Prebeta-1 HDL in plasma of normolipidemic individuals: influences of plasma lipoproteins, age, and gender

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Abstract Prebeta-1 HDL is a molecular species of plasma HDL of approximately 67 kDa mass that contains apolipoprotein A-I, phospholipids, and unesterified cholesterol. It participates in a cyclic process involved in the retrieval of cholesterol from peripheral tissues. In this cycle, unesterified cholesterol from cells is incorporated into prebeta-1 HDL, providing a substrate for esterification of cholesterol by lecithin:cholesterol acyltransferase. Prebeta-1 HDL then becomes incorporated into larger HDL species of alpha mobility as esterification proceeds and is regenerated during the transfer of cholesteryl esters from alpha HDL particles to acceptor lipoproteins. Thus the steady state level of prebeta-1 HDL in plasma reflects the relative efficiencies of the major metabolic processes involved in its generation and removal. We have used an isotope dilution technique to measure prebeta-1 HDL levels in the plasmas of 136 normolipidemic individuals (46 M, 90 F). The mean absolute concentration of prebeta-1 HDL as apolipoprotein A-I was 68 ± 40 μg/ml for women, and 84 ± 49 m/ml for men. Prebeta-1 HDL represented 5.5 ± 3.3% of total apolipoprotein A-I in women, and 7.2 ± 4.0% in men. The distributions of both absolute and percent prebeta-1 HDL are highly asymmetric, with skew toward higher values. However, the skew appears not to be attributable to either plasma cholesterol or triglyceride levels which are also skewed in population samples. The percent prebeta-1 HDL was negatively correlated with HDL cholesterol levels ($P < 0.0001$), whereas absolute levels of prebeta-1 HDL were positively correlated with apolipoprotein A-I and negatively correlated with HDL cholesterol (*P*, for both, < 0.0001). Multiple linear regression analysis revealed effects of age and gender, but no association with lipoprotein fractions other than HDL. Lower levels of prebeta-1 HDL were associated with female gender in all models.**—O'Connor, P. M., B. R. Zysow, S. A. Schoenhaus, B. Y. Ishida, S. T. Kunitake, J. M. Naya-Vigne, P. N. Duchateau, R. F. Redberg, S. J. Spencer, S. Mark, M. Mazur, D. C. Heilbron, R. B. Jaffe, M. J. Malloy, and J. P. Kane.** Prebeta-1 HDL in plasma of normolipidemic individuals: influences of plasma, age, and gender. *J. Lipid Res.* 1998. **39:** 670–678.

Supplementary key words apolipoprotein A-I • lecithin:cholesterol acyltransferase • cholesteryl ester transfer protein • cholesterol transport

Prebeta-1 HDL is a molecular species of high density lipoproteins (HDL) that was not recognized until 1985 (1). Because of its high modal density it was not included in HDL recovered from serum by ultracentrifugation in the traditional density interval of 1.063–1.21 g/ml. Also, its rapid conversion to HDL species of larger diameter, ex vivo, by lecithin:cholesterol acyltransferase, impeded its recognition by other techniques. The advent of a minimally perturbing chromatographic technique, selected affinity immunosorption (2) that recovers apolipoprotein A-I-containing particles quantitatively from human plasma, permitted the subsequent recognition of HDL species of prebeta electrophoretic mobility, distinguishing them from the predominant mass of HDL which has alpha mobility (1). The chief molecular species among the prebeta HDL (prebeta-1 HDL) is a particle of circa 67 kDa mass, containing two copies of apolipoprotein A-I. No other protein species is present in the particles. The lipid moiety represents but 10% of mass, comprised of phospholipid and unesterified cholesterol. Prebeta-1 HDL has now been identified in the plasma of monkeys (3) and mice (4) in addition to humans.

Abbreviations: HDL, high density lipoproteins; LDL, low density lipoproteins; VLDL, very low density lipoproteins; LCAT, lecithin:cholesterol acyltransferase; CETP, cholesteryl ester transfer protein; kDA, kilodaltons; PLTP, phospholipid transfer protein; SR-BI, scavenger receptor, type B, one; BMI, body mass index; ELISA, enzyme-linked immunosorbent assay; ESTRSTAT, estrogen-based statistical variable; GENESTR, gender- and estrogen-based statistical variable; PCTPREB, percent of total apoA-I in prebeta-1 HDL; RPCTPREB, square root of PCTPREB; ABSPREB, absolute amount of apoA-I in prebeta-1 HDL in μg/ml; RABSPREB, square root of ABSPREB.

