

Physiologic mechanisms for reduced apolipoprotein A-I concentrations associated with low levels of high density lipoprotein cholesterol in patients with normal plasma lipids¹

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Abstract Low plasma concentrations of high density lipoprotein (HDL) cholesterol and apolipoprotein A-I (apoA-I) are major risk factors for coronary heart disease (CHD). Low HDL levels are common in patients with hypertriglyceridemia, but they also occur in those with normal plasma lipids; the latter include obese patients and cigarette smokers, though other patients with low HDL levels are neither obese nor smokers. The present study was designed to define metabolic causes of low apoA-I levels in normal-weight, normolipidemic patients. ApoA-I tracer studies were carried out in two groups of normolipidemic patients having low HDL levels to determine input rates and residence times for apoA-I; these patients included 11 nonobese nonsmokers and 11 nonobese cigarette smokers. Their results were compared to those of 20 normal-weight, normolipidemic controls with normal HDL levels and 12 obese nonsmokers also having low HDL. In all three groups manifesting low HDL-cholesterol and low apoA-I levels, residence times for plasma apoA-I were reduced by approximately 30%, compared to control subjects with normal HDL levels. In contrast, average input rates for apoA-I were similar among the three low-HDL patients and control subjects. No differences in apoA-I kinetics were observed among any of the three groups with low apoA-I concentrations. Within each of the four groups of the study, however, input rates for apoA-I were highly correlated with plasma concentrations of apoA-I. Thus, for individuals with normal levels of plasma lipids, both residence times and input rates for apoA-I appeared to be important determinants of apoA-I levels. Residence times for apoA-I were reduced in almost all patients with low apoA-I levels, regardless of concomitant factors, whereas input rates were highly variable among individuals. —Gylling, H., G. L. Vega, and S. M. Grundy. Physiologic mechanisms for reduced apolipoprotein A-I concentrations associated with low levels of high density lipoprotein cholesterol in patients with normal plasma lipids. *J. Lipid Res.* 1992. 33: 1527–1539.

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Plasma concentrations of high density lipoprotein (HDL) cholesterol are inversely and independently cor-

related with risk for coronary heart disease (CHD) (1–3). The mechanisms whereby low HDL-cholesterol levels are linked to increased CHD risk are not fully understood, although three possibilities have been proposed. First, low HDL levels can be secondary to other CHD risk factors—smoking (4, 5), sedentary life style (6), obesity (7, 8), and diabetes mellitus (9)—and these other factors, independently of low HDL, may be the true determinants of CHD risk. Second, low HDL-cholesterol concentrations commonly accompany other lipoprotein abnormalities, notably increases in triglyceride-rich lipoproteins (10, 11); these latter lipoproteins, in addition to low HDL levels, could be atherogenic agents. And third, a low HDL concentration per se may directly promote atherogenesis, either by interfering with the atherogenicity of LDL or by promoting reverse cholesterol transport.

If a low HDL level is directly atherogenic, which component of HDL plays the most direct role in atherogenesis? A strong candidate is apolipoprotein A-I (apoA-I), the major apolipoprotein of HDL. When HDL-cholesterol levels are low, plasma concentrations of apoA-I usually are reduced as well; moreover, a series of studies (12–14) indicate that apoA-I levels, like HDL-cholesterol concentrations, are inversely correlated with CHD risk. In fact, apoA-I may act directly within the arterial wall to prevent oxidation (15) or self-aggregation (16) of LDL or to promote mobilization of cholesterol from arterial-wall cells (17, 18). Recent studies (19) in transgenic animals provide additional support for the concept that apoA-I directly

Abbreviations: HDL, high density lipoprotein; CHD, coronary heart disease; LDL, low density lipoprotein; VLDL, very low density lipoprotein; LPL, lipoprotein lipase; HTGL, hepatic triglyceride lipase.

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protects against development of atherosclerosis. If apoA-I is directly protective, factors determining apoA-I concentrations could indirectly affect the atherosclerotic process.

Low levels of HDL cholesterol and apoA-I have been noted in two types of patients, i.e., those with hyperlipidemia and those with normal plasma lipids. Among the former, hypertriglyceridemia is the most frequent concomitant abnormality (10, 11). In normolipidemia patients two factors that apparently contribute to low HDL levels are obesity (7, 8) and cigarette smoking (4, 5); but in addition, some normolipidemic patients with low HDL levels are neither obese nor smokers, and determinants of their low levels remain obscure. One approach to investigating factors causing low concentrations of apoA-I is to use isotopes to trace the kinetic behavior of this apolipoprotein. Two key kinetic parameters that affect apoA-I levels are the rate of input of apoA-I into plasma and the time apoA-I resides in plasma. Changes in both have been reported to affect apoA-I concentrations under various circumstances (20–39). In a recent report, Brinton, Eisenberg, and Breslow (39) indicated that a reduced residence time of apoA-I in plasma is the primary cause for low apoA-I concentrations in obese subjects, whether they are normolipidemic or hypertriglyceridemic.

