albumin, filtered, and injected into each patient. Three days before the injection the subjects started to take peroral potassium iodide. The total amount of radioactivity did not exceed 60 μCi. After the injection, blood samples were collected for 14 days and counted. The die-away curves were constructed from plasma for ^{131}I-labeled LDL and ^{125}I-labeled dense LDL. Fractional catabolic rates (FCRs) for total and dense LDL apoB were determined using a two-pool model (26). Transport rates (TRs) were calculated by multiplying the FCRs by the pool size, which was the apoprotein plasma concentration multiplied by approximated plasma volume, i.e., 4.5% of body weight.

TABLE 1. Clinical characteristics in CAD^- and CAD^+ groups and in the combined group

Variable	CAD^- (n = 42)	CAD^+ (n = 56)	Combined (n = 98)
Age, years[a]	52.5 ± 1.1	55.1 ± 0.8	54.0 ± 0.7
BMI, kg/m^2[a]	27.4 ± 0.7	26.9 ± 0.6	27.1 ± 0.4
Waist-to-hip ratio[a]	0.91 ± 0.02	0.89 ± 0.01	0.90 ± 0.01
Females/males	27/15	26/30	53/45
ApoE phenotype			
3/2, 4/2	1	3	4
3/3	24	31	55
4/3, 4/4	17	22	39

[a] Values represent means ± SE.

Statistics

Groups were tested for significant differences by analysis of variance, Chi-square test, and Student's two-tailed *t* test. Correlations were analyzed by calculating the Spearmans rank-order correlation coefficient. A stepwise logistic regression analysis was carried out with the presence of CAD as the dependent variable, and LDL cholesterol and triglyceride concentrations, the LDL sialic acid ratio, and the FCR and TR for LDL apoB as the independent variables. To explain variability in the LDL sialic acid ratio, a stepwise regression analysis was used with the presence of CAD, the concentrations of cholesterol and triglycerides in LDL, and the FCR and TR for LDL apoB as the independent variables. A *P* value less than 0.05 was considered significant.

RESULTS

The sialic acid ratios of total LDL and its subfractions were similar in males and females and in smokers and nonsmokers (data not shown). In the whole study group and in the CAD^+ group, the sialic acid ratios of total LDL and its subfractions were not affected by beta-blocker treatment (39.0 ± 1.6 vs. 37.1 ± 2.1 μg/mg in total LDL in users vs. nonusers), but in total LDL of hypertensive CAD^- subjects taking beta-blockers (n = 6) it was lower than in CAD^- subjects not taking beta-blockers (n = 34)

TABLE 2. Sialic acid ratio per apoB in total LDL and its subfractions in CAD^- and CAD^+ subjects[a]

	Sialic Acid Ratio	
	CAD^- (n = 42)	CAD^+ (n = 56)
	μg/mg apoB	
Total LDL	43.7 ± 2.0	38.4 ± 1.2[b]
Light LDL	40.2 ± 3.8	38.7 ± 2.3
Dense LDL	39.9 ± 2.5	34.0 ± 1.4[b]
Very dense LDL	181.5 ± 14.8	131.7 ± 7.1[c]

[a] Values represent means ± SE.
[b] $P < 0.05$.
[c] $P < 0.01$.

TABLE 3. Sialic acid ratios per apoB in total and dense LDL of CAD$^-$ and CAD$^+$ subjects in quartiles (Q_1–Q_4)[a] divided for both groups separately according to the sialic acid ratios of total and dense LDL, respectively

	Sialic Acid Ratio	
	CAD$^-$	CAD$^+$
	μg/mg apoB	
Total LDL		
Q_1	31.0 ± 1.2	31.2 ± 0.6
Q_2	38.5 ± 0.6	34.5 ± 0.2[d]
Q_3	44.5 ± 0.9	37.4 ± 0.3[d]
Q_4	61.9 ± 3.6	50.4 ± 3.1[b]
Dense LDL		
Q_1	23.8 ± 1.8	24.8 ± 0.5
Q_2	32.5 ± 0.7	30.0 ± 0.5[c]
Q_3	40.6 ± 1.4	34.4 ± 0.4[d]
Q_4	64.1 ± 3.2	47.9 ± 2.8[d]

[a] Values represent means ± SE.
[b] $P < 0.05$.
[c] $P < 0.01$.
[d] $P < 0.001$.

