Apolipoprotein L-I is positively associated with hyperglycemia and plasma triglycerides in CAD patients with low HDL

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Abstract **Apolipoprotein L-I (apoL-I) is present on a subset of HDL particles and is positively correlated with plasma triglycerides (TGs). We measured plasma apoL-I levels in coronary artery disease (CAD) subjects with low HDL who were enrolled in an angiographic CAD prevention trial. At baseline, apoL-I levels (n = 136; range, 2.2–64.1 μg/ml) were right skewed with a large degree of variability. Multivariate analysis for biological determinants of apoL-I revealed that the log of VLDL-TG (+0.17; $P < 0.05$) and hyperglycemia (HG; +0.26; $P < 0.005$) independently predicted apoL-I level. Hyperglycemic patients (n = 24) had mean apoL-I levels >50% higher than normoglycemic subjects (n = 112; 13.2 vs. 8.3 μg/ml, respectively; $P < 0.001$). No relationship between apoL-I level and change in CAD was found ($r = 0.06$, $P = 0.49$). Simvastatin-niacin therapy did not alter apoL-I levels (n = 34; $P = 0.27$), whereas antioxidant vitamins alone increased apoL-I by >50% (n = 36; $P < 0.01$). Genotyping of a known apoL-I polymorphism (Lys166Glu) did not independently account for any of the variability in apoL-I levels. In conclusion, we found TG and HG to be the strongest predictors of apoL-I within a dyslipidemic CAD population. These data provide further characterization of the novel HDL-associated apoL-I.**—Albert, T. S. E., P. N. Duchateau, S. S. Deeb, C. R. Pullinger, M. H. Cho, D. C. Heilbron, M. J. Malloy, J. P. Kane, and B. G. Brown. **Apolipoprotein L-I is positively associated with hyperglycemia and plasma triglycerides in CAD patients with low HDL.** ***J. Lipid Res.*** **2005.** 46: **469–474.**

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We originally identified, cloned, and characterized a novel apolipoprotein termed apolipoprotein L (apoL) in 1997 (1). Subsequently, our group and another have identified a cluster of apoL genes on chromosome 22 that encode up to six apoL proteins (apoL-I to apoL-VI) (2, 3). These genes have putative sterol response elements in their promoters, and sequence analysis has revealed conserved amphipathic helices, suggesting that they may be involved in lipid metabolism (2, 3). ApoL proteins are found in plasma and a variety of tissues, including liver, lung, brain, pancreas, placenta, and vascular endothelium (2–4). In addition, apoL-I was recently found to be the trypanosome lytic factor in human serum (5), suggesting that the apoL family may play an important role in biology.

ApoL-I, the original protein described in this family, is a 42 kDa protein that is found in the plasma mainly associated with large-diameter HDL particles (found on ~10% of apoA-I-containing lipoproteins) (1). Recently, apoL-I protein levels were found to be positively associated with plasma triglycerides (TGs) in both normolipidemic and dyslipidemic subjects (6). Because apoL-I is found on HDL particles, is associated with TG, and as yet has no clear function, we elected to measure apoL-I in the HDL Atherosclerosis Treatment Study (HATS) (7) population. HATS was a coronary artery disease (CAD) prevention trial that enrolled patients with documented CAD who, as a group, had dyslipidemia characterized by low plasma HDL-cholesterol (HDL-C) concentrations and mildly increased TGs. Our goals were to determine what metabolic factor(s) were most closely associated with apoL-I plasma levels, to determine the distribution of apoL-I in a dyslipidemic population with CAD, and to determine if apoL-I was associated with the progression or regression of CAD and/or affected by drug therapy. After a skewed, bimodal-appearing distribution of apoL-I levels was observed, genetic analysis of a polymorphism of the apoL-I gene (the Lys166Glu variant) (2) was also undertaken to evaluate the relationship between this gene variant and apoL-I level.