In the present study, tracer kinetics were used to examine the metabolic abnormalities responsible for low apoA-I levels in patients with reduced HDL-cholesterol levels who were neither obese nor hyperlipidemic. Patients of this type, although commonly found among those with premature CHD, have not been studied specifically for their apoA-I kinetics. Two groups of patients with low HDL levels were studied: *a*) normolipidemic, nonobese nonsmokers (low-HDL nonsmokers) and *b*) normolipidemic, nonobese smokers (low-HDL smokers). For comparison, two other groups were examined: *a*) nonobese, normolipidemic nonsmokers with normal HDL-cholesterol levels (normal-HDL controls), and *b*) obese, normolipidemic nonsmokers with low HDL-cholesterol levels (low-HDL obese patients). The primary questions under study were 1) whether nonobese, normolipidemic patients with low-HDL concentrations have decreased apoA-I levels on the basis of reduced input of apoA-I; 2) whether there is a difference in mechanisms for low apoA-I levels between smokers and nonsmokers; and 3) whether apoA-I kinetics differ between obese and non-obese patients who have normal plasma lipids.

METHODS

Patients

A total of 54 male subjects participated in this investigation. They were recruited from outpatient clinics of the Veterans Affairs Medical Centers, Dallas and Bonham,

Texas and the University of Texas Southwestern Medical Center, Dallas, Texas. Ages for the subjects ranged from 43 to 68 years. Four groups of subjects were recruited: *a*) 20 subjects with normolipidemia (total cholesterol < 250 mg/dl and triglycerides < 250 mg/dl), normal HDL cholesterol (≥ 40 mg/dl), and absence of obesity [body mass index (BMI) < 27 kg/m²] (normal-HDL controls); *b*) 11 patients with hypoalphalipoproteinemia (HDL cholesterol < 40 mg/dl) who were nonobese (as defined above), normolipidemic (as defined above) and nonsmokers (low-HDL nonsmokers); *c*) 11 patients with hypoalphalipoproteinemia who were nonobese and normolipidemic, but who also were cigarette smokers (at least 20 cigarettes per day) (low-HDL smokers); and *d*) 12 patients with hypoalphalipoproteinemia who were normolipidemic nonsmokers, but also were obese (≥ 27 kg/m²) (low-HDL obese patients). The patients in the latter three categories were selected first because they had low HDL-cholesterol levels, and they were then divided into the three categories on the basis of smoking habits and body weight. Hypoalphalipoproteinemia was defined as an HDL-cholesterol level below 40 mg/dl; this value was chosen because of data of the Framingham Heart Study (40) that showed that a striking increase in risk for CHD occurs below this level; 31 of 34 with hypoalphalipoproteinemia had HDL-cholesterol concentrations below 35 mg/dl levels during the study, the latter level being defined as "low HDL cholesterol" by the National Cholesterol Education Program (41). For simplicity, the term HDL is used generically in this paper to denote both HDL-cholesterol and apoA-I; this seems appropriate as the two parameters generally change in parallel.

Control men generally were healthy without a history of angina pectoris or myocardial infarction. None of the control subjects were smokers, nor were they taking beta-adrenergic blocking agents (beta-blockers) during the study. Many of the patients in the low HDL groups had a history of CHD, but they were excluded if a myocardial infarction had occurred in the 6 months preceding the study; they also were excluded if they had recent unstable angina pectoris or a history of chronic congestive heart failure. None of the patients had clinically detectable diseases of the liver, kidneys, or endocrine system; none had diabetes mellitus. Thirty-one percent of patients with low HDL levels had a history of hypertension. None of these patients took beta-blockers during the study; however some were taking other drugs that have no known effect on lipoprotein metabolism (e.g., calcium channel inhibitors and angiotensin-converting enzyme inhibitors). Patients taking thiazide diuretics were not excluded, but if taken, the dose of drug was not changed throughout the study; thiazides generally are not believed to affect HDL levels. Each subject gave written, informed consent to participate in a protocol that had been approved by the appropriate institutional review board.

Experimental design

The study on each patient lasted for 5 weeks. Upon entrance into study, patients were started on a solid-food diet containing 40% of energy as fat (17% saturates, 18% monounsaturates, and 5% polyunsaturates), 45% as carbohydrate, and 15% as protein. Daily cholesterol intake was in the range of 350 to 400 mg. Alcohol was proscribed for the duration of the study. This diet has been used previously in our lipoprotein turnover studies (42-44), and it resembled a "typical" American diet in composition. Patients were instructed to maintain their exercise at a constant level throughout the study. They generally had a sedentary lifestyle; none were engaged in an exercise program.

After 2 weeks of equilibration on this diet in the out-patient setting, the patients underwent a one-unit plasmapheresis to obtain plasma for isolation of HDL; blood cells were returned to the patients. Autologous apolipoprotein A-I (apoA-I) was isolated, radiolabeled with ^{131}I , and reinjected into the patients 5 days later. Patients were admitted to the metabolic ward the night before reinjection, and most remained in the hospital for 14 days completing their turnover study as inpatients. A few patients completed the last part of the turnover study as outpatients; in these, as in the others, the diet described above was consumed throughout the turnover study. Twenty-three blood samples were obtained over a period of 2 weeks after isotope injection. All samples were collected after patients had been in the sitting position for 10 min. Patients were started on supersaturated potassium iodide (0.5 mg/day) 3 days before isotope injection and remained on this dose throughout the entire turnover study. A plasma die-away curve for radioactive apoA-I was constructed, and kinetic parameters were estimated by multicompartamental analysis.