(33.5 ± 3.1 μg/mg vs. 44.9 ± 2.3 μg/mg, respectively, $P < 0.01$). Use of thiazide diuretics or, among females, that of hormone replacement therapy had no effect on the LDL sialic acid ratio (data not shown).

Age, body mass index (BMI), sex, or apoE phenotype distributions were not associated with the sialic acid ratios of total LDL and its subfractions, and they were similar in the CAD$^-$ and CAD$^+$ groups (**Table 1**). The mean sialic acid ratios of total LDL and its dense and very dense subfractions were lower in CAD$^+$ than CAD$^-$ subjects (**Table 2**). When the CAD$^-$ and CAD$^+$ subjects were both divided into quartiles according to the sialic acid ratios of total and dense LDL, the ratios were markedly lower in CAD$^+$ than CAD$^-$ subjects of the three highest quartiles but not in those of the lowest quartile (**Table 3**).

In very low density lipoprotein (VLDL) and LDL, the concentrations of all the lipids, and those of cholesterol and phospholipids also in serum, were negatively associated with the sialic acid ratio of total LDL. Concentrations of apoB in serum and LDL also had strong inverse associations with the sialic acid ratio of total LDL, while the respective correlations with high density lipoprotein (HDL) lipids and serum Lp[a] were insignificant (**Table 4**). Similar negative correlations with lipid and apoB concentrations were seen in the light and dense LDL subfractions, but not in the very dense fraction (**Table 5**, **Fig. 1** for dense LDL apoB). The lipids per apoB in total, dense, and very dense LDL were positively associated with the sialic acid ratio of LDL. CAD$^+$ subjects had a higher cholesterol level in total and light LDL than CAD$^-$ subjects, but otherwise the lipid levels of the two groups were roughly similar.

The ratios of noncholesterol sterols to cholesterol did not differ between CAD$^-$ (n = 33) and CAD$^+$ (n = 49) subjects (data not shown), and did not associate with the sialic acid ratio of total LDL. However, the sialic acid ratio of dense LDL was negatively correlated with Δ^8-lathosterol, desmosterol, and lathosterol ratios ($r = -0.389$, $P < 0.001$; $r = -0.244$, $P < 0.05$; and $r = -0.311$, $P < 0.01$, respec-

TABLE 4. Serum and lipoprotein lipid and apoB concentrations and serum Lp[a] concentration in CAD$^-$ and CAD$^+$ subjects and in all subjects combined, and their correlation coefficients with the sialic acid ratio of total LDL for the combined group

Variable	CAD$^-$[a] (n = 42)	CAD$^+$[a] (n = 56)	Combined[a] (n = 98)	Correlation Coefficient (n = 98)
Cholesterol, mmol/L				
Serum	6.13 ± 0.18	6.45 ± 0.14	6.31 ± 0.11	−0.382[d]
VLDL	0.70 ± 0.09	0.68 ± 0.07	0.69 ± 0.05	−0.245[b]
IDL	0.32 ± 0.03	0.29 ± 0.02	0.30 ± 0.02	−0.114
LDL	3.45 ± 0.12	3.98 ± 0.10[c]	3.75 ± 0.08	−0.377[d]
HDL	1.30 ± 0.05	1.18 ± 0.03	1.23 ± 0.03	0.044
Triglycerides, mmol/L				
Serum	1.85 ± 0.15	1.92 ± 0.13	1.89 ± 0.10	−0.187
VLDL	1.28 ± 0.13	1.32 ± 0.12	1.31 ± 0.09	−0.224[b]
IDL	0.12 ± 0.01	0.11 ± 0.01	0.12 ± 0.00	−0.139
LDL	0.29 ± 0.01	0.30 ± 0.01	0.29 ± 0.01	−0.296[c]
HDL	0.18 ± 0.01	0.16 ± 0.01	0.17 ± 0.00	−0.104
Phospholipids, mg/dL				
Serum	253 ± 6.5	245 ± 5.3	248 ± 4.1	−0.208[b]
VLDL	36.8 ± 3.9	36.4 ± 3.3	36.5 ± 2.5	−0.232[b]
IDL	10.2 ± 0.8	8.9 ± 0.6	9.5 ± 0.5	−0.099
LDL	88.6 ± 2.9	98.4 ± 2.5[b]	94.2 ± 1.9	−0.333[d]
HDL	102.2 ± 3.9	90.4 ± 2.3[b]	95.5 ± 2.2	0.044
Serum apoB, mg/dL	127.7 ± 7.0	136.5 ± 4.4	132.9 ± 3.7	−0.428[d]
LDL apoB, mg/dL	83.4 ± 3.8	89.3 ± 2.4	86.8 ± 2.1	−0.526[d]
Serum Lp(a), U/L	317 ± 62	213 ± 50	274 ± 31	−0.011

[a] Value represent means ± SE.
[b] $P < 0.05$.
[c] $P < 0.01$.
[d] $P < 0.001$.