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METHODS

Study design

HATS (7) was a quantitative angiographic clinical trial aimed at assessing the effects of lipid-altering drugs and/or antioxidant vitamins on CAD progression and regression. HATS enrolled patients with clinical and anatomic CAD, low HDL-C (≤35 mg/dl for men, ≤40 mg/dl for woman), and LDL-C ≤145 mg/dl. There were four treatment arms: niacin plus simvastatin; antioxidant vitamin supplements (vitamins C and E, β-carotene, and selenium); combination of niacin-simvastatin plus vitamins; or placebo for all drugs. Patients were followed on average for 3 years. The primary patient end point was the average change in percentage diameter stenosis for nine proximal coronary artery segments (%ΔS). Of the 160 individuals who were enrolled in HATS, 146 completed the protocol. Plasma was available for on- and off-treatment apoL-I measurement for 137 subjects. At the time of enrollment in HATS, patients read and signed a consent form that had been approved by the university human subjects committee.

ApoL-I measurements

On-therapy apoL-I measurements were obtained after 24 months of treatment, and off-therapy measurements were obtained 2 months (SD < 2 days) after stopping all treatments. ApoL-I was quantified by a competitive ELISA, described previously (1). Briefly, samples were prepared by serially diluting previously frozen plasma (−70°C) and incubating with a constant amount of rabbit anti-human apoL-I antibody. Samples were then added to a 96-well plate coated with immunopurified apoA-I-containing lipoprotein to quantify uncoupled antibody. Purified apoL-I was used as a standard. The coefficients of variation, interassay and intra-assay, respectively, were 12.5% and 11%. The anti-apoL-I antibody does not have any reactivity with the other apoL proteins.

Lipid, lipoprotein, and metabolic measurements

Fasting plasma lipid and lipoprotein levels, fasting glucose (FG) and fasting insulin, and fibrinogen levels were determined before, during, and at the completion of the study. The lipid and lipoprotein measurements were made at the Northwest Lipid Research Laboratories as previously described (7). Measurements included cholesterol and TGs in whole serum and in VLDL, intermediate density lipoprotein, LDL, and HDL (including HDL_2 and HDL_3) (8). ApoB, apoA-I, apoA-II, and lipoprotein [a] were measured by immunochemical means.

Patient characteristics

Body mass index (BMI), age, smoking and hypertension history, and hyperglycemia (HG) status were considered in our analysis. A person was considered a smoker if actively smoking at the time of apoL-I off-therapy measurement, whereas hypertension was based on history at enrollment. A patient was classified as hyperglycemic if he or she had either type 2 diabetes mellitus (DM2) or impaired fasting glucose (IFG). DM2 patients had a prior clinical diagnosis and treatment with hypoglycemic agents or at least two separate FG values ≥ 126 mg/dl around the time of study initiation (9). IFG patients were defined by repeated FG ≥ 110 mg/dl around the time of study initiation. Normoglycemic (NG) subjects had FG < 110 mg/dl. One HATS patient had type 1 diabetes mellitus and was excluded from analysis of apoL-I in this study after independent associations of HG with apoL-I were found. Thus, apoL-I levels for only 136 patients are presented.

ApoL-I genotyping

Of the 160 HATS subjects, DNA was available for genotyping in 142. The apoL-I gene has four known single nucleotide polymorphisms in exon 7, which are in linkage disequilibrium and together describe two distinct haplotypes (2). We genotyped the Lys166Glu polymorphism of exon 7, which changes the lysine residue at position 166 to glutamic acid. Briefly, a 580 bp fragment of genomic DNA was amplified by PCR using the following primers: 7F, 5′-GTCAGGATGGTCTCAATCTCCTG-3′; and 4R, 5′-CACCTCCTTCAATTTGTCAAGGC-3′. PCR was performed for 35 cycles at 94°C for 10 s, 66°C for 30 s, and 72°C for 50 s. The PCR product was digested with the restriction enzyme *Hin*dIII overnight, and the resulting fragments were separated on a 1% agarose gel. The A allele is cut with *Hin*dIII to give 265 and 315 bp fragments. The G allele is uncut.

Statistical analysis

Quantitative variables within or between groups were compared using paired or unpaired *t*-tests, respectively. Comparisons of multiple means were done by ANOVA. Proportions were compared using Chi-square analysis. Variables exhibiting right skewness in distribution (skewness coefficient > 2) generally were transformed to $\log_{10}$ for analysis. Means are presented with 95% confidence intervals (CIs). For variables requiring logarithmic transformations, geometric means (GMs) and approximate CIs based on antilogs are presented. Univariate correlations were done using Spearman's rank correlation. Multivariate regression modeling used a forward stepwise regression algorithm (F-to-enter = 4). Normality of distribution was tested using SAS procedure UNIVARIATE, and SAS procedure CLUSTER was used for cluster analysis. Determination of off-therapy apoL-I predictors was done using metabolic variables measured at the same time. $P < 0.05$ was considered significant.