Turnover of autologous apoA-I

The procedure used in this study for estimating turnover rates of apoA-I has been described in detail recently (45). Briefly, HDL (d 1.090-1.21 g/ml) was isolated from plasma by sequential preparative ultracentrifugation by the method of Lindgren, Jensen, and Hatch (46). The lipoprotein was resuspended at its native density and ultracentrifuged in a TV-865B vertical rotor (Dupont-Sorvall, Wilmington, DE) for 2.5 h at 65,000 rpm to remove any contaminating proteins. An aliquot of this HDL was used to purify autologous A-I according to the following procedure: HDL containing approximately 9 mg protein was dialyzed against 150 mM NaCl, 10 mM Tris-chloride, and 0.01% disodium EDTA, pH 8.0. The dialyzed solution containing HDL was transferred into a sterile, pyrogen-free vial and 1.146 mg of guanidine hydrochloride was added to bring the solution to 4 M. This solution was incubated for 3 h at 37°C, and then it was dialyzed against 150 mM NaCl, 10 mM Tris-chloride,

and 0.01% EDTA, pH 8.0. After dialysis, the solution was transferred into a sterile 6-ml Ultraclear centrifuge tube and the density was adjusted to 1.21 g/ml with solid NaBr. The solution was overlaid with an equal volume of NaBr solution of similar density, and the mixture was ultracentrifuged at 50,000 rpm for 24 h in a 50.3 fixed-angle rotor (Beckman Instruments, Palo Alto, CA) at 10°C. The bottom 1.5 ml was collected and dialyzed extensively against 150 mM NaCl, 0.01% EDTA, pH 7.4. The concentration of protein was determined by the Lowry et al. (47) procedure, as modified by Markwell et al. (48) and described previously (49). No apoA-II was detected immunochemically (50), and no apolipoprotein bands other than apoA-I were detected on polyacrylamide gels (51).

Approximately 1 mg of autologous apoA-I was radiolabeled with ^{131}I by the method of McFarlane (52), as modified in our laboratory (42). Free iodine was removed from the preparation by dialysis against 150 mM NaCl, 10 mM Tris-chloride, 0.01% EDTA, pH 8.0. In the labeled apoA-I preparation, 99% of the radioactivity was precipitable by 15% trichloroacetic acid. When the preparation was subjected to sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE), all of the radioactivity was associated with a single band that corresponded to apoA-I.

An injection mixture containing 4% human serum albumin, and 25-30 μCi of the tracer, was injected intravenously. Twenty three blood samples of 15 ml each were collected over a period of 14 days. After injection, samples were drawn at 10, 20, 30, and 60 min and at 4, 8, 12, and 24 h. Subsequently, fasting samples were obtained daily over the next 13 days. These samples were collected into tubes containing 0.005% chloramphenicol, 0.005% gentamicin sulfate, and 0.1% sodium azide.

Model selection: determination of kinetic parameters for apoA-I

Several different multi-compartmental models have been used previously to estimate kinetic parameters for plasma apoA-I. The simplest and most widely used model is that proposed by Matthews (53)—a two-pool model consisting of one intravascular compartment and one extravascular compartment. This model postulates that the two compartments are in equilibrium across the capillary membrane and that all new input and exit of apoA-I occurs via the intravascular compartment. A modification of this model assumes that exit of apoA-I can occur via both intra- and extravascular compartments; this alternate model was used by Blum et al. (20) and has been discussed in detail by Foster (54). Evidence for the validity of this model was the finding that the urine-to-plasma (U/P) ratio of radioactivity was not constant at every time point after injection of tracer apoA-I, as would be predicted by the Matthews model (53). However, estimate of parameters for this model require assumptions about the

half-life of the free iodine pool and estimates based on incomplete recovery of radioactivity in the urine; uncertainties about these estimates represent a limitation in the use of this model. A still more complex model is that of Gurbide, Mann, and Sandberg (55); according to this model, both inputs and outputs of apoA-I occur via both intravascular and extravascular compartments. Although it is not possible to rule out the existence of dual inputs and exits, the available data, even with urine collection, are insufficient to identify all the parameters of this model. Finally, Zech et al. (56) added new complexity by postulating a model in which there are two intravascular pools, one of which is rapidly catabolized and the other decays more slowly. This model likewise will cause an inconstant U/P ratio that makes differentiation from the model of Blum et al. (20) difficult. The rapidly catabolized, intravascular pool of A-I might be related in part to HDL particles that contain only apoA-I, in contrast to particles having both apoA-I and apoA-II. Indeed, Rader et al. (57) recently reported that HDL particles having only apoA-I in fact are catabolized at a somewhat faster rate than HDL particles having both apoA-I and apoA-II, and they suggested that the residence time of whole apoA-I lies between residence times of apoA-I on the two types of particles.

In the present study, we chose to use the Matthews model (53) for several reasons. Foremost is a lack of validation of the more complex models. A major question about these other models is whether the kinetics of the isolated tracer are identical to the natural tracee. Several studies using these more complex models have radio-labeled very small quantities of isolated apoA-I to serve as a tracer for the whole apoA-I pool. However, using this labeling technique, Osborne et al. (58) were able to separate radioiodinated apoA-I into two fractions; the first was indistinguishable in physical properties from unlabeled apoA-I, whereas the second seemingly was partially denatured. In vivo kinetic studies revealed that the second, partially denatured fraction was catabolized more rapidly than the first, suggesting that it did not reassociate completely with lipoproteins. Results of a similar type have been reported by Patterson and Lee (59). The method of isolation of apoA-I in the present study differed from those of previous reports in that relatively large amounts were isolated for labeling; this should minimize the overlabeling and denaturation of apoA-I as may occur when only small quantities of apoprotein are labeled. This method should minimize the denaturation of apoA-I by the labeling procedure. Indeed, in the report (45) describing our current method, it was shown that the isolated, pure apoA-I decayed at the same rate as apoA-I on whole HDL particles, when both were labeled and injected intravenously. Thus, until the possibility of introducing artificial isotopic heterogeneity in the tracer has been ruled out, we propose that use of the Matthews model (53)

is more appropriate than more complex models; it is possible that the latter were developed partly in response to artifacts of labeling. Further, it should be noted that absolute differences in residence time values for apoA-I between the Matthews model (53) and more complex models are relatively small and should not affect the overall interpretation of the results.