TABLE 5. Lipid and apoprotein concentrations of LDL subfractions and lipid-to-apoB ratios of total LDL and its subfractions in CAD$^-$ and CAD$^+$ subjects and in the combined group, and their correlation coefficients with the sialic acid ratio of total LDL for the combined group

Variable	CAD$^-$[a] (n = 42)	CAD$^+$[a] (n= 56)	Combined[a] (n = 98)	Correlation Coefficient (n= 98)
Light LDL				
Cholesterol, mmol/L	1.54 ± 0.09	1.84 ± 0.10[b]	1.72 ± 0.07	−0.126
Triglycerides, mmol/L	0.15 ± 0.01	0.16 ± 0.01	0.15 ± 0.00	−0.274[c]
Phospholipids, mg/dL	39.7 ± 2.1	45.6 ± 2.3	43.1 ± 1.6	−0.134
ApoB, mg/dL	36.4 ± 2.2	38.6 ± 2.0	37.6 ± 1.5	−0.246[b]
Dense LDL				
Cholesterol, mmol/L	1.70 ± 0.11	1.92 ± 0.09	1.83 ± 0.07	−0.305[c]
Triglycerides, mmol/L	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.00	−0.225[b]
Phospholipids, mg/dL	42.2 ± 2.6	46.6 ± 2.0	44.7 ± 1.6	−0.283[c]
ApoB, mg/dL	42.6 ± 2.9	46.2 ± 2.1	44.7 ± 1.7	−0.372[d]
Very dense LDL				
Cholesterol, mmol/L	0.20 ± 0.02	0.22 ± 0.03	0.21 ± 0.02	0.066
Triglycerides, mmol/L	0.023 ± 0.002	0.024 ± 0.002	0.024 ± 0.001	−0.003
Phospholipids, mg/dL	6.7 ± 0.4	6.2 ± 0.6	6.4 ± 0.4	0.160
ApoB, mg/dL	4.4 ± 0.4	4.5 ± 0.3	4.5 ± 0.2	−0.144
Total LDL, per apoB				
Cholesterol	1.65 ± 0.04	1.74 ± 0.03	1.70 ± 0.02	0.268[c]
Triglycerides	0.32 ± 0.01	0.30 ± 0.01	0.31 ± 0.01	0.226[b]
Phospholipids	1.11 ± 0.03	1.11 ± 0.02	1.11 ± 0.02	0.385[d]
Light LDL, per apoB				
Cholesterol	1.86 ± 0.03	1.94 ± 0.03	1.91 ± 0.02	0.188
Triglycerides	0.42 ± 0.02	0.42 ± 0.02	0.42 ± 0.01	0.062
Phospholipids	1.21 ± 0.02	1.23 ± 0.02	1.22 ± 0.01	0.192
Dense LDL, per apoB				
Cholesterol	1.76 ± 0.04	1.73 ± 0.04	1.75 ± 0.03	0.227[b]
Triglycerides	0.28 ± 0.01	0.25 ± 0.01	0.26 ± 0.01	0.125
Phospholipids	1.10 ± 0.02	1.06 ± 0.02	1.08 ± 0.01	0.302[c]
Very dense LDL, per apoB				
Cholesterol	2.11 ± 0.10	1.89 ± 0.06	1.98 ± 0.06	0.351[d]
Triglycerides	0.57 ± 0.04	0.54 ± 0.03	0.55 ± 0.02	0.132
Phospholipids	1.96 ± 0.20	1.43 ± 0.06[b]	1.66 ± 0.10	0.395[d]

[a] Values represent means ± SE.
[b] $P < 0.05$.
[c] $P < 0.01$.
[d] $P < 0.001$.

tively) (in **Fig. 2** shown for lathosterol), and positively with campesterol, sitosterol, and cholestanol ratios (r = 0.219, $P < 0.05$; r = 0.239, $P < 0.05$; and r = 0.407, $P < 0.001$, respectively) (in Fig. 2 shown for cholestanol). In Fig. 2 it can also be seen that at high dense LDL sialic acid ratios (>40 μg/mg), CAD$^+$ subjects had lower lathosterol ratios than CAD$^-$ subjects (135.9 ± 14.4 vs. 191.0 ± 15.4, $10^2 \times$ mmol/mol cholesterol, $P < 0.05$).