RESULTS

Baseline characteristics

Table 1 shows the clinical and lipid/lipoprotein/metabolic characteristics of 136 HATS subjects at the time of their off-treatment apoL-I measurement. Overall, this group was similar to the whole HATS population (7). Approximately 18% of the patients had DM2 or IFG, 26% were active smokers, and 45% had a history of hypertension.

ApoL-I is right skewed with a bimodal appearance

Figure 1 shows the right skewed, nonnormal distribution of apoL-I in the HATS population (all four of the tests examining for normality of log apoL-I produced $P < 0.01$). The GM of plasma off-therapy apoL-I concentration was 9.0 μg/ml (95% CI, 8.1–9.9; range, 2.2–64.1 μg/ml). As illustrated by Fig. 1B, there appeared to be two distinct modes in the apoL-I distribution. Using a variety of clustering methods, the predominant finding for log apoL-I was of two clusters, each containing one of the modes, whose boundary was between the observed apoL-I values of 16.7 and 23.1 μg/ml. Corresponding to those clusters, 88% (120 of 136) of the patients had apoL-I levels < 17 μg/ml ["low"; GM = 7.9 μg/ml (CI, 7.4–8.4)] and 12% (16 of 136) had levels > 23 μg/ml ["high"; GM = 36.4 μg/ml (CI, 31.4–41.4)].

TABLE 1. Off-therapy characteristics of 136 HATS subjects

Age (years)	56 (55–58)
Male (%)	118 (87)
Type 2 diabetes mellitus (%)	15 (11)
Impaired FG (%)	9 (7)
Active smoker (%)	36 (26)
Hypertension (%)	61 (45)
FG (mg/dl)	104 (99–108)
BMI (kg/m^2)	30 (29–31)
Total cholesterol (mg/dl)	199 (193–205)
Total TG (mg/dl)	179 (164–196)
LDL-cholesterol (mg/dl)	129 (123–135)
HDL-cholesterol (mg/dl)	33 (32–34)
HDL_2 (mg/dl)	3.9 (3.6–4.2)
ApoB (mg/dl)	113 (110–117)
ApoA-I (mg/dl)	114 (111–116)
ApoA-II (mg/dl)	29 (29–30)
Lipoprotein [a] (mg/dl)	9.0 (6.8–11.9)

ApoB, apolipoprotein B; BMI, body mass index; FG, fasting glucose; HATS, HDL Atherosclerosis Treatment Study; TG, triglyceride. Quantitative variables are presented as arithmetic means [95% confidence intervals (CIs)] except for FG, total TG, and lipoprotein [a], which were logarithmically transformed and presented as geometric means [GMs (95% CIs)]. n = 135 for ApoA-II and n = 133 for Lipoprotein [a].

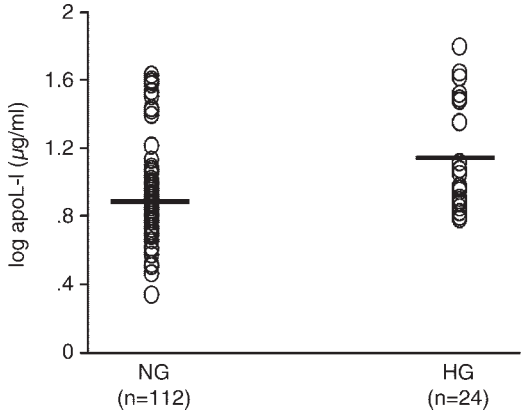

Fig. 2. Distribution of off-therapy apoL-I in normoglycemic (NG) and hyperglycemic (HG) patients. The geometric mean (thick lines) was greater for the HG group than for the NG group. The large range of apoL-I values in both the NG and HG groups is shown.

VLDL-TG and HG independently predict apoL-I level

Multivariate regression analysis of log apoL-I vs. 24 predictors revealed log VLDL-TG ($r = +0.17$, $P = 0.042$) and HG ($r = +0.26$, $P = 0.003$) to be the only significant, independent predictors of log apoL-I. Combined, they generated a multiple $R = 0.34$ with $P < 0.0005$. If HG was excluded from the model, FG became an independent predictor along with log VLDL-TG, but the model was weaker (multiple $R = 0.32$, $P < 0.001$).