To perform the two-pool kinetic analysis, 4 ml of plasma was counted for each time point, and the fraction of injected dose was calculated for each sample. The residence time for apoA-I was calculated from the resulting die-away curve using the Matthews model (53). The input rate for apoA-I was calculated by dividing the plasma pool size of apoA-I by the residence time. The former was estimated as the product of plasma apoA-I concentration and plasma volume (i.e., volume of distribution of apoA-I as determined by the 10-min sample).

Concentrations of plasma lipids, lipoprotein cholesterol and apoA-I

These concentrations were determined on five samples that were collected every 3 days throughout the turnover study. Total cholesterol and triglyceride concentrations were measured colorimetrically using enzymatic methods (60, 61); plasma HDL-cholesterol was measured after precipitation of apoB-containing lipoproteins with heparin-manganese (62). Levels of very low density lipoprotein (VLDL) + intermediate density lipoprotein (IDL) cholesterol and low density lipoprotein (LDL) cholesterol were determined by sequential ultracentrifugation, as described previously (42).

Concentrations of plasma apoA-I were determined by a modification of the electroimmunoassay of Laurell (EIA) (63). Polyclonal anti-human apoA-I antisera was obtained from Boehringer-Mannheim Corporation (Biochemical Products, Indianapolis, IN). The electrophoresis gel consisted of 1.25% antisera, 2% (w/v) agarose (Seakem agarose) (0.16–0.19 relative mobility electroendosmosis); (Marine Colloids Division, FM Corporation, Rockland, ME), 2% (w/v) dextran (mol wt ca. 70,000, Sigma Chemical Company, St. Louis, MO), and a non-barbital buffer (64). The standard used was a serum calibrator for apoA-I obtained from Boehringer-Mannheim Diagnostics. This standard was recalibrated in our laboratory by electroimmunoassay using pure apoA-I as the primary standard. Concentrations were calculated from peak heights. The coefficient of variation for each assay was determined; each assay had 5% or less variation within and between assays.

Statistical analysis

Means of each variable were compared between each group by analysis of variance (ANOVA) using Dunnett's multiple comparison of means. Linear regression analysis was carried out using linear statistical packages available in CLINFO.

RESULTS

The clinical characteristics, plasma total lipids, and lipoprotein-cholesterol levels obtained during the turn-over studies for the four different groups are presented in **Table 1**. All of the groups had similar levels of total cholesterol. However, average triglyceride levels for all groups with low HDL-cholesterol levels were significantly higher than normolipidemic controls, although none of the patients with low HDL had triglyceride concentrations exceeding 250 mg/dl. VLDL-cholesterol levels were somewhat higher in the low-HDL groups, but LDL-cholesterol levels for all of the groups were similar. Because of the criteria of selection, HDL-cholesterol levels for all low-HDL groups were significantly lower than the normal-HDL control group.

Mean values for concentrations and kinetic parameters (residence times and input rates) for apoA-I are presented for the four groups in **Table 2**. Input rates for apoA-I are presented both in absolute terms (mg/day) and after normalization for total body weight (mg/kg-d). For all groups with low HDL-cholesterol levels, plasma concentrations of apoA-I were significantly lower than those of the normal-HDL group. In the next section the mean results for each low-HDL group will be compared to the control group (**Table 2**), and correlation coefficients between key parameters within each of the four groups will be presented.

In **Fig. 1**, concentrations of apoA-I are plotted against residence time and total input rates for apoA-I for control subjects. In these subjects, residence times and concentrations for apoA-I were not significantly correlated ($r = 0.403$; $P = 0.087$), but input rate for apoA-I was significantly correlated with concentration ($r = 0.682$; $P = 0.001$). Thus, within the group of men having HDL-cholesterol concentrations in the normal range, the input rate for apoA-I appeared to be a more important determinant of apoA-I concentrations than was apoA-I residence time.

Low-HDL nonsmokers had significantly lower concentrations of apoA-I than control men (**Table 2**). Residence times for apoA-I were significantly lower for these patients than for controls; however, between the two groups, average input rates for apoA-I were not significantly different. As shown in **Fig. 2**, for low-HDL nonsmokers, residence times for apoA-I were not significantly correlated with concentrations of apoA-I ($r = 0.075$; $P = 0.826$), whereas input rates were highly correlated with concentrations ($r = 0.788$, $P < 0.004$). Thus, within this group, input rates apparently explained the variation in concentrations of apoA-I much more than did residence times of apoA-I.

Low-HDL smokers also had significantly lower concentrations of apoA-I than normal-HDL controls (**Table 2**). Again, residence times for apoA-I were significantly reduced in this group, compared to control, whereas average input rates were not different from controls. As in the preceding group, residence times for apoA-I were not correlated with concentrations ($r = -0.268$, $P = 0.454$), but input rates and concentrations of apoA-I were significantly correlated ($r = 0.664$, $P = 0.026$) (**Fig. 3**). Thus, input rates again explained more of the variability in apoA-I concentration within this group than did residence times. No significant differences in apoA-I kinetics were found between low-HDL smokers and nonsmokers.