A total of 58 study subjects volunteered for the kinetic studies. They had higher BMI, higher triglyceride levels in serum and all lipoproteins (2.17 ± 0.15 vs. 1.48 ± 0.10 mmol/L in serum, $P < 0.001$), and a higher mean sialic acid ratio in total LDL than the nonparticipants (43.8 ± 1.7 vs. 36.0 ± 0.8 μg/mg, $P < 0.001$). Similar to the total study group, in the kinetic study participants cholesterol and apoB concentrations of both total and dense LDL had inverse associations with the sialic acid ratio of total LDL (**Table 6**). FCRs were not significantly associated with LDL sialic acid ratios, while the TRs for total and dense LDL apoB were negatively correlated with the sialic acid ratios of total and dense LDL (**Fig. 3**). The associations of the kinetic parameters with the sialic acid ratio were similar in dense as in total LDL, and in dense LDL the correlation between the sialic acid ratio and the TR was even stronger than in total LDL. Furthermore, the TR for dense LDL apoB had significant positive correlations with Δ^8-lathosterol and

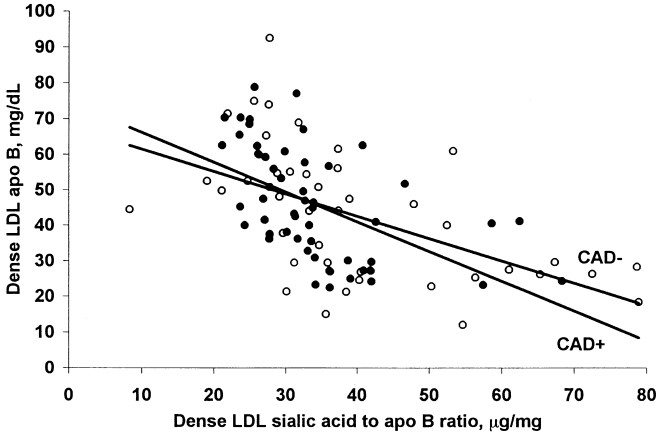

Fig. 1. Correlation between dense LDL sialic acid-to-apoB ratio and dense LDL apoB concentration. CAD$^+$ (*closed circles*), n = 56, r = −0.657, $P < 0.001$, $y = -0.84x + 74.7$; CAD$^-$ (*open circles*), n = 42, r = −0.619, $P < 0.001$, $y = -0.63x + 67.9$; combined group, N = 98, r = −0.633, $P < 0.001$, $y = -0.70x + 70.0$.