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It is now well recognized that cholesterol from cells in the periphery is incorporated into prebeta-1 HDL in the first step of the centripetal transport pathway in which cholesterol is moved through plasma to the liver (5, 6). The next step is the esterification of cholesterol by lecithin:cholesterol acyltransferase (LCAT)(7). The cholesterol passes through two other HDL species of larger molecular weight, prebeta-2 and prebeta-3 HDL, ultimately appearing as cholesteryl esters in HDL particles of alpha electrophoretic mobility (5). A fraction of cholesteryl esters is then transferred to acceptor lipoproteins containing apolipoprotein B-100 or apoB-48, a transfer catalyzed by cholesteryl ester transfer protein (CETP) (8–10). The endocytosis of remnants of chylomicrons and a portion of remnants of very low density lipoproteins (VLDL) and also of a portion of LDL, itself a terminal product of the apoB-100 cascade, provides a means of delivery of cholesterol to hepatocytes. Whereas prebeta-1 HDL becomes incorporated into alpha HDL particles as its free cholesterol is esterified by LCAT, it is regenerated by the depletion of cholesteryl esters transferred by CETP. Thus a new metabolic cycle, the prebeta-1 HDL cycle, is identified that is an integral part of cholesterol recovery from the periphery (11, 12) (**Fig. 1**). The simultaneous expression of human apoA-I and CETP transgenes in mice results in a doubling of prebeta-1 HDL levels, consistent with these in vitro observations (13). Compound transgenic animals expressing human LCAT and human apoA-I have a 4-fold increase in plasma cholesterol levels including a 2-fold increase in HDL cholesterol levels (14). The transgenic animals have HDL of increased diameter, in accord with an increased content of cholesteryl esters, and the content of prebeta-1 HDL in plasma is significantly decreased, in agreement with the results of in vitro incubation.

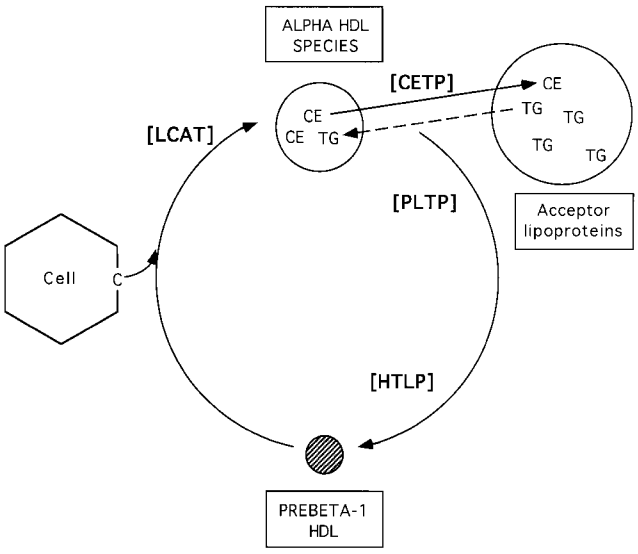

Fig. 1. The prebeta-1 HDL cycle. LCAT, lecithin: cholesterol acyltransferase; CETP, cholesteryl ester transfer protein; PLTP, phospholipid transfer protein; HTLP, hepatic triglyceride lipase. Acceptor lipoproteins can be very low density lipoproteins, chylomicrons, remnant particles derived from both, and low density lipoproteins.

The newly discovered scavenger receptor, type B1 (SR-BI) also appears to participate in the reverse transport pathway, endocytosing cholesteryl esters from HDL into hepatocytes (15). The overexpression of SR-BI in mice, transiently transfected with an adenovirus transgene, resulted in dramatic reduction of HDL levels and a large increase in elimination of cholesterol in the bile, suggesting that this receptor may channel HDL-derived cholesterol into bile (16).