ApoL-I in hyperglycemic subjects

As predicted by the multivariate analysis and shown in **Fig. 2**, HG patients had higher apoL-I levels than NG subjects. The GM of apoL-I in HG patients [n = 24, GM = 13.2 μg/ml (CI, 9.9–17.8)] was ~60% greater than that of NG subjects [n = 112, GM = 8.3 μg/ml (CI, 7.5–9.1)] ($P = 0.0004$). HG patients were also more prevalent in the high apoL-I cluster (7 of 16) than in the low apoL-I cluster (17 of 120) ($P = 0.004$). After correcting for the difference in VLDL-TG between groups, apoL-I levels were still ~50% greater in HG patients than in NG subjects ($P = 0.003$).

ApoL-I levels and CAD progression

Positive associations of in-treatment levels of a variable with change in CAD (%ΔS) are suggestive that the variable, or another highly correlated variable, is proatherogenic (7, 8, 10). ApoL-I on-treatment levels did not correlate with %ΔS in this population (n = 136; $r = 0.06$, $P = 0.49$).

Effect of lipid-altering and antioxidant vitamin therapy on apoL-I levels

Table 2 shows mean apoL-I levels on and off therapy for each of the four treatment groups. The antioxidant vitamin-only group (n = 36) was the only one to have a significant change in log apoL-I level on treatment ($P = 0.007$), with a 56% increase in the GM of apoL-I on therapy. The

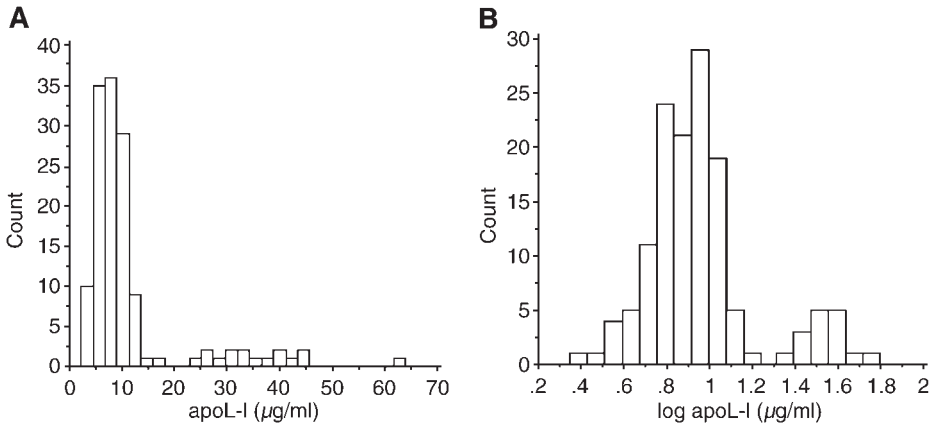

Fig. 1. Off-therapy apolipoprotein L-I (apoL-I) values in the HDL Atherosclerosis Treatment Study population presented in histograms as normal and $\log_{10}$-transformed values. The right skewed distribution of apoL-I values is shown, with the log apoL-I histogram suggesting a bimodal distribution of apoL-I.

TABLE 2. ApoL-I levels on and off therapy by treatment group

Group	N	Off Therapy	On Therapy	P
		μg/ml		
Placebo	32	9.0 (1.1)	10.2 (1.1)	0.31
Simvastatin-niacin	34	9.7 (1.1)	11.0 (1.1)	0.27
Simvastatin-niacin + antioxidant vitamins	34	8.4 (1.1)	10.0 (1.1)	0.18
Antioxidant vitamins	36	8.9 (1.1)	13.9 (1.2)	0.007

ApoL-I plasma levels are presented as GMs and SEM based on antilogs of logarithmically transformed data.

groups taking placebo, simvastatin + niacin, and simvastatin + niacin + antioxidant vitamins showed no significant change in apoL-I levels. The antioxidant vitamin-only group did not show an increase in VLDL-TG or FG level while on therapy (data not shown).

