Plasma apolipoprotein L concentrations correlate with plasma triglycerides and cholesterol levels in normolipidemic, hyperlipidemic, and diabetic subjects

Philippe N. Duchateau,[1,*] Irina Movsesyan,* Shizuya Yamashita,[†] Naohiko Sakai,[†] Ken-Ichi Hirano,[†] Samantha A. Schoenhaus,* Patricia M. O'Connor-Kearns,* Susan J. Spencer,[§] Robert B. Jaffe,[§] Rita F. Redberg,*[,] Brian Y. Ishida,* Yugi Matsuzawa,[†] John P. Kane,* and Mary J. Malloy***

Cardiovascular Research Institute,* University of California, San Francisco, CA 94143; Second Department of Internal Medicine,[†] Osaka University Medical School, Osaka 565, Japan; Reproductive Endocrinology Center,[§] University of California, San Francisco, CA 94143; and Division of Cardiology,** University of California, San Francisco, CA 94143

Abstract **Apolipoprotein L is a newly recognized component of human plasma lipoproteins. Mainly associated with apoA-I-containing lipoproteins, it is a marker of distinct HDL subpopulations. In an effort to gain inference as to its as yet unknown function, we studied biological determinants of apoL levels in human plasma. The distribution of apoL in normal subjects is asymmetric, with marked skewing toward higher values. No difference was found in apoL concentrations between males and females, but we observed an elevation of apoL in primary hypercholesterolemia (10.1 vs. 8.5 μg/mL in control), in endogenous hypertriglyceridemia (13.8 μg/mL, $P < 0.001$), combined hyperlipidemia phenotype (18.7 g/mL, $P < 0.0001$), and in patients with type II diabetes (16.2 μg/mL, $P < 0.02$) who were hyperlipidemic. Significant positive correlations were observed between apoL and the log of plasma triglycerides in normolipidemia (0.446, $P < 0.0001$), endogenous hypertriglyceridemia (0.435, $P < 0.01$), primary hypercholesterolemia (0.66, $P < 0.02$), combined hyperlipidemia (0.396, $P < 0.04$), hypoalphalipoproteinemia (0.701, $P < 0.005$), and type II diabetes with hyperlipidemia (0.602, $P < 0.01$). Apolipoprotein L levels were also correlated with total cholesterol in normolipidemia (0.257, $P < 0.004$), endogenous hypertriglyceridemia (0.446, $P = 0.001$), and non-insulin-dependent diabetes mellitus (NIDDM) (0.548, $P < 0.02$). No significant correlation was found between apoL and body mass index, age, sex, HDL-cholesterol or fasting glucose and glycohemoglobin levels. ApoL levels in plasma of patients with primary cholesteryl ester transfer protein deficiency significantly increased (7.1 ± 0.5 vs. 5.47 ± 0.27, $P < 0.006$).**—Duchateau, P. N., I. Movsesyan, S. Yamashita, N. Sakai, K-I. Hirano, S. A. Schoenhaus, P. M. O'Connor-Kearns, S. J. Spencer, R. B. Jaffe, R. F. Redberg, B. Y. Ishida, Y. Matsuzawa, J. P. Kane, and M. J. Malloy. **Plasma apolipoprotein L concentrations correlate with plasma triglycerides and cholesterol levels in normolipidemic, hyperlipidemic, and diabetic subjects.** ***J. Lipid Res.*** **2000.** 41: **1231–1236.**

Supplementary key words apolipoprotein L • triglycerides • hypercholesterolemia • hypertriglyceridemia • combined hyperlipidemia • diabetes • CETP deficiency

A protective effect against the development of atherosclerotic disease is attributed to high density lipoproteins (HDL) on the basis of their key roles in reverse cholesterol transport (1–5) and perhaps also their antioxidant properties *vis à vis* other lipoproteins (6–8). However, these processes will not be fully understood until the components of HDL and their architecture are better defined at a molecular level.