Another element in the regulation of levels of prebeta-1 HDL in plasma is the activity of phospholipid transfer protein (PLTP) (17). Overexpression of PLTP results in a substantial increase in prebeta HDL (18), in agreement with the apparent formation of prebeta HDL when larger HDL particles are incubated with PLTP (19). PLTP appears to facilitate transfer of unesterified cholesterol in addition to phospholipid (20). Hepatic lipase may also be involved in formation of prebeta HDL. As cholesteryl esters are transferred from alpha HDL species to acceptor lipoproteins, there is some countertransfer of triglycerides to alpha HDL particles. Hepatic lipase is known to hydrolyze triglycerides and phospholipids of HDL (21). HDL that contain apoE are triglyceride-rich (22) and apoE may be acquired in response to enrichment with triglycerides. The acquisition of apoE by triglyceride-rich HDL thus may function to improve the particles' substrate properties for hepatic lipase. Observations in the perfused liver suggest that hepatic lipase activity may be involved in the generation of prebeta-1 HDL particles from such precursors (23).

Quantitation of prebeta-1 HDL particles in plasma would provide a means of studying the relative efficiency of the processes catalyzing the esterification of cholesterol and the transfer of cholesteryl esters to acceptor lipoproteins in the prebeta-1 HDL cycle. Because cholesterol recovery is potentially one of the mechanisms linking HDL levels in an inverse risk relationship with atherosclerosis, measurement of this metabolically active HDL species could possibly emerge as an indicator of risk.

The measurement of prebeta-1 HDL heretofore has been technically difficult, usually requiring two-dimensional separations. The development of a facile isotope dilution technique (24) now permits the examination of the behavior of prebeta-1 HDL in human populations. In this report we describe the distribution of prebeta-1

HDL in the plasma of normal humans and evaluate its association with lipoprotein levels, age, and gender.

METHODS

Subjects

One hundred thirty-six healthy subjects (90 F, 46 M) were studied. Total cholesterol and triglyceride levels were below 240 mg/dl and 180 mg/dl, respectively, in all subjects. Their ages ranged from 16 to 66, with a mean of 37.9 years. Sixteen of the women were postmenopausal and seventy-four were menstruating or pre-menopausal. Eight women were receiving exogenous estrogens, four in the reproductive years, and four postmenopausal. None of the 136 subjects was receiving medications known to affect lipids, other than estrogens. Accurate data for calculation of body mass index (BMI) were available on 92 subjects. BMI ranged from 17.9 to 41.6, mean 22.6. Only four values exceeded 30. The study was approved by the Committee on Human Research of the University of California, San Francisco. Written consent was obtained for venipuncture and the collection of personal data from the individuals studied.

Lipoprotein measurements

Blood was drawn, after a minimum 10 h fast, into tubes containing sodium EDTA, 1.7 mg/ml final concentration. The blood was chilled immediately in ice and was kept at 0°C during the separation of plasma and during storage up to 24 h before lipoprotein measurements were made. The triglyceride and cholesterol contents of lipoprotein fractions were measured on lipoproteins separated by sequential ultracentrifugation in 92 samples (25). Total triglyceride and cholesterol contents were measured in all samples by automated fluorescence analysis (Hoffman-LaRoche, Inc., Nutley, NJ) and HDL was measured by precipitation with heparin and manganese (26) in forty-four samples. For those samples the LDL cholesterol content was calculated by the formula:

$$\text{LDL-C} = \text{Total Chol} - \left(\frac{\text{TG}}{5} + \text{HDL-C}\right)$$

Where LDL cholesterol and HDL cholesterol measurements were available, VLDL cholesterol was approximated as the difference between total cholesterol levels and the sum of HDL cholesterol and LDL cholesterol levels.

Prebeta-1 HDL was measured by isotope dilution (24). Briefly, isotopically labeled prebeta-1 HDL was prepared as follows. The total apoA-I-containing lipoprotein fraction was sequestered from normal human plasma by selected affinity immunosorption (2). The prebeta-1 HDL fraction was separated from other apoA-I lipoproteins by electrophoresis in starch block (1), avoiding the lipoproteins of the anodic shoulder of the prebeta HDL peak that contains prebeta lipoprotein species of higher molecular weight. Purity of the prebeta-1 lipoproteins was verified by electrophoresis in 3–34% non-denaturing gradient acrylamide gels where the apparent molecular weight was 67 kDa and by demonstration of prebeta electrophoretic mobility in non-gradient gel electrophoresis (27). The prebeta-1 HDL to be used as the dilution probe was labeled as a tritiated adduct using N-succinimidyl [2,3-^{3}H] propionate (Amersham). The unbound label was removed by extensive dialysis at 0°C.