The apoL-I Lys166Glu variant

The Lys166Glu variant was genotyped in 142 HATS patients to assess for a relationship of this variant with apoL-I level. The Lys166 allele frequency was 0.796 and the Glu166 allele frequency was 0.204. The genotype frequencies were in Hardy-Weinberg equilibrium, with baseline patient characteristics by genotype shown in **Table 3**. ApoL-I levels were available for 124 of the 142 genotyped patients (87%). There was a tendency toward higher apoL-I levels in the Glu166 allele homozygotes, although these patients also had higher FG levels. The Glu166 allele was not more common in the cluster of patients with high apoL-I levels (frequency of Glu166 allele low cluster = 0.200 vs. high cluster = 0.269; $P = 0.43$), and the Lys166Glu genotype did not provide any independent predictive value when added to our multivariate apoL-I model (data not shown).

DISCUSSION

We present a series of interesting findings regarding the newly described apoL-I (1–3, 6) among patients enrolled in HATS (7). In this population, apoL-I levels were right skewed, with a bimodal-appearing distribution. Of the many metabolic variables measured, VLDL-TG and hyperglycemic state were the strongest independent predictors of apoL-I. No association was found between in-treatment apoL-I levels and progression of CAD. Interestingly, apoL-I levels were significantly increased by antioxidant vitamin therapy.

Although a right skewed distribution of apoL-I has been seen previously in a population of normolipidemic individuals (6), apoL-I appeared to have a bimodal distribution in our population of dyslipidemic CAD patients. The presence of bimodality is supported by the fact that log transformation of apoL-I did not normalize the apoL-I distribution, as would be expected for most skewed variables with a single mode. In addition, cluster analysis suggested that there were two distinct clusters of apoL-I values. The fact that there may be two apoL-I modes may be biologically important, as there appeared to be a group of patients with mean apoL-I levels >4-fold higher than the rest of this population.

A genetic explanation for the observed apoL-I variability was investigated. Genetic variation is known to play a role in the distribution of some factors important in lipid metabolism (e.g., hepatic lipase, paraoxonase, cholesteryl ester transfer protein) (11–15). The Lys166Glu apoL-I polymorphism did not, however, appear to account for the high degree of apoL-I variability that we observed. Although apoL-I levels were higher in the Glu homozygotes, this occurred in the setting of correspondingly higher FG levels, making an independent relationship difficult to support. In addition, the lack of an independent association of the Lys166Glu genotype with apoL-I levels in our multivariate model further suggests that a strong relationship between the Lys166Glu polymorphism and apoL-I plasma level did not exist in our study population. These findings, however, must be considered in light of the fact that our sample was significantly underpowered. Using our observed apoL-I variability, we calculate that ~500 patients would need to be studied to have an 80% power of showing a difference in apoL-I levels between Lys homozygotes and Glu carriers at the $P \leq 0.05$ confidence level.

As off-therapy apoL-I was measured at the end of the study, it is possible that some lingering effect of randomization might explain our findings of a skewed apoL-I distribution. This, however, is unlikely because randomization was proportionally similar between the high and low apoL-I groups ($P = 0.88$). In addition, measurement of pretreatment apoL-I levels in a subgroup (n = 28) of high and low apoL-I patients revealed that, even before ran-

TABLE 3. Baseline characteristics of the HATS population by apoL-I genotype

Variable	Lys166/Lys166	Lys166/Glu166	Glu166/Glu166	P
Number (%)	91 (64.1)	44 (31.0)	7 (4.9)	
Men/women	79/12	39/5	5/2	0.46
Age (years)	57 (55–58)	55 (53–58)	61 (57–66)	0.17
BMI (kg/m^2)	30 (29–30)	31 (29–32)	34 (29–38)	0.05
TG (mg/dl)	195 (176–216)	193 (169–220)	169 (127–225)	0.75
FG (mg/dl)	99 (95–103)	102 (95–111)	144 (109–191)	0.001
ApoL-I (μg/ml)	8.4 (7.4–9.6)	9.0 (7.7–10.5)	14.5 (8.4–25.1)	0.05

For ApoL-I, n = 79 for Lys166/Lys166 and n = 38 for Lys166/Glu166. Chi-square analysis and ANOVA were used for proportions and quantitative variables, respectively. Data are presented as arithmetic means (95% CIs), except for logarithmically transformed data, which are presented as GMs (95% CIs). For FG, TG, and ApoL-I, statistical tests were computed on logarithmic transformations. FG was averaged over the course of the study.

domization, there was a significant difference in apoL-I level between the two groups (data not shown). At this point, no clear explanation is available for our observation. The question of whether apoL-I has a bimodal distribution in the general population, or only in select populations, will need to be investigated with larger numbers of patients.