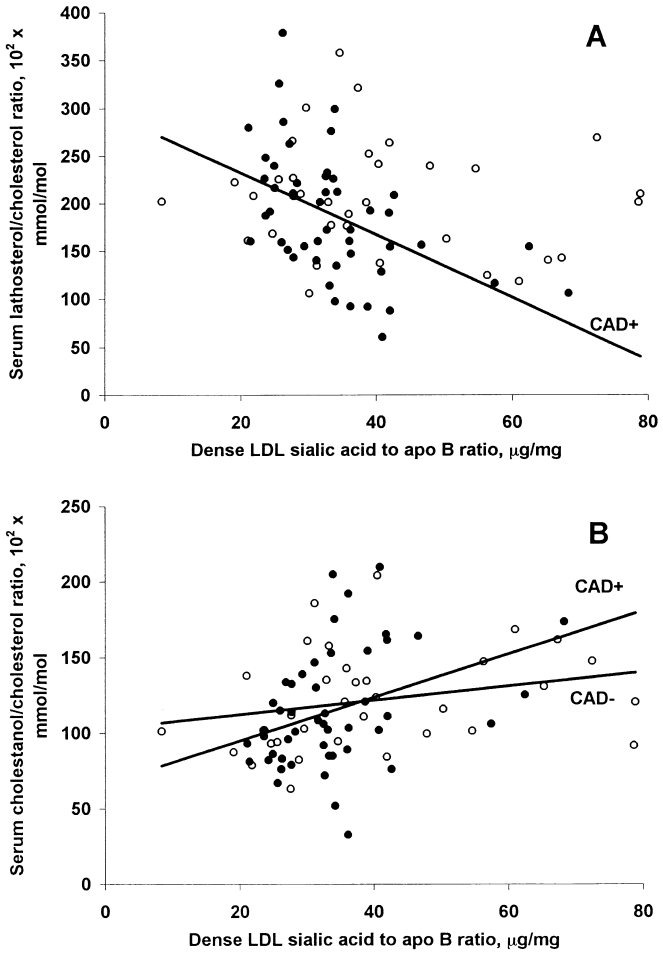

Fig. 2. Correlation between dense LDL sialic acid-to-apoB ratio and (A) serum lathosterol-to-cholesterol ratio (CAD^+ subjects [*closed circles*], n = 49, r = −0.547, $P < 0.001$, $y = -3.26x + 297$; CAD^- subjects [*open circles*], n = 33, r = −0.107, NS; combined group, N = 82, r = −0.311, $P < 0.01$, $y = -1.09x + 235$) and (B) serum cholestanol-to-cholesterol ratio (CAD^+ subjects [*closed circles*], n = 49, r = 0.395, $P < 0.01$, $y = 1.44x + 66.0$; CAD^- subjects [*open circles*], n = 33, r = 0.356, $P < 0.05$, $y = 0.47x + 102.9$; combined group, N = 82, r = 0.407, $P < 0.001$, $y = 0.79x + 88.6$).

lathosterol ratios (r = 0.322 and r = 0.307, respectively, $P < 0.05$ for both), and a negative correlation with the cholestanol ratio (r = −0.410, $P < 0.01$).

In contrast to the total study group, the sialic acid ratios of total and dense LDL did not differ significantly in the kinetic study between CAD^- and CAD^+ subjects ($P = 0.07$ in dense LDL), but the CAD^+ subjects had higher cholesterol and apoB concentrations in both total and dense LDL (Table 6). They also had higher TRs for total and dense LDL apoB, this being seen only in the two highest sialic acid quartiles. The FCRs did not differ between CAD^- and CAD^+ groups.

The stepwise regression analysis showed that both the TR (step 1, $R^2 = 0.120$) and the FCR (step 2, $R^2 = 0.241$) for total LDL apoB accounted for the variability in LDL sialic acid ratio, but the presence of CAD and the concentrations of cholesterol and triglycerides in LDL did not contribute to the result. Furthermore, when the presence of CAD was the dependent variable, the only step explaining its variability was the LDL cholesterol concentration ($R^2 = 0.195$), whereas the sialic acid ratio of total LDL and the TR or FCR for LDL apoB did not contribute.

DISCUSSION

The main new observations in the present study were that the LDL sialic acid-to-apoB ratio was lower in CAD^+ subjects than in CAD^- subjects especially at high sialic acid ratios, and that it was negatively associated with serum and LDL cholesterol and apoB levels and with the TR for LDL apoB, and the ratio of dense LDL also with cholesterol synthesis.

The result of the present study partly supports the theory that the sialic acid content of LDL might affect its atherogenicity and thus predispose to the development of CAD. However, at low total and dense LDL sialic acid ratios (in Q_1), CAD^+ and CAD^- subjects had similar mean sialic acid ratios, while at higher values (Q_2–Q_4) CAD^+ patients had lower mean sialic acid ratios than did CAD^- subjects. This implies that in populations with low LDL sialic acid ratios CAD^+ and CAD^- subjects have similar sialic acid ratios in LDL. This could, in fact, partly explain the inconsistencies of previous studies in showing differences in the LDL sialic acid ratio between CAD^+ and CAD^- subjects. A relatively weak association of the sialic acid ratio with CAD was also indicated by the finding that in stepwise logistic regression analysis only the concentration of cholesterol in LDL, and not its sialic acid ratio, was associated with the presence of CAD.

In agreement with the present findings, but in contrast to many other observations (18, 27), negative correlations of LDL sialic acid ratios with serum and LDL cholesterol levels have been reported in one earlier study (28). Serum and LDL apoB levels, remarkable risk factors for CAD (29), and triglyceride concentrations also exhibited inverse correlations with sialic acid ratios, the latter finding observed earlier also by others (15, 28).