A labeled probe sample was added to plasma samples at 0°C with gentle but thorough mixing. The diluted prebeta-1 HDL pool of plasma was sampled by ultrafiltration using a centrifugal ultrafilter that discriminates between prebeta-1 HDL and other apoA-I-containing lipoproteins (microcon-100, Amicon). The specific activity of the ultrafiltrate was determined by scintillation counting and the apoA-I contents of the plasma and the ultrafiltrate were measured by an ELISA technique developed to quantitate apoA-I equally in alpha and prebeta-1 HDL species (28). The content of prebeta-1 HDL in plasma was then calculated by the isotope dilution equation:

$$\frac{\text{probe radioactivity added}}{\text{specific activity of ultrafiltrate}} = \text{mass of original prebeta-1 HDL pool in plasma}$$

All measurements were made in duplicate. Paired values that did not agree within 10 μg were repeated. Values were rejected if final duplicates did not agree within 10 μg. Prebeta-1 HDL levels were expressed as absolute levels of prebeta-1 HDL apoA-I and as the percent of total plasma apoA-I represented by prebeta-1 HDL.

Statistical methods

The SAS (1990) system for statistical analysis was used, principally procedure REG for multiple linear regression models. On candidate sets of predictors, subset regression models were ranked by the Cp criterion. Models having the minimal value of AICC (the bias-corrected Akaike information criterion (29) were selected. Partial regression plots, residual plots, and Cook's distance were examined to check analysis assumptions and detect outliers. Power transformations of response variables were examined using the methods of Box and Cox (30). Covariate-adjusted means for levels of categorical factors were generated using procedure GLM (least-squares means).

TABLE 1. Lipid and Lipoprotein levels

Subjects (n)	ApoA-I	% Prebeta	Abs. Prebeta	HDL Chol	HDL TG	Total TG	Total Chol	LDL Chol	LDL TG	VLDL Chol	VLDL TG
	mg/ml		*μg/ml*				*mg/dl*				
All (136)	1.23 ± 0.3	6.1 ± 3.6	73 ± 44	54 ± 13	17 ± 7	79 ± 29	178 ± 29	109 ± 25	34 ± 6	8 ± 5	36 ± 20
Women (90)	1.25 ± 0.31	5.5 ± 3.3	68 ± 40	58 ± 12	19 ± 6	77 ± 28	179 ± 29	107 ± 25	34 ± 6	7 ± 4	31 ± 18
Men (46)	1.16 ± 0.27	7.2 ± 4.0	84 ± 49	48 ± 11	11 ± 3	81 ± 27	175 ± 28	112 ± 24	33 ± 6	10 ± 5	44 ± 21
Post-menopausal women, no estrogen (12)	1.53 ± 0.37	5.8 ± 4.4	80 ± 47	61 ± 15	18 ± 6	94 ± 29	198 ± 27	124 ± 23	34 ± 6	8 ± 5	35 ± 18
Menstruating women, no estrogen (70)	1.16 ± 0.20	5.5 ± 3.2	63 ± 37	56 ± 11	19 ± 7	71 ± 27	173 ± 28	103 ± 24	32 ± 5	6 ± 3	27 ± 14

Values given as mean ± SD. ApoA-I, total plasma apolipoprotein A-I; % prebeta, percent of total apoA-I in prebeta-1 HDL; Abs. prebeta, concentration of prebeta-1 HDL in plasma; HDL, LDL, VLDL, high, low, and very low density lipoproteins; chol, cholesterol; TG, triglycerides.

RESULTS

Summary measures

Prebeta-1 HDL as percent of total plasma apoA-I ranged from 0.47 to 19.65, with an average of 6.10, median 5.39 (10th percentile 1.79 and 90th percentile 10.0) (**Table 1**). Absolute levels of prebeta-1 HDL-associated apoA-I ranged from 5 to 257 μg/ml, with an average of 73 μg/ml, median 66 μg/ml (10th percentile 22 and 90th percentile 131 μg/ml). As is evident from the asymmetry with respect to the median, both of these measures had distributions that were substantially skewed toward higher levels (skewness coefficients were 1.06 and 1.31, respectively. Non-normality of distribution was highly significant ($P < 0.0001$ for both measures) (**Fig. 2** and **Fig. 3**).