Understanding of the native molecular speciation of HDL is organic to the development of mechanistic models for the critical function of HDL in lipid transport and in the prevention of arteriosclerosis. The historical classification of HDL has been based on ultracentrifugation that resolves two major classes: HDL_2 and HDL_3 (9, 10). However this technique has been shown to bring about the decomposition and loss of some native molecular species of HDL (11, 12). A newer and less disruptive method of isolation is immunoaffinity chromatography, capable of separating lipoproteins on the basis of differences in apolipoprotein content (12, 13). This technique has been used to study two major classes of apolipoprotein A-I (apoA-I)-containing lipoproteins: particles containing apoA-I without apoA-II [Lp(A-I)] or particles containing apoA-I and apoA-II [Lp(A-I:A-II)] (14–17). However the apolipoprotein content of HDL is still more complex, and this dichotomous subdivision of HDL [Lp(A-I), Lp(A-I:A-II)] is insufficient to reveal the true array of HDL particles in plasma. Indeed,

Abbreviations: apo, apolipoprotein; BMI, body mass index; BSA, bovine serum albumin; CETP, cholesteryl ester transfer protein; ELISA, enzyme-linked immunosorbent assay; HbA1c, glycohemoglobin; HDL, high density lipoprotein; LCAT, lecithin–cholesterol acyltransferase; LDL, low density lipoprotein; PBS, phosphate-buffered saline; PLTP, phospholipid transfer protein; TG, triglycerides; TMB, 3,3′,5,5′-tetramethylbenzidine.

[1] To whom correspondence should be addressed.

HDL particles containing apoA-I and apoA-IV or apoA-I and apoE have been separated by immunoaffinity (18–20). Moreover, important constituents such as lecithin–cholesterol acyltransferase (LCAT), cholesterol ester transfer protein (CETP), and phospholipid transfer protein (PLTP) are also associated with subspecies of HDL (14, 21, 22).

We have reported a new HDL apolipoprotein, apolipoprotein L (23). The product of a gene on chromosome 22 is a single peptide of 372 amino acids in its secreted form that has no sequence homology with any of the previously recognized apolipoprotein families. ApoL cDNA was cloned from pancreas RNA. Entirely bound to lipoproteins, apoL was found to be a marker of a distinct subpopulation of HDL, representing about 10% of the apoA-I-containing lipoproteins, and distributed in two HDL subpopulations of large particle diameter (12.2–17 and 10.4–12.2 nm). No function has yet been found for apoL. However, its strong association with apoA-I-containing lipoproteins suggests a significant role in HDL metabolism. Study of the biological determinants of levels of apoL in human plasma may provide clues to its role in lipoprotein metabolism.

In the current study, we have explored the influence of gender, body mass index, age, total cholesterol, triglycerides, apoA-I, HDL-cholesterol, and low density lipoprotein (LDL)-cholesterol levels on the concentration of apoL in normolipidemic plasma as well as in plasma from individuals with lipoprotein disorders and type II diabetes.

MATERIALS AND METHODS

Study population

Two hundred and twenty-nine subjects were selected for this study. Of these, 135 were healthy normolipidemic subjects (64 males, 71 females) ranging in age from 23 to 90 years of age. Their mean total plasma cholesterol and triglyceride levels were below 250 and 180 mg/dL, respectively. Ninety-six were patients with primary dyslipoproteinemia. Among them, 14 (11 males, 3 females) had primary hypercholesterolemia with LDL-cholesterol >190 mg/dL and plasma triglycerides <160 mg/dL. Thirty-two (26 males, 6 females) had predominant increases in plasma triglycerides (endogenous lipidemia) (mean level, 1,007.3 ± 156.6 mg/dL), all with LDL-cholesterol <160 mg. Twenty-six (12 males, 14 females) had combined lipidemia with LDL-cholesterol of 198 ± 16.5 mg/dL and triglycerides of 412.8 ± 73.7 mg/dL. Thirteen (12 males, 1 female) were classified as having hypoalphalipoproteinemia because they had HDL-cholesterol <35 and <30 mg/dL for females and males, respectively. Finally, 21 (5 males, 16 females) were patients with type II diabetes, treated with hypoglycemic drugs. Total plasma cholesterol and triglycerides among the diabetic subjects ranged from 145 to 360 mg/dL and from 103 to 582 mg/dL, respectively. None of the subjects in this study were receiving drugs known to affect lipoprotein metabolism. Subjects deficient in cholesteryl ester transfer protein (CETP) were also studied. Plasma from 6 Japanese subjects (4 males, 2 females) with homozygosity or combined heterozygosity were studied. Their CETP activity ranged from 0 to 23% of normal control values (11.5 ± 4.5%). All six had at least one allele for the intron 14 (+1 G:A) mutation, and one was homozygous. Three were also heterozygous for the D442G mutation and one for G181X. A second mutation was not detected in the remaining patient, but his CETP activity was extremely low (4.6% of normal). Because it is well documented that lipid profiles differ between Japanese and American subjects six normolipidemic subjects (4 males, 2 females) from the same geographic area were studied as controls. These 12 subjects were not included in the population study. The study was approved by the Committee on Human Research of the University of California, San Francisco (San Francisco, CA). Written consent was obtained for venipuncture and collection of personal data from the individual studied.