Multivariate analysis showed that there were significant associations between apoL-I and VLDL-TG and HG. Associations of apoL-I with total plasma TGs in normolipidemic individuals, and in those with dyslipidemias that include hypertriglyceridemia, have been reported previously (6). We now extend this finding to patients with low HDL-C and CAD and provide evidence that VLDL-TG is the specific TG component associated with apoL-I. Because a small fraction of total plasma apoL-I has been found on the VLDL particle (1), it is possible that the independent association we are seeing with VLDL-TG is directly related to changes in VLDL particle number or composition, or to another highly correlated variable. The lack of an association of apoL-I with HDL-C and/or plasma apoA-I content has been noted before (6). This may be related to its presence on only ~10% of HDL particles and suggests that apoL-I-containing particles constitute a subpopulation that is not influenced by the cholesterol content of HDL.

An association of apoL-I with HG has not been previously reported. This finding adds another lipid variable to the list of abnormalities seen in the dyslipidemia of diabetes and the metabolic syndrome (16–19). Because the association between apoL-I and HG was stronger than that with FG, it is possible that the relationship between apoL-I and the hyperglycemic state is not directly related to glucose level itself but to some other metabolic abnormality associated with, or contributing to, the hyperglycemic phenotype. We tried to account for known predictors of HG by including many of them in our multivariate model (age, fasting insulin, BMI, and TGs), although we obviously could not account for all of them. The fact that almost 50% of patients with high apoL-I levels had a HG phenotype compared with <15% of those in the low apoL-I cluster further highlights the strong association between apoL-I level and a hyperglycemic phenotype.

We did not find an association between apoL-I level and the progression of CAD in our population with low HDL-C. This suggests that apoL-I itself is not strongly proatherogenic. Our finding that apoL-I levels are positively associated with other known atherogenic factors (TG and HG), however, suggests that high apoL-I levels may be a novel marker of an atherogenic phenotype. The discovery that apoL proteins are present in human atherosclerotic vascular tissue (4, 20) is consistent with this hypothesis and argues for further investigation into apoL-I's role in lipid metabolism.

Interestingly, the lipid-altering drugs simvastatin and niacin did not significantly alter apoL-I levels. This is surprising considering that these drugs significantly decreased TG and increased HDL-C in HATS (7). One possibility is that the simvastatin-niacin drug combination influences competing factors important in apoL-I regulation. Niacin itself has the potential to do this through its somewhat unusual effect of increasing insulin resistance while decreasing plasma TG levels. Another possibility is that the subset of apoL-I-containing HDL particles is not influenced by these lipid-altering drugs.

The increase in apoL-I seen in the antioxidant vitamin group was unanticipated. These data suggest that antioxidant vitamins can alter factors important in apoL-I regulation. This finding is supportive of recent work showing that antioxidant vitamins can alter various aspects of lipid metabolism (21, 22). Cheung et al. (21) showed that antioxidant vitamins can interfere with the beneficial effects of niacin on HDL metabolism, a finding that has challenged the assumption that antioxidant vitamins are, at worst, benign. Our study again raises the question of whether antioxidant vitamins in their current formulations are benign by showing that they can increase levels of an apolipoprotein that is associated with known atherogenic factors. Although we are unable to provide a mechanistic explanation for this finding, we anticipate that this observation will be helpful in guiding further investigations of apoL-I metabolism.

In conclusion, we present a series of interesting observations regarding apoL-I in patients with dyslipidemia and CAD. ApoL-I has previously been found to be right skewed and associated with plasma TG level (6). In our CAD population, we also found a right skewed distribution of apoL-I, but with a tendency toward a bimodal distribution. Although much of the variability in apoL-I was unaccounted for by the variables we measured, the HATS population showed a significant association of apoL-I with both hyperglycemic phenotype and VLDL-TG. In this study, lipid-altering therapies did not change apoL-I levels, whereas antioxidant vitamins increased apoL-I plasma levels. We feel that these data highlight some unique characteristics of the novel HDL-associated apoL-I and should facilitate further investigation into its role in lipid metabolism.

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