Preliminary screening

The potential predictors of the percentage of prebeta and absolute prebeta-1 HDL were the absolute level of apoA-I, triglycerides, HDL cholesterol, LDL cholesterol, VLDL cholesterol, BMI, age, and gender. Of these, apoA-I, total cholesterol, HDL cholesterol, and gender (coded as male = 1, female = 2) had Pearson correlations of some significance $P < 0.1$) with at least one of the response variables (**Table 2**).

Two alternatives to the gender categories were considered that utilized information on endogenous or supplemental estrogen. An "estrogen status" variable (ESTRSTAT) had category 1 for males and postmenopausal females without estrogen supplementation, and category 2 for menstruating females and postmenopausal females with estrogen supplementation. A second recoding with three categories (GENESTR) separated the females from category 1. Preliminary models also including apoA-I, triglycerides, and HDL cholesterol showed no significant differences for gender or the alternatives ($P > 0.16$), and neither of the latter exhibited substantial superiority to gender. The alternative codings are reconsidered below using final models developed to include gender.

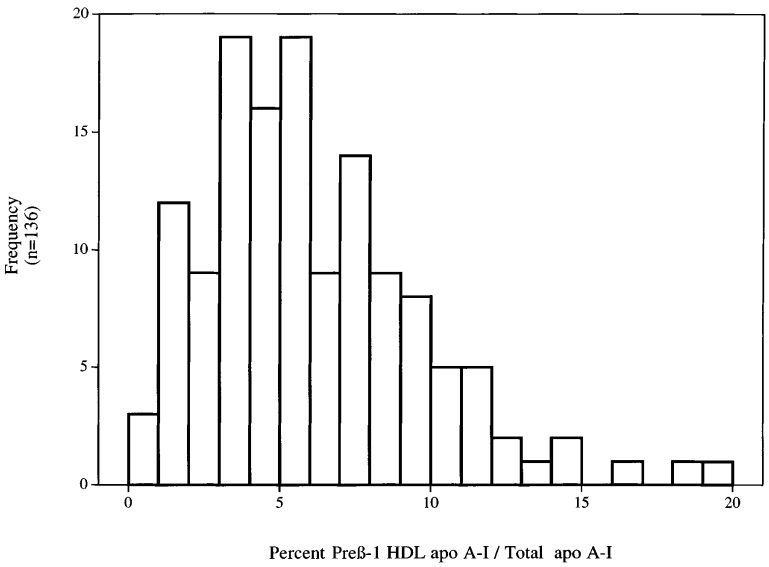

Fig. 2. Frequency distribution of percent prebeta-1 HDL as percent of total apolipoprotein A-I in plasma samples from normolipidemic subjects.

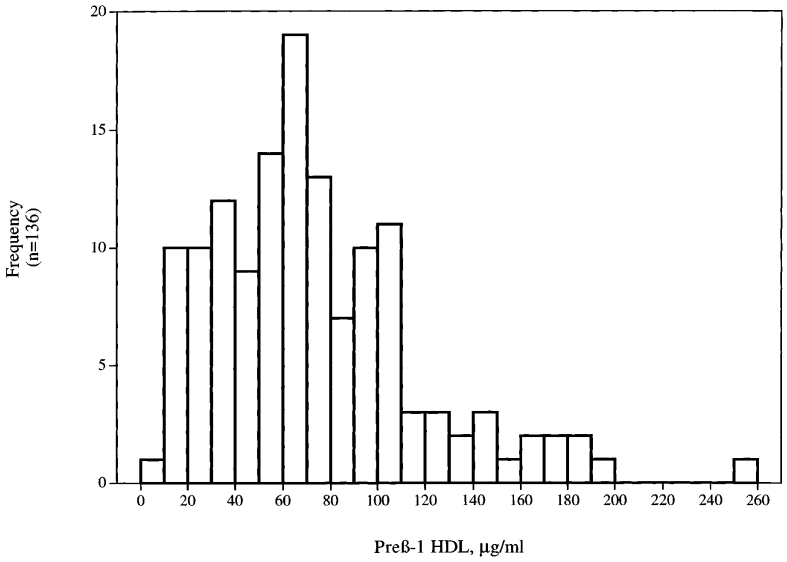

Fig. 3. Frequency distribution of absolute levels of prebeta-1 HDL in plasma samples from normolipidemic subjects, µg/ml.