In low-HDL, obese patients, apoA-I levels were significantly lower than in control subjects (**Table 2**). The low apoA-I concentrations in this group on the average were due to a decrease in residence time for apoA-I and not to a decrease in input rates. Within this group, concentrations for apoA-I were positively correlated both with input rates ($r = 0.908$, $P < 0.001$) and residence times ($r = 0.832$, $P < 0.001$) (**Fig. 4**). Within this group, when the residence time of apoA-I was prolonged, concentrations of apoA-I tended to be higher. This might be the expected result, although it was not observed among the other three groups. No differences were noted between apoA-I

TABLE 1. Levels of plasma lipids and lipoprotein cholesterol

Group	n	Age ^a yr	Body Mass Index kg/m ²	Triglycerides mg/dl	Cholesterol			
					Total	VLDL + IDL	LDL	HDL
Normolipidemic controls	20	56 ± 2	23.6 ± 0.7	99 ± 8	213 ± 6	30 ± 4	130 ± 5	54 ± 3
Normolipidemic Low HDL								
Nonsmokers	11	63 ± 2	24.6 ± 0.5	153 ± 19	204 ± 9	44 ± 6	128 ± 6	34 ± 1 ^c
Smokers	11	57 ± 4	24.4 ± 0.6	157 ± 15	189 ± 9 ^c	35 ± 3	122 ± 8	29 ± 2 ^{c,d}
Obese	12	54 ± 3	30.7 ± 0.6 ^b	166 ± 14	205 ± 8	39 ± 5	133 ± 7	29 ± 1 ^{c,d}

Values are given as mean ± standard error. Abbreviations: VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein.

^aThere were no significant differences in age.

^bSignificantly higher than control and other low HDL groups; $P < 0.001$.

^cSignificantly lower than controls; $P < 0.003$.

^dSignificantly lower than nonsmokers; $P < 0.03$.

TABLE 2. Kinetic parameters of apoA-I

Group	n	Conc <i>mg/dl</i>	Residence Time <i>days</i>	Total Input Rate <i>mg/day</i>	Input Rate <i>mg/kg-day</i>
Normolipidemic controls	20	122 ± 4	5.05 ± 0.16	757 ± 33	10.5 ± 0.5
Normolipidemic low-HDL					
Nonsmokers	11	88 ± 4 ^a	3.51 ± 0.13 ^a	791 ± 54	10.6 ± 0.7
Smokers	11	91 ± 5 ^a	3.78 ± 0.12 ^a	786 ± 58	9.7 ± 0.7
Obese	12	86 ± 4 ^a	3.36 ± 0.08 ^a	866 ± 32	9.0 ± 0.3

Values are expressed as mean ± standard error.

^aSignificantly lower than controls; $P < 0.001$.

kinetic parameters for obese subjects and the other two nonobese groups (smokers and nonsmokers) with low HDL levels.

Table 3 shows additional correlation coefficients (and P values) in comparisons of other key parameters. Triglyceride levels were significantly and inversely correlated with apoA-I levels, HDL-cholesterol levels, and apoA-I residence times for all subjects of the study, but none of these correlations were significant for individual groups. There was a significant and positive correlation between apoA-I levels and HDL-cholesterol levels for all subjects (and for controls), but not within any of the low-HDL groups. It should be noted that the number of patients in the latter three groups was relatively small, which might explain a lack of significant correlation. For all subjects, HDL-

cholesterol concentrations were significantly correlated with apoA-I residence time, but not in individual groups. HDL-cholesterol levels did not significantly correlate with apoA-I input rates in any comparisons.

DISCUSSION

The purpose of this study was to evaluate the kinetics of apoA-I in patients with low HDL-cholesterol concentrations who did not have elevated plasma lipids. Primary focus was given to low-HDL patients who did not have secondary causes of low HDL-cholesterol, i.e., low-HDL nonsmokers. However, results in low-HDL nonsmokers were compared to low-HDL patients who were *a*) non-

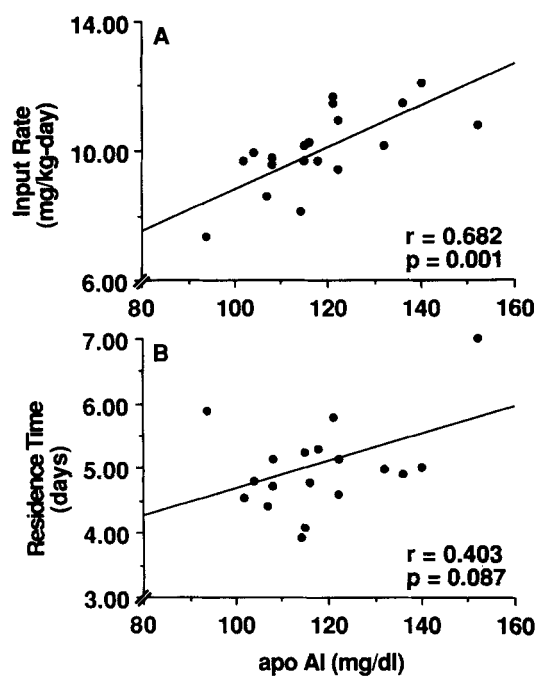


Fig. 1. Correlations between total input rates and plasma concentrations for apoA-I (top) and between residence time and concentrations for apoA-I (bottom) for control subjects.