Blood collection and lipid determination

Blood was drawn from subjects who had fasted for 10 h and the blood was immediately cooled to 4°C in the presence of the following substances: EDTA, 0.08% (w/v); sodium azide, 0.1% (w/v); benzamidine, 50 μg/mL; ε-amino caproic acid, 300 μg/mL; and gentamicin sulfate, 10 μg/mL. The plasma was separated by centrifugation of the blood at 1000 *g* at 4°C for 20 min and aliquots were kept at −80°C until analysis. Total cholesterol and triglyceride levels in plasma were determined by standard chemical techniques. The cholesterol content of lipoprotein fractions was measured on lipoproteins separated by sequential ultracentrifugation (9).

ELISA methods

Total apoA-I was measured by enzyme-linked immunosorbent assay (ELISA) as described previously (24). ApoL was quantified by a competitive ELISA designed in our laboratory. Briefly, 96-well plates were coated with 100 μL per well of immunopurified A-I-containing lipoproteins diluted to 5 μg/mL in saline–phosphate buffer (NaCl, 0.15 M; Na_2HPO_4, 10 mM; $NaH_2PO_4 \cdot H_2O$, 1 mM; pH 7.5) (PBS) and incubated for 16 h at 4°C. The plates were then washed 6 times with wash buffer (PBS, 0.05% Tween 20) and blocked with 3% bovine serum albumin (BSA) in PBS for 45 min at 25°C with shaking. Before being used, plates were then washed 6 times with wash buffer. Samples and standards were prepared as follows: A series of dilutions of plasma was incubated with a constant amount of rabbit anti-human apoL antibody (diluted 4000 times in PBS, 0.1% BSA) for 16 h at 4°C. To measure the uncoupled antibodies, each sample was then added to the plate in a volume of 100 μL per well for 30 min at 25°C. After incubation, plates were washed 6 times with wash buffer and bound antibodies were detected by addition of 100 μL of anti-rabbit antibody conjugated to horseradish peroxidase (30 min at 25°C). After 6 washes, secondary antibodies were quantitated colorimetrically with 100 μL per well of 3,3′,5,5′-tetramethylbenzidine (TMB) (Kirkegaard & Perry, Gaithersburg, MA). After the reaction was stopped, the optical density of the reaction product was measured at 450 nm with an automated plate reader (Molecular Devices, Menlo Park, CA). Typically, the apoL ELISA exhibits coefficients of variation, inter- and intraassay respectively, of 12.5 and 11%. We did not find any systematic difference between the concentration of apoL measured in fresh plasma and frozen plasma (−70°C).

Statistical methods

The results are given as means ± standard error. Data were analyzed to determine whether they were normally distributed. When they were not, log transformation was applied to correct skewness and kurtosis. Correlations were determined by univariate linear regression analysis. When necessary, multiple stepwise regression was used to confirm significant independent correlation. Nonparametric tests (Mann–Whitney) were performed to determine significant differences between two groups.

TABLE 1. Lipids, lipoproteins, and other characteristics of normolipidemic men and women

	TC	TG	HDL-C	LDL-C	BMI	AGE	A-I	L
		mg/dL			*kg/m²*	*yrs*	*mg/mL*	*μg/mL*
Male (n = 64)	171.6 ± 6.7	94.1 ± 7.6	46.2 ± 1.8	115.4 ± 6.18	28.5 ± 1.17	43 ± 2.1	1.21 ± 0.03	7.2 ± 0.4
Female (n = 71)	181.5 ± 6.5	77.1 ± 6.1	68.6 ± 5.7	105.9 ± 4.7	30.9 ± 2.21	56 ± 2.1	1.35 ± 0.04	9.7 ± 0.9
P value	0.09	0.48	0.0004	0.9	0.11	0.5	0.19	0.68

Abbreviations: TC, total plasma cholesterol; TG, plasma triglycerides; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; BMI, body mass index; A-I, apolipoprotein A-I; L, apolipoprotein L.

Values given as means ± SE

RESULTS

Normolipidemic population

One hundred and thirty-five healthy subjects were studied (71 females, 64 males). Plasma lipid and lipoprotein values are presented in **Table 1**. No significant difference in apoL levels between men and women was found for age ($P \leqslant 0.5$), triglycerides ($P \leqslant 0.48$), LDL-cholesterol ($P \leqslant 0.9$), apoA-I ($P \leqslant 0.19$), or total cholesterol ($P \leqslant 0.09$). HDL-cholesterol was significantly higher ($P \leqslant 0.0004$) in the female population. No significant difference in plasma apoL level was found between women and men ($P < 0.68$). Moreover, apoL exhibited a distribution (**Fig. 1**) in the normal population with significant skew toward higher levels (skewness, 1.48). Among the women, 30 were postmenopausal. No difference in the plasma apoL concentration was found between pre- and postmenopausal females. Therefore we did not differentiate these groups for the remainder of the study.