On the subset on which BMI could be computed (n = 92), BMI was not significant in preliminary models also including apoA-I, triglycerides, HDL cholesterol, and gender ($P > 0.7$). Further, there were no significant differences in means or medians of the response variables between the subsets with and without BMI ($P > 0.2$). Therefore, BMI was not further considered as a predictor.

Final Models

Candidate predictors for final models included apoA-I, triglycerides, HDL cholesterol, LDL cholesterol, VLDL cholesterol, age, and gender. Using the methodology of Box and Cox (30), power transformations of the response variables in models including all candidate predictors showed the 0.4 power to be optimal, to the nearest 0.1. For convenience, the 0.5 power or square root transformation was examined. Models ranked as first using the Cp criterion also had minimum AICC (**Table 3**).

For PCTPREB (percent prebeta-1 HDL) and RPCT PREB (square root of percent of total apoA-I contained in prebeta-1 HDL) the selected models included only one predictor, HDL cholesterol. For ABSPREB (absolute prebeta-1 HDL), the selected predictors were apoA-I, HDL cholesterol, and age, while for RAB SPREB (square root of the absolute level of prebeta-1 HDL), the same predictors were included as well as gender. For each of these four response variables, models with and without gender but otherwise including the same predictors were ranked 1st or 2nd. Thus, while gender was not a significant predictor in any of these models ($0.15 < P < 0.23$), models including gender were the best or next to best that could be produced according to the selection criterion used.

The models excluding gender are summarized in Table 3. These show that HDL cholesterol was negatively related to the response in all instances ($P < 0.0001$), while age was at most a weakly significant negative predictor ($P =$

TABLE 2. Correlations of response variables with potential predictors

Variable	ApoA-I	TG	HDLC	LDLC	VLDLC	Age	BMI	Gender
Percent prebeta-1 HDL								
r	0.162	0.069	−0.354	−0.006	0.021	−0.138	0.112	−0.218
P	0.060	0.424	0.001	0.942	0.812	0.109	0.286	0.011
n	136	136	136	136	136	136	92	136
Absolute prebeta-1 HDL								
r	0.222	0.156	−0.161	0.101	0.063	−0.007	0.129	−0.175
P	0.010	0.070	0.062	0.243	0.469	0.939	0.220	0.041
n	136	136	136	136	136	136	92	136

(Pearson Correlation Coeffs./Prob> |R| under Ho: Rho 0/Number of Obs.)

TG, triglycerides; HDLC, LDLC, and VLDLC, HDL cholesterol, LDL cholesterol, and VLDL cholesterol, respectively, in mg/dl; BMI, body mass index; *r*, Pearson Correlation Coefficients; *P*, *P* value; n, number of subjects.

TABLE 3. Parameter estimates for final models

Parameter Estimates for	Parameter Estimates	Standard Error	Prob > T
PCTPREB			
INTERCEP	11.53	1.279	0.0001
HDL-Chol	−0.1001	0.023	0.0001
Square root of percent prebeta-1 HDL			
INTERCEP	3.500	0.257	0.0001
HDL-Chol	−0.0209	0.005	0.0001
ABSPREB			
INTERCEP	0.0803	0.019	0.0001
ApoA-I	0.0700	0.015	0.0001
HDL-Chol	−0.0013	0.000	0.0001
Age	−0.0005	0.000	0.1178
Square root of absolute prebeta-1 HDL			
INTERCEP	0.2785	0.034	0.0001
ApoA-I	0.1300	0.027	0.0001
HDL-Chol	−0.0025	0.000	0.0001
Age	−0.0010	0.000	0.0825

PCTPREB, percent of total apoA-I in prebeta-1 HDL; ABSPREB, absolute level of apoA-I in prebeta-1 HDL.

0.12 for ABSPREB, $P = 0.082$ for RABSPREB). The same findings hold for models including gender, with only minor changes in significance levels. In all instances, the estimated effect of being female was negative.

For the final models for the untransformed response variables, the three recordings of gender and estrogen status were reexamined. **Table 4** presents adjusted means for gender and ESTRSTAT. Adjusted means for GENESTR suggest that postmenopausal women without estrogen (category 3) are more similar to males (category 1) in these responses than they are to women with estrogen (category 2). Also, the differences between categories 1 (males) and 2 are similar to the differences between males and all females. Results for ESTRSTAT were similar to those for gender.