In the light and dense LDL subfractions, the number of particles as indicated by apoB concentration was negatively associated with the LDL sialic acid ratio. In the dense and very dense LDL subfractions the size of the particles, shown by their lipid-to-apoB ratios, was positively related to the sialic acid ratios. These differences in the LDL subfractions resulted in the sialic acid ratio of total LDL being negatively related to the lipid and apoB concentrations and positively to the ratios of lipids to apoB. This suggests that a low LDL sialic acid ratio is associated with a large number of relatively small LDL particles, generally known to be atherogenic (30), and is in concordance with an earlier finding that subjects with small dense LDL have a lower LDL sialic acid content (28).

Lp[a] is highly sialylated (31) and overlaps with the density range of LDL (32). High levels of Lp[a] have been associated with atherosclerosis (33). If LDL was contaminated with even small amounts of Lp[a], the sialic acid content of LDL would probably be increased. Accord-

TABLE 6. Kinetics of total and dense LDL apoB in CAD$^-$, CAD$^+$, and all study subjects participating in the kinetic study, and their correlation coefficients with sialic acid-to-apoB ratio of total LDL

Variable	CAD$^-$[a] (n = 37)	CAD$^+$[a] (n = 21)	Combined[a] (n = 58)	Correlation Coefficient (n = 58)
Age, years	53.7 ± 1.1	55.9 ± 1.2	54.5 ± 0.9	0.009
BMI, kg/m^2	27.8 ± 0.7	28.5 ± 1.1	28.1 ± 0.6	−0.100
Total LDL				
Cholesterol, mmol/L	3.48 ± 0.14	4.15 ± 0.14[c]	3.72 ± 0.11	−0.341[c]
ApoB, mg/dL	80.0 ± 3.8	94.0 ± 3.4[c]	85.1 ± 2.8	−0.583[d]
Sialic acid ratio, μg/mg	44.7 ± 2.2	42.3 ± 2.8	43.8 ± 1.7	
FCR, pools/day	0.289 ± 0.008	0.283 ± 0.015	0.287 ± 0.007	0.241
TR, mg/kg/day	10.1 ± 0.5	11.9 ± 0.7[b]	10.8 ± 0.4	−0.384[c]
Dense LDL				
Cholesterol, mmol/L	1.72 ± 0.12	2.26 ± 0.12[c]	1.92 ± 0.09	−0.369[c]
ApoB, mg/dL	41.2 ± 3.1	54.7 ± 2.4[c]	46.1 ± 2.3	−0.476[d]
Sialic acid ratio, μg/mg	40.7 ± 2.8	33.8 ± 2.4	38.3 ± 2.0	
FCR, pools/day	0.269 ± 0.011	0.274 ± 0.009	0.271 ± 0.008	−0.003
TR, mg/kg/day	3.88 ± 0.28	5.09 ± 0.36[c]	4.32 ± 0.23	−0.351[c]

FCR, Fractional catabolic rate; TR, transport rate.
[a] Values represent means ± SE.
[b] $P < 0.05$.
[c] $P < 0.01$.
[d] $P < 0.001$.

ingly, it could be assumed that CAD patients would have a higher LDL sialic acid content. However, even though the CAD$^+$ patients tended to have a higher mean serum Lp[a] level, they had lower LDL sialic acid ratios than did CAD$^-$ subjects, and there was no correlation between the LDL sialic acid ratio and serum concentration of Lp[a] in the present study, a finding consistent with previous results (15). This strongly suggests that the possible contamination of LDL with Lp[a] was negligible or, in the case of a real contamination, the difference in LDL sialic acid ratio between CAD$^+$ and CAD$^-$ subjects would be even higher. Furthermore, our measurements of Lp[a] in LDL subfractions showed that only a minority of subjects with very high serum Lp[a] levels had small amounts of Lp[a] in the dense and very dense LDL subfractions.