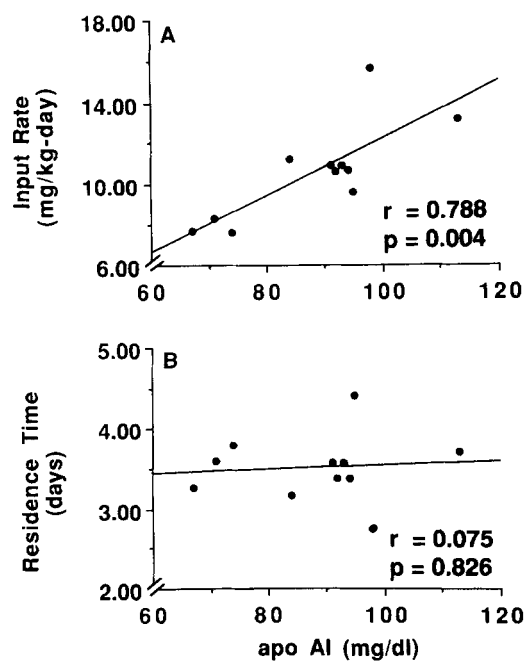


Fig. 2. Correlations between total input rates and plasma concentrations for apoA-I (top) and between residence time and concentrations for apoA-I (bottom) for normolipidemic, nonobese smokers.

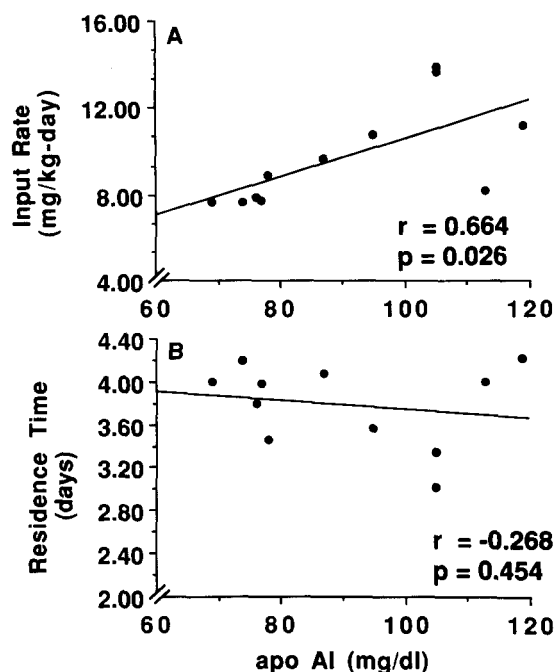


Fig. 3. Correlations between total input rates and plasma concentrations for apoA-I (top) and between residence time and concentrations for apoA-I (bottom) for normolipidemic, nonobese, nonsmokers.

obese but smokers and *b*) obese but nonsmokers. Patients in the latter two categories were chosen first for having low HDL-cholesterol levels, and then were divided into different groups on the basis of either smoking or weight. The possibility thus cannot be ruled out that their low HDL levels may have been caused, at least in part, by factors other than smoking or obesity. Examination of these two groups nonetheless might reveal unique effects of smoking

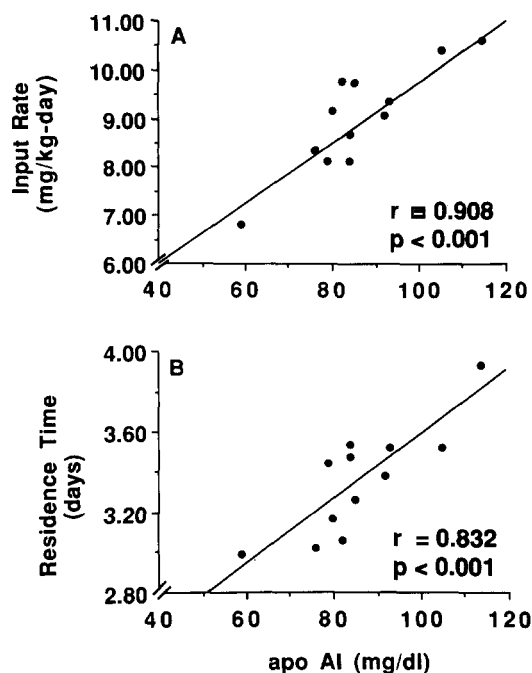


Fig. 4. Correlations between total input rates and plasma concentrations for apoA-I (top) and between residence time and concentrations for apoA-I (bottom) for normolipidemic, obese subjects.

or obesity on apoA-I metabolism that are not present in patients who are devoid of these factors.