Univariate linear analysis showed that three parameters correlated significantly with apoL. The two strongest were total cholesterol (r = 0.306, $P < 0.0005$) and log of triglycerides (log TG) (r = 0.446, $P < 0.0001$). Plasma LDL-cholesterol was also significantly correlated with apoL (r = 0.257, $P < 0.004$). On the other hand, no correlation between apoL and apoA-I, body mass index (BMI), or age was found. To determine which of these three parameters were independently correlated with apoL we performed multiple stepwise regression. All seven parameters were used in this model. We found that total cholesterol and log TG were independent covariates influencing plasma apoL concentration in our normolipidemic population.

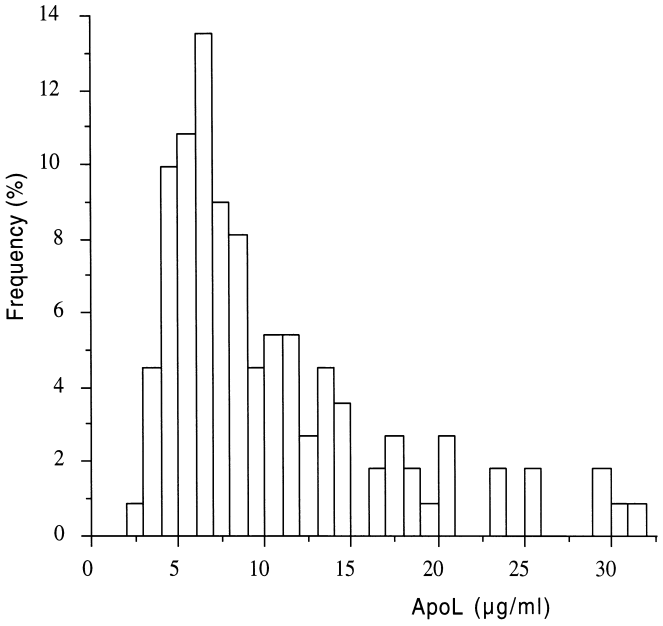

Fig. 1. Frequency distribution of apolipoprotein L concentration in plasma samples from 135 normolipidemic subjects.

Dyslipoproteinemic populations

As shown in **Table 2**, patients with endogenous hypertriglyceridemia had elevated mean plasma triglycerides (1,007.3 ± 156 mg/dL) and normal mean levels of LDL-cholesterol (96.4 ± 5.4 mg/dL). Total cholesterol was also significantly higher ($P < 0.0001$) whereas HDL-cholesterol was lower than that of the normal population ($P < 0.0001$). Patients with primary hypercholesterolemia had elevated mean total cholesterol and LDL-cholesterol levels, 307.2 and 229.9 mg/dL, respectively. Plasma triglycerides, although within the normal range, were also significantly higher than in the control group (138.3 mg/dL). The group with combined lipidemia exhibited elevated mean levels of total cholesterol, LDL-cholesterol, and triglycerides (348.1, 198.2, and 412.8 mg/dL, respectively). Patients with hypoalphalipoproteinemia had lower plasma apoA-I and HDL-cholesterol levels (1.04 and 28.1 mg/dL, respectively) and slightly higher mean triglyceride levels (148.2 mg/dL) than the normolipidemic subjects ($P < 0.001$).

Except in the HDL-deficient population, plasma apoL concentrations were higher than in the normolipidemic individuals, particularly in primary endogenous hypertriglyceridemia and combined hyperlipidemia (13.8 μg/mL, $P < 0.02$; 18.7 μg/mL, $P < 0.0001$, respectively). Plasma apoL levels were also significantly higher among the patients with type II diabetes (16.2 μg/mL, $P < 0.03$). Moreover those patients also had higher levels of total cholesterol, triglycerides, and LDL-cholesterol (239.1 mg/dL, $P < 0.0001$; 250.6 mg/dL, $P < 0.0001$; and 159.1 mg/dL, $P < 0.001$, respectively) than did normal subjects.