To more fully examine effects of plasma triglyceride

TABLE 4. Adjusted means of response variables by gender, ESTRSTAT, and GENESTR

Variable	LSMEAN	Std Err	*P* Value		
Gender	PCTPREB				
Males	6.678	0.524		0.22	
Females	5.869	0.371			
	ABSPREB				
Males	0.081	0.006		0.15	
Females	0.069	0.004			
ESTRSTAT	PCTPREB				
Cat. 1	6.626	0.456		0.17	
Cat. 2	5.781	0.396			
	ABSPREB				
Cat. 1	0.080	0.006		0.11	
Cat. 2	0.068	0.005			
GENESTR	PCTPREB		1	2	3
Cat. 1 (Males)	6.674	0.525		0.18	0.85
Cat. 2	5.778	0.398	0.19		0.51
Cat. 3	6.463	0.993	0.85	0.52	
	ABSPREB				
Cat. 1 (Males)	0.081	0.006		0.12	0.88
Cat. 2	0.068	0.005	0.12		0.48
Cat 3	0.078	0.014	0.88	0.48	

All values are least squares means (LSMEAN). Category 1 (Cat. 1) includes males and postmenopausal females without supplemental estrogen; category 2 (Cat. 2) includes premenopausal females and postmenopausal females treated with estrogen; category 3 (Cat. 3) includes postmenopausal females without estrogen. Abbreviations: ESTRSTAT, estrogen-based statistical variable; GENESTR, gender- and estrogen-based statistical variable; PCTPREB, percent of total apoA-I in prebeta-1 HDL; ABSPREB, absolute amount of apoA-I in prebeta-I HDL.

level, that predictor was added to final models for the untransformed response variables. In models without gender, triglycerides had a slope of 0.00516 ($P = 0.96$) for PCTPREB and a slope of 0.000046 ($P = 0.75$) for ABSPREB. Results in models including gender were quite similar.

DISCUSSION

Contemporary models of HDL metabolism assign an important role to prebeta-1 HDL (Fig. 1). It is an early acquisitor of free cholesterol from peripheral tissues (5). The demonstration that the process is cyclic (12), involving both the conversion of prebeta-1 HDL to alpha HDL species and its regeneration during the transfer of cholesteryl esters to acceptor lipoproteins, suggests that the steady-state level of prebeta-1 HDL in plasma should reflect the relative rates of LCAT-mediated esterification, and CETP-mediated transfer. Indeed elevated levels of prebeta-1 HDL, measured by two-dimensional electrophoresis have been reported in individuals with hypertriglyceridemia (31). A large mass of triglycerides in plasma, reflecting increased levels of VLDL or chylomicrons, would be expected to provide an entropic sink, favoring the transfer of cholesteryl esters, thus leading to increased formation of prebeta-1 HDL. This is consistent with findings in compound transgenic animals with hypertriglyceridemia due to overexpression of human apolipoprotein C-III in which the addition of the human CETP gene resulted in depletion of cholesteryl esters in HDL (32). The resulting HDL had smaller diameters and the fractional catabolic rate for apoA-I was significantly increased. The weak association between absolute or percent prebeta-1 HDL levels with triglycerides in the present study suggests that such an effect is overshadowed by other determinants within the limited range of plasma triglycerides in normolipidemic subjects. It is possible that the entropic term for cholesteryl ester transfer may be larger per unit of triglyceride mass in large diameter VLDL and chylomicrons than in smaller particles typically found in normolipidemic individuals. The lack of association of prebeta-1 HDL levels or percentage prebeta-1 HDL with LDL cholesterol may reflect a relatively small entropic term due to the ordered nature of cholesteryl esters in LDL.