The primary hypothesis set forth for this study was that low apoA-I levels in nonobese nonsmokers can be explained by a decreased input rate for apoA-I, possibly reflecting a decreased synthesis of apoA-I. This hypothesis seemed plausible because patients of this type do not have factors that previously have been reported to enhance

TABLE 3. Correlation coefficients for various comparisons

Comparison	Group				
	Normal-HDL Controls	Low-HDL Nonsmokers	Low-HDL Smokers	Low-HDL Obese	All Groups
	<i>Correlation Coefficient (P value)</i>				
TG conc. vs. apoA-I conc.	0.154 (0.546)	-0.034 (0.921)	-0.357 (0.281)	0.077 (0.811)	-0.425 (0.002)
TG Conc vs. HDL-cholesterol conc.	-0.387 (0.102)	-0.144 (0.672)	-0.357 (0.281)	0.198 (0.537)	-0.499 (0.001)
TG vs. apoA-I residence time	-0.353 (0.151)	-0.131 (0.701)	0.34 (0.306)	-0.076 (0.815)	-0.46 (0.001)
TG vs. apoA-I input rate	0.073 (0.774)	0.062 (0.847)	-0.664 (0.026)	-0.042 (0.897)	0.0192 (0.893)
ApoA-I conc. vs. HDL-cholesterol conc.	0.587 (0.013)	0.443 (0.172)	0.471 (0.172)	0.22 (0.491)	0.714 (0.001)
HDL-cholesterol conc. vs. apoA-I residence time	0.394 (0.106)	-0.322 (0.355)	-0.314 (0.348)	0.082 (0.799)	0.734 (0.001)
HDL-cholesterol conc. vs. apoA-I input rate	0.172 (0.494)	0.508 (0.111)	0.303 (0.365)	0.383 (0.220)	0.204 (0.147)

Abbreviations: HDL, high density lipoprotein; TG, triglyceride; apoA-I, apolipoprotein A-I.

catabolism of apoA-I, namely, obesity (39) and hypertriglyceridemia (27, 34, 39). On the other hand, since smoking has been reported to raise triglyceride levels (5), an increased catabolism of apoA-I was considered a probable mechanism in smokers. We therefore hypothesized that kinetic patterns of apoA-I would differ between smokers and nonsmokers, both of whom had low HDL levels but who otherwise were not obese or hypertriglyceridemic.

A common feature of many patients with low HDL levels is the presence of high-normal triglyceride levels even in the absence of frank hypertriglyceridemia. Absence of hypertriglyceridemia in all of the low-HDL groups was defined as total triglyceride levels below 250 mg/dl. The level of 250 mg/dl for total triglycerides is approximately the 95th percentile for men of the current age group. Further, various expert panels (41, 65) have defined hypertriglyceridemia as levels exceeding 250 mg/dl. For this reason, it seems appropriate to define triglyceride levels in the range of 150 to 250 mg/dl as "high normal." Although it is possible that high-normal triglyceride levels contribute to lowering of HDL-cholesterol levels, previous studies (66, 67) have shown that most patients with triglyceride levels in this range do not have frank hypolipoproteinemia. Moreover, as shown in Table 4, less than 20% of the variance in apoA-I levels in all of the patients could be explained by total triglyceride levels. This is not to say that defects in triglyceride metabolism (e.g., increased VLDL-triglyceride flux) are not an important cause of low HDL levels, but it cannot be said that the magnitude of the reduction in HDL levels observed in current patients can be explained primarily by high-normal triglyceride levels per se.

Before considering the data in patients with low HDL levels, the findings in normal control men will be examined, because the findings in these subjects are of interest independent of comparisons with low-HDL groups.

Normal male subjects

Twenty middle-aged, healthy men, who were nonsmokers, served as controls for this study. The mean HDL-cholesterol level in these subjects, 54 ± 3 mg/dl,

was about 8 mg/dl higher than the average of middle-aged men in the general U.S. population (68). There are two reasons for these higher values. First, entry criteria required an HDL-cholesterol for this age group of 40 mg/dl or higher, which corresponds to the upper 65% of the male population (68). And second, we excluded obese individuals, and obesity is known to lower HDL levels; the BMIs of our controls averaged 23.6 ± 0.7 kg/m², whereas the mean BMI for middle-aged American men is 26.3 kg/m² (68). Thus, the HDL-cholesterol levels of our control subjects were typical of nonobese American men having average HDL concentrations above 40 mg/dl, although they were not representative of all middle-aged American men; many of the latter have relatively low HDL-cholesterol levels because of obesity or other factors (e.g., smoking).

As shown in Fig. 1, the variation in plasma concentrations of apoA-I in control men was influenced much more by the input rate for apoA-I than by residence time for apoA-I. Input rates for apoA-I in these patients presumably reflect synthesis rates of apoA-I, although this identity has not been proven for humans. It is interesting to compare and contrast these findings in normal men with those of 15 normal women reported recently by Brinton, Eisenberg, and Breslow (38). In Table 4, comparisons between these two studies are expressed in the same units. On the average, the two groups had similar average input rates and residence times for apoA-I, but women had higher plasma levels of apoA-I, presumably because of a smaller plasma volume. For the women, however, the variation in apoA-I concentrations among individuals appeared to be determined more by residence time for apoA-I than by input rate. The opposite was found for our normal men; in them, apoA-I concentrations were correlated highly with input rate, whereas no correlation was found for residence time of apoA-I. Women possibly possess hormonal factors that delay clearance of apoA-I and thereby increase plasma apoA-I levels; these factors may be absent in men. For example, it is possible that women have a lower hepatic triglyceride lipase, possibly because of estrogens (69) or lack of androgens, which could account for their

TABLE 4. Comparison of apoA-I kinetics in normal women and men

	ApoA-I			Correlation Coefficients	
	Conc.	Residence Time	Input	Concentration versus	
				Residence Time	Input Rate
	mg/dl \pm SE	days \pm SE	mg/kg \cdot d \pm SE	Coefficient R (p value)	
Normal women (Brinton et al.) (38)	163 \pm 8	5.0 \pm 0.3	11.9 \pm 0.4	0.64 (0.01) ^c	0.30 (0.28) ^b
Normal men (current study)	122 \pm 4	5.05 \pm 1.5	10.6 \pm 0.7	0.24 (0.31) ^b	0.87 (0.001) ^c

^cStatistically significant $P < 0.001$.