To determine the existence of any correlation between apoL and these parameters in the dyslipoproteinemic population, we performed univariate linear regression analysis. There was a significant correlation between plasma apoL concentration and log TG (r = 0.66, $P < 0.026$; r = 0.435, $P < 0.01$; r = 0.39, $P < 0.04$; r = 0.70,

TABLE 2. Plasma parameters of dislipoproteinemic populations

	TC	TG	HDL-C	LDL-C	A-I	L	HbA1	Glc
	mg/dL				*mg/mL*	*μg/mL*	%	*g/L*
Normal (n = 135)	180.6 ± 4.4	83.6 ± 4.3	59.1 ± 3.14	113.3 ± 3.8	1.29 ± 0.04	8.5 ± 0.5	ND	ND
Endogenous hypertriglyceridemia (n = 32)	345.3 ± 35.9[c]	1007.3 ± 156.6[c]	31.3 ± 1.4[c]	96.4 ± 5.4	1.34 ± 0.05	13.8 ± 2[a]	ND	ND
Primary hypercholesterolemia (n = 14)	307.2 ± 19.8[c]	138.3 ± 7.98[c]	55 ± 4.1	229.9 ± 17.9[c]	1.65 ± 0.1	10.1 ± 1.1	ND	ND
Combined hyperlipidemia (n = 27)	348.1 ± 23.3[c]	412.8 ± 73.7[c]	43.7 ± 2.1[c]	198 ± 16.5[c]	1.53 ± 0.07	18.7 ± 2.9[c]	ND	ND
Hypoalphalioproteinemia (n = 13)	185.2 ± 13.2	148.2 ± 14.4[c]	28.1 ± 2.1[c]	135.6 ± 12	1.04 ± 0.07	9.2 ± 1.1	ND	ND
NIDDM (n = 21)	239.1 ± 13.5[c]	250.6 ± 29.9[c]	42.2 ± 2.5[c]	159.1 ± 13.1[b]	1.36 ± 0.03	16.2 ± 3.8[a]	8.06 ± 0.4	1.77 ± 0.11

Abbreviations: TC, total plasma cholesterol; TG, plasma triglycerides; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; A-I, apolipoprotein A-I; L, apolipoprotein L; HbA1, glycohemoglobin; Glc, glucose; ND, not determined.

Values given as means ± SE.

[a] $P < 0.03$.

[b] $P < 0.001$.

[c] $P < 0.0002$.

$P < 0.005$; and r = 0.60, $P < 0.01$; for primary hypercholesterolemia, endogenous hyper-triglyceridemia, combined hyperlipidemia, hypoalphalipoproteinemia, and type II diabetes, respectively). Moreover, we also found a relationship between apoL and total cholesterol in patient groups with primary endogenous hypertriglyceridemia and with type II diabetes (r = 0.446, $P < 0.001$; r = 0.548, $P < 0.02$, respectively) and LDL-cholesterol was correlated with apoL in the combined hyperlipidemia population (r = 0.554, $P < 0.002$). No significant correlation was found between apoL and glycohemoglobin (HbA1c) levels or glycemia among the type II diabetes patients. Multiple stepwise regression analysis showed that log TG was in all groups an independent covariate influencing plasma apoL concentration. Moreover, in the combined hyperlipidemia population LDL-cholesterol was found to be an independent covariate as total cholesterol was in the group with type II diabetes.

CETP-deficient population

Because of the difference existing between Japanese and western populations regarding their lipid profiles and cardiovascular risk, the CETP-deficient subjects were studied separately and were compared with 6 normolipidemic subjects from the same region of Japan. As shown in **Table 3**, CETP-deficient patients had markedly higher plasma levels of HDL-cholesterol and apolipoprotein A-I than did normal subjects (131.8 ± 16.7 vs. 53.3 ± 3.6, $P < 0.003$ and 2.69 ± 0.2 vs. 1.57 ± 0.1, $P < 0.01$, respectively). Plasma apoL concentrations were also significantly increased (7.1 ± 0.5 vs. 5.47 ± 0.25, $P < 0.006$).