One of the most prominent predictors (negative) of percent prebeta-1 HDL and absolute prebeta-1 HDL levels is HDL cholesterol. This is consistent with the model in Fig. 1, in which the level of prebeta-1 HDL would be expected to be relatively low when there is a high content of cholesteryl esters in alpha HDL particles. On the other hand, high levels of apoA-I are correlated positively with absolute levels of prebeta-1 HDL, suggesting that increasing the availability of apoA-I may favor the maintenance of a higher steady state level of prebeta-1 HDL, even in the presence of increased levels of alpha HDL particles. This possibility is supported by the observation that expression of a human apoA-I transgene results in increased levels of prebeta HDL in rabbits (33). It is interesting that both the absolute levels of prebeta-1 HDL and the percent of total apoA-I found in the prebeta-1 fraction distribute with significant skew toward higher levels, similar to total cholesterol levels, LDL cholesterol levels, and total triglycerides in the general population. The skew is apparently not attributable to the levels of triglycerides or cholesterol per se, however, because of the lack of association between these variables observed in our statistical models.

In our group of normolipidemic subjects, no association was found between either of the prebeta-1 HDL values and body mass index. The lack of significantly obese subjects in this cohort limits the interpretation of this finding. It is possible that alterations in lipoprotein metabolism associated with significant obesity may well affect one or more of the determinants of levels of prebeta-1 HDL in plasma. The mechanisms underlying the negative trend detected with age are not readily apparent. This correlation was observed almost entirely with percent prebeta-1 HDL.

Both percent prebeta-1 HDL and absolute prebeta-1 HDL were significantly correlated with gender in bivariate relationships. In both single and multiple predictor models female gender was associated with lower values. Though the numbers of subjects limit the strength of the observation, the finding that adjusted mean levels for postmenopausal women without estrogen supplementation more closely resemble those of males suggest that the effect is mediated by ovarian steroid hormones. This could reflect the effect of estrogen in down-regulating the SR-BI receptor (34). If the SR-BI receptor contributes significantly to the removal of cholesteryl esters from HDL, its down-regulation by estrogen could increase the plasma content of HDL cholesteryl esters and hence the content of alpha HDL particles in plasma. The latter is associated negatively with plasma levels of prebeta-1 HDL in this study. Indeed, down-regulation of the SR-BI receptor could be a major determinant of the differences in HDL cholesterol levels between men and menstruating women.

Another mechanism that could underlie the lower levels of prebeta-1 HDL in women is their relatively lower levels of hepatic lipase activity (35). Hepatic lipase has been demonstrated to promote the formation of prebeta HDL in vitro (23). Also, prebeta HDL appears to be formed during the perfusion of liver with HDL_2

(23). Decreased activity of either SR-BI or hepatic lipase could contribute to the observed increase in mean diameters of HDL particles among women (36).

The ability to measure prebeta-1 HDL levels readily in plasma can yield important insights into the determinants of HDL speciation. Prebeta-1 HDL mass levels in plasma represent a kinetic steady state in which diverse determinants operate to increase or decrease levels. Thus the import for net cholesterol recovery may depend on the kinetic context. For example, a high rate of transfer of cholesteryl esters to acceptor lipoproteins may elevate prebeta HDL levels, but if esterification rates are also relatively high, prebeta-1 HDL levels would tend to be reduced, and net reverse transport of cholesterol could be favored. Furthermore, the inference that prebeta-1 HDL is formed from phospholipid and unesterified cholesterol from the surface monolayers of triglyceride-rich lipoproteins during intravascular lipolysis suggests that high levels could be the result of a high lipolytic rate as is observed in endurance athletes. On the other hand, a slow rate of esterification by LCAT would result in elevated levels of prebeta-1 HDL and impaired recovery of cholesterol. This may be the case in many individuals who have accelerated atherosclerosis as suggested by a preliminary study by Miida et al. (37). They found higher levels of prebeta-1 HDL in twenty patients with coronary disease than among twenty control subjects. The LCAT concentrations were lower among the patients with coronary disease than among controls.

Clearly, relative changes in the activities of LCAT, CETP, PLTP, and also lipoprotein lipase and hepatic lipase could individually alter the steady state levels of prebeta-1 HDL in plasma. Modulating effects of apolipoproteins such as apoA-II on the LCAT reaction (14) and other processes (38) and upon the molecular speciation of HDL may also have significant impact on prebeta-1 HDL levels and upon the rate of reverse cholesterol transport. Thus, measurement of prebeta-1 HDL levels in plasma will provide a functional measurement of value in the interpretation of the complex processes involved in HDL metabolism and the cholesterol recovery process. It may also emerge as a significant predictor of risk of atherosclerosis among patients with impaired cholesterol retrieval.

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