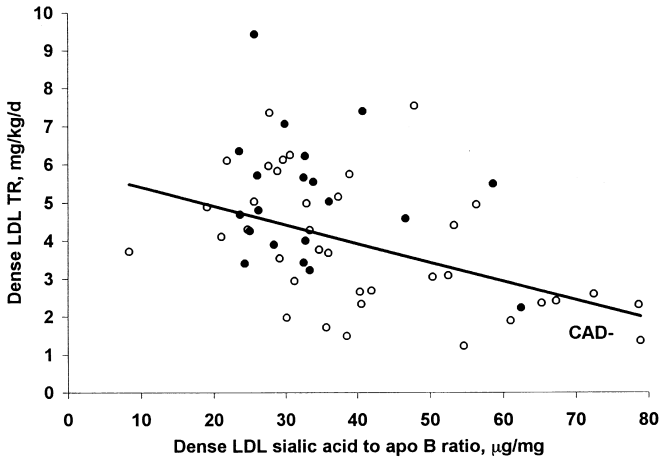

Fig. 3. Correlation between dense LDL sialic acid-to-apoB ratio and dense LDL transport rate (TR). CAD$^+$ subjects (*closed circles*), n = 20, r = −0.147, NS; CAD$^-$ subjects (*open circles*), n = 37, r = −0.543, $P < 0.001$, $y = -0.049x + 5.89$; combined group, N = 57, r = −0.456, $P < 0.001$, $y = -0.053x + 6.36$.

Serum ratios of the cholesterol precursor sterols Δ^8-lathosterol, desmosterol, and lathosterol to cholesterol reflect cholesterol synthesis, while the ratios of the plant sterols campesterol and sitosterol and that of cholestanol reflect cholesterol absorption (34). The correlations of the dense LDL sialic acid ratio with serum noncholesterol sterol ratios in the whole study group indicate that a low sialic acid ratio in dense LDL was associated with high synthesis and low absorption of cholesterol, which in turn were associated with a high TR for dense LDL apoB. This opposes the results of earlier studies, where a high TR for LDL apoB was associated with high cholesterol absorption (35, 36). At high dense LDL sialic acid ratios, CAD$^+$ subjects had lower cholesterol synthesis than did CAD$^-$ subjects, in accordance with an earlier finding that low cholesterol synthesis predisposes to CAD (37).

The association of high-level LDL production with a low LDL sialic acid ratio, seen also in our earlier study (18), could indicate that more sialic acid-poor than sialic acid-rich VLDL and intermediate density lipoprotein (IDL) were converted to LDL, or that sialic acid-rich lipoproteins were cleared more avidly from the circulation before conversion to LDL. The fact that the negative association between LDL production and the LDL sialic acid ratio is even stronger for dense LDL can explain, in part, the association between the dense LDL sialic acid ratio and cholesterol metabolism, as cholesterol synthesis and the TR for dense LDL apoB were positively correlated. Thus, the more cholesterol is synthesized, the more dense LDL is produced, and the lower is its sialic acid ratio.

The finding that the TRs for total and dense LDL apoB were higher in CAD$^+$ subjects compared with CAD$^-$ subjects is consistent with an earlier study (38), and suggests that high production of LDL apoB can be considered atherogenic. The difference between CAD$^+$ and CAD$^-$ subjects being found only in the highest two sialic acid quar-

tiles can be explained by the fact that the negative association between the sialic acid ratio and the TR for dense LDL apoB was significant only for the CAD^- subjects (Fig. 3).

FCRs for total and dense LDL apoB were not significantly associated with their sialic acid ratios, although there was a tendency toward a positive correlation between the LDL sialic acid ratio and the FCR for LDL apoB ($r = 0.241$, $P = 0.08$). Such a correlation was seen in our previous study (14), in which the sialic acid ratio and the FCR for dense LDL apoB were positively correlated in diabetic subjects, but compared with the present nondiabetic population they had exceptionally high LDL sialic acid ratios. Interestingly, however, in the stepwise regression analysis of the present study, the FCR for LDL apoB, on entering the model, explained the variability of the LDL sialic acid ratio along with the TR for LDL apoB. This suggests that both the production and catabolism of LDL are associated to some extent with its sialic acid ratio.

A reason for the lower sialic acid ratios in the LDL of CAD^+ rather than CAD^- patients could be in the differing lipoprotein metabolism in these groups. Specifically, the higher production and similar clearance of LDL apoB in CAD^+ subjects compared with CAD^- subjects, which indicates longer residence time in the circulation for LDL, could allow more time for desialylation of LDL. The mechanism of this desialylation is not known, but sialidase is an enzyme with the ability to remove sialic acid residues from carbohydrate chains, and its concentration in serum has been found to be higher in CAD^+ than in CAD^- subjects (39).

In conclusion, a low sialic acid ratio in total and dense LDL was associated with CAD, with high numbers of small LDL particles, and with a high transport rate for total and dense LDL apoB. In addition, a low sialic acid ratio in dense LDL seemed to be associated with high synthesis and low absorption of cholesterol.

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