DISCUSSION

Knowledge of the molecular speciation of HDL and of the protein constituents of HDL particles is crucial for our understanding of HDL metabolism. Indeed, even low-abundance proteins can be extremely important in HDL metabolism, for example, lecithin–cholesterol acyltransferase (LCAT), cholesteryl ester transfer protein (CETP), or phospholipid transfer protein (PLTP). We reported a new HDL apolipoprotein, apoL (23). Virtually associated only with HDL particles, apoL is a marker of HDL subpopulation. Having no homology with any known protein, no prediction of its function based on primary structure has been possible. In this report we quantified apoL mass in plasma from normolipidemic subjects and dyslipoproteinemic patients and identified some determinants of apoL levels. Among normolipidemic subjects, no significant difference was found between males and females, yielding a mean value of 8.5 μg of apoL per mL of plasma, in agreement with a preliminary report in which only 5

TABLE 3. Plasma parameters of CETP deficiency subjects

	Plasma		VLDL		LDL		HDL			
	C	TG	C	TG	C	TG	C	TG	ApoA-I	ApoL
									mg/mL	*μg/mL*
CETP def. (n = 6)	265.6 ± 34.2	132.8 ± 38.3	13.1 ± 6	89.6 ± 38.5	120.6 ± 14.4	32 ± 4.3	131.8 ± 16.7	11.1 ± 1.1	2.69 ± 0.2	7.1 ± 0.5
Control (n = 6)	202.5 ± 19.7	65.6 ± 9.4	6 ± 1.5	27.5 ± 5.8	143.1 ± 16.3	20.5 ± 3.7	53.3 ± 3.6	17.6 ± 1.8	1.57 ± 0.11	5.47 ± 0.27
P value	0.05	0.29	0.63	0.17	0.17	0.05	0.003	0.01	0.006	0.006

See Table 2 footnote for abbreviations; values given as means ± SE.

subjects were studied (23). Moreover, apoL was found to be distributed asymmetrically, with a pronounced skew to higher values, resembling the distribution of total cholesterol, triglycerides, and pre-β_1-HDL in the general population. Interestingly, we found a highly significant and independent correlation between apoL and total plasma cholesterol and triglyceride levels in normolipidemic subjects. In contrast, HDL-cholesterol was not correlated with apoL even though HDL particles are the main carriers of apoL in plasma (23). This suggests that apoL-containing particles constitute a subpopulation that is not influenced by the cholesterol content of HDL among normolipidemic subjects. A lack of correlation with the plasma content of apoA-I is consistent with this finding and the observation that apoL-containing particles account for no more than 10% of HDL mass, distributed among two HDL populations of large particle diameter. No significant correlation was found between apoL and BMI or age.

On the basis of the relationship that exists between apoL and total cholesterol and triglycerides in normolipidemic subjects, we analyzed plasma from four groups of subjects with hyperlipidemia. Except for the hypoalphalipoproteinemia population, where the plasma apoL concentration was normal, endogenous hypertriglyceridemia, primary hypercholesterolemia, and combined hyperlipidemia populations had higher levels of apoL in plasma. However, data concerning patients with primary hypercholesterolemia failed to reach significance, probably because of the low number of subjects in this population. Moreover, similar to the finding among the control subjects, apoL was significantly correlated with plasma triglyceride in all lipoprotein disorders studied, suggesting that the levels of apoL in these phenotypes is not a reflection of the dyslipoproteinema per se, but a result of the increase of triglyceride levels in plasma.

LCAT is believed to be crucial in HDL metabolism. Cholesterol is esterified by this enzyme and transferred to the hydrophobic cores of HDL particles, which then become larger. It has been reported that plasma LCAT activity is positively correlated with plasma triglyceride (25). It is then tempting to speculate on the existence of a relationship between apoL, present in large HDL, and LCAT activity. ApoL-containing lipoproteins could be an intermediate HDL population generated by LCAT activity. This hypothesis is supported by our study of CETP-deficient plasma. Indeed, CETP deficiency presents a unique opportunity to analyze apoL mass in plasma where HDL catabolism is reduced. The hyperalphalipoproteinemia of CETP deficiency is characterized by the presence of large HDL_2-like particles (26) enriched in cholesteryl ester by LCAT activity. Our finding shows that in this disorder, plasma apoL levels are significantly increased. Moreover, as previously reported (26), plasma from CETP-deficient individuals shows a significant increase in HDL-cholesterol and apoA-I. This suggests a possible role of LCAT in the generation of apoL-containing HDL particles.

Finally, we analyzed apoL mass in the plasma of subjects with type II diabetes. We found significant increases in plasma apoL levels in this population, in which total cholesterol and triglycerides were also increased. It is likely that the significant increase in apoL in these patients is related to the dyslipidemia per se. Indeed, we observed, as in the normal population, a significant relationship between apoL and plasma triglyceride and cholesterol, whereas no correlation was found with either fasting glycemia or HbA1c. Furthermore, the increase in plasma apoL concentration in type II diabetes disappears when apoL levels are normalized for the plasma triglyceride concentrations. Increased LCAT activity has been observed in diabetic subjects with hypertriglyceridemia (27). This observation is consistent with our hypothesis involving LCAT activity in the metabolism of apoL-containing HDL particles. LCAT activity may favor the accumulation of apoL in large cholesterol-enriched HDL particles.

The increase in apoL content of plasma with increasing triglyceride levels cannot be explained by LCAT activity alone and must be a reflection of some process that is unrelated to the accumulation of cholesteryl ester-rich HDL, because it is known that there is increased transfer of cholesteryl ester from HDL to the core region of triglyceride-rich lipoproteins with increasing plasma triglyceride levels. Indeed, the lipoproteins in which apoL is carried are still unknown in patients with hypertriglyceridemia.

In conclusion, we have found a significant correlation between apoL and triglycerides in plasma in normolipidemic subjects as well as patients with primary hypercholesterolemia, endogenous hypertriglyceridemia, combined hyperlipidemia, hypoalphalipoproteinemia, and type II diabetes with hypertriglyceridemia. To a lesser extent, apolipoprotein L levels were also correlated with plasma cholesterol levels, possibly reflecting in part increases in total plasma cholesterol levels that are associated with increased content of triglyceride-rich lipoproteins. No relationship was found with HDL-cholesterol or apoA-I levels, suggesting that apoL content of HDL is dependent on some pathway that leads selectively to the generation of a subpopulation HDL particles of large diameter with which apoL is associated, perhaps involving the generation of cholesteryl ester by LCAT. The mechanism underlying the relationship between triglyceride and apoL is still unknown; however, the presence of a minor portion of apoL on triglyceride-rich lipoproteins suggests that the generation of apoL-containing HDL species could be linked to lipolysis.

The authors acknowledge support by NIH grants HL-31210, HL-50779, HL-50782, and AA-11205, and by a gift from the Joseph Drown Foundation and Donald Yellon.

Manuscript received 17 December 1999 and in revised form 11 April 2000.

REFERENCES

1. Miller, G. S., and N. F. Miller. 1975. Plasma high density lipoprotein and development of ischemic heart disease. *Lancet.* **1:** 16–19.
2. Gordon, T., W. P. Castelli, M. C. Hjortland, W. B. Kannel, and T. R. Dawber. 1977. Predicting coronary heart disease in middle-aged and older persons. The Framington study. *J. Am. Med. Assoc.* **238:** 497–499.
3. Glomset, J. A. 1968. The plasma lecithin:cholesterol acyltransferase reaction. *J. Lipid Res.* **9:** 155–167.

4. Kunitake, S. T., K. J. La Sala, C. Mendel, G. C. Chen, and J. P. Kane. 1987. Some unique properties of apo A-I-containing lipoproteins with pre-beta electrophoretic mobility. National Institutes of Health Workshop on Lipoprotein Heterogeneity. *NIH Publ.* **2646:** 419–427.
5. Fielding, C. J., and P. E. Fielding. 1995. Molecular physiology of reverse cholesterol transport. *J. Lipid Res.* **36:** 211–228.
6. Parthasarathy, S., J. Barnett, and L. G. Fong. 1990. High-density lipoprotein inhibits the oxidative modification of low-density lipoprotein. *Biochim. Biophys. Acta.* **1044:** 275–283.
7. Kunitake, S. T., M. Jarvis, R. L. Hamilton, and J. P. Kane. 1992. Binding of transition metals by apolipoprotein A-I-containing plasma lipoproteins: inhibition of oxidation of low density lipoproteins. *Proc. Natl. Acad. Sci. USA.* **89:** 6993–6997.
8. Ohta, T., K. Takata, S. Horiuchi, Y. Morino, and I. Matsuda. 1989. Protective effect of lipoproteins containing apoprotein A-I on Cu2+-catalyzed oxidation of human low density lipoprotein. *FEBS Lett.* **257:** 435–438.
9. Havel, R. J., H. Eder, and J. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345–1353.
10. Lindgren, F., and A. Nichols. 1960. The Plasma Lipoproteins: Biosynthesis, Metabolism, Alterations in Disease. Academic Press, New York. 1–58.
11. Kunitake, S. T., and J. P. Kane. 1982. Factors affecting the integrity of high density lipoproteins in the ultracentrifuge. *J. Lipid Res.* **23:** 936–940.
12. Cheung, M. C., and A. C. Wolf. 1988. Differential effect of ultracentrifugation on apolipoprotein A-I-containing lipoprotein subpopulations. *J. Lipid Res.* **29:** 15–25.
13. Alaupovic, P. 1980. The concepts, classification systems and nomenclatures of human plasma lipoproteins. *In* CRC Handbook of Electrophoresis, L. A. Lewis and J. J. Opplt, editors. CRC Press, Boca Raton, FL. 27–46.
14. Cheung, M. C., A. C. Wolf, K. D. Lum, J. H. Tollefson, and J. J. Albers. 1986. Distribution and localization of lecithin:cholesterol acyltransferase and cholesteryl ester transfer activity in A-I-containing lipoproteins. *J. Lipid Res.* **27:** 1135–1144.
15. Duverger, N., N. Ghalim, N. Theret, P. Duchateau, G. Aguie, G. Ailhaud, G. Castro, and J. C. Fruchart. 1991. Lipoprotrein A-I containing particles. *In* Advances in Experimental Medicine and Biology. L. Malmendier, P. Alaupovic, and H. B. Brewer, editors. Plenum Press, New York. 93–99.
16. Broutin, H., P. Puchois, G. Ailhaud, R. Barbaras, G. Torpier, and J. C. Fruchart. 1988. Receptors and molecular heterogeneity of lipoprotein particles containing apolipoprotein A-I. *Ann. Biol. Clin.* **46:** 16–23.
17. Hennessy, L. K., S. T. Kunitake, and J. P. Kane. 1993. Apolipoprotein A-I-containing lipoproteins, with or without apolipoprotein A-II, as progenitors of pre-beta high-density lipoprotein particles. *Biochemistry.* **32:** 5759–5765.
18. Duchateau, P., D. Rader, N. Theret, N. Duverger, C. Degeitere, H. B. Brewer, Jr., J. C. Fruchart, and G. Castro. 1993. Reverse cholesterol transport in Tangier disease. Isolation and characterization of LpA-I, LpA-II, LpA-I:A-II and LpA-IV particles. *Biochim. Biophys. Acta.* **1182:** 30–36.
19. Hennessy, L. K., S. T. Kunitake, M. Jarvis, R. L. Hamilton, G. Endeman, A. Protter, and J. P. Kane. 1997. Isolation of subpopulations of high density lipoproteins: three particle species containing apoE and two species devoid of apoE that have affinity for heparin. *J. Lipid Res.* **38:** 1859–1868.
20. Duverger, N., N. Ghalim, N. Theret, J. C. Fruchart, and G. Castro. 1994. Lipoproteins containing apolipoprotein A-IV: composition and relation to cholesterol esterification. *Biochim. Biophys. Acta.* **1211:** 23–28.
21. Kunitake, S. T., C. T. Carilli, K. Lau, A. A. Protter, J. Naya-Vigne, and J. P. Kane. 1994. Identification of proteins associated with apolipoprotein A-I-containing lipoproteins purified by selected-affinity immunosorption. *Biochemistry.* **33:** 1988–1993.
22. Fielding, P. E., and C. J. Fielding. 1980. A cholesteryl ester transfer complex in human plasma. *Proc. Natl. Acad. Sci. USA.* **77:** 3327–3330.
23. Duchateau, P. N., C. R. Pullinger, R. E. Orellana, S. T. Kunitake, J. Naya-Vigne, P. M. O'Connor, M. J. Malloy, and J. P. Kane. 1997. Apolipoprotein L, a new human high density lipoprotein apolipoprotein expressed by the pancreas. Identification, cloning, characterization, and plasma distribution of apolipoprotein L. *J. Biol. Chem.* **272:** 25576–25582.
24. Gaver, A., N. K. Weedy, B. R. Maldonado, S. Huang, and L. Wong. 1992. Procedure for development of an enzyme-linked immunosorbent assay. Development of an assay for human apolipoprotein A-I. *Clin. Chim. Acta.* **208:** 23–37.
25. Dullaart, R. P. F., W. J. Sluiter, L. D. Dikkeschei, K. Hoogenberg, and A. Van Tol. 1994. Effect of adiposity on plasma lipid transfer protein activities: a possible link between insulin resistance and high density lipoprotein metabolism. *Eur. J. Invest.* **24:** 188–194.
26. Tall, R. A., and J. L. Breslow. 1996. Plasma high density lipoprotein and atherogenesis. *In* Atherosclerosis and Coronary Artery Disease. Vol. 1. V. Fuster, R. Ross, and E. J. Topol, editors. Lippincott-Raven, New York. 105–128.
27. Riemens, S. C., A. Van Tol, W. J. Sluiter, and R. P. F. Dullaart. 1998. Elevated plasma cholesteryl ester transfer in NIDDM: relationships with apolipoprotein B-containing lipoproteins and phopholipid transfer protein. *Atherosclerosis.* **140:** 71–79.