^bNot statistically significant.

slowed apoA-I catabolism. Consequently, in contrast to women, the input rate emerged as the major determinant of variations in apoA-I concentrations in normal men. In another report in which male endurance athletes were studied (70), apoA-I concentrations were correlated with input rates, as in the present study.

The question can be raised whether it is meaningful to correlate input rates and plasma concentrations of apoA-I since input rate is a function of pool size, derived from concentration of apoA-I. Certainly, for every individual, the input rate is one determinant of that person's apoA-I concentration. Still, the question to be asked is whether differences in apoA-I concentrations among individuals within a group can be explained in small or large part by differences in input rates. According to Brinton, Eisenberg, and Breslow (37), for women in their study, residence times for apoA-I varied to a much greater extent than did input rates; consequently, residence times were the major factor determining the variation in apoA-I levels. In contrast, in our control men, input rates for apoA-I varied considerably, whereas residence times were restricted to a relatively narrow range; thus, input rates for apoA-I had a much greater impact on differences in concentrations of apoA-I than did residence times.

Obese patients

Previous studies (7, 8) have shown that obesity frequently is accompanied by low HDL levels, whereas weight reduction raises HDL levels (8); these findings indicate that the obese state is directly responsible for a reduced HDL concentration. The current investigation included 12 obese subjects who had normal lipid levels and were nonsmokers. The consistent abnormality responsible for low apoA-I levels in these obese subjects was a decreased residence time for apoA-I. Seemingly, obesity per se enhances clearance of apoA-I. A similar finding was reported recently by Brinton et al. (39). These workers (39) examined apoA-I kinetics in a group of six patients with normolipidemia in whom the mean BMI was 30.0 kg/m², somewhat higher than the average for our obese group. They too found that the primary cause for a low apoA-I level was a decreased residence time for apoA-I. In their patients, input rates for apoA-I were not decreased, and they found no correlation between input rates and apoA-I levels. In our patients, however, the latter correlation was positive and highly significant (Fig. 4); this finding indicates that inherent input rates for apoA-I of individuals affect their apoA-I concentrations independently of the effect of obesity to lower the residence time for apoA-I. In our group of obese patients, residence times for apoA-I also were variable, and the pool sizes also were positively correlated with the duration of apoA-I residence time. This relationship was not observed in the other groups.

Let us ask how obesity without hypertriglyceridemia might promote the clearance of apoA-I. Although a reduced lipoprotein lipase (LPL) activity has been reported to reduce HDL levels (71, 72), obesity usually is accompanied by increased synthesis of LPL (73). The possibility might be considered that increased release of free fatty acids from adipose tissue can inhibit LPL activity, but previous studies have shown that obese, normolipidemic patients usually have increased rates of clearance of both VLDL triglycerides (74) and chylomicron triglycerides (75), suggesting that obese patients in general do not have a reduced functional activity of LPL. A second possible cause for low HDL levels is an increase in hepatic triglyceride lipase (HTGL). Previous studies (76) revealed that HDL-cholesterol levels are inversely correlated with postheparin HTGL activity. Whether obesity specifically increases synthesis of HTGL is not clear, but Brinton et al. (39) found that their obese subjects with low HDL levels had slightly higher postheparin HTGL activity than normal-weight controls. In addition, their low-HDL patients definitely had higher HTGL/LPL ratios than controls (39). An alternate mechanism, however, might be entertained. If adipose tissue has the capacity to catabolize HDL (or apoA-I), the excess of adipose tissue of obese subjects might clear apoA-I levels at an increased rate. This latter mechanism is attractive because of the finding of Wolf and Grundy (8) that obese patients undergoing weight reduction do not show a rise of HDL-cholesterol levels immediately after starting caloric restriction, but only after most excess adipose tissue had been eliminated. Indeed, there are reports that adipose tissue can directly degrade HDL (77, 78).

Low-HDL nonsmoking men

The mechanisms for low HDL-cholesterol levels in patients without obvious secondary factors are not understood. At least two possibilities, however, might be considered. First, these patients could have subtle defects in triglyceride metabolism, which are not manifest by fasting hypertriglyceridemia, but nonetheless can enhance catabolism of apoA-I. Alternatively, a low apoA-I level could result from a decreased synthesis of apoA-I. The current study aimed primarily at determining whether patients of this type have deficient synthesis of A-I causing low apoA-I levels. Contrary to our hypothesis, however, residence times for apoA-I were reduced and average apoA-I input rates were in the normal range. The reason for an increased fractional clearance of apoA-I is not apparent. Although plasma triglycerides were significantly higher in low-HDL patients than in control subjects, low-HDL nonsmokers did not have triglyceride levels in a range that could be classified as abnormally high. Still, differences in triglyceride levels between the control and low HDL groups raise the possibility that subtle abnormalities in

triglyceride metabolism, yet to be identified, account for the increased catabolism of apoA-I in these low-HDL patients. The increased postprandial response in triglyceride levels reported in some patients with lower levels of HDL cholesterol may reflect such defects (79).

Of interest, within this low-HDL group, apoA-I levels correlated more highly with input rates than with residence times for apoA-I (Fig. 1). This finding again emphasizes that both input rates and residence times are determinants of apoA-I levels in individual subjects. If a particular patient has a low apoA-I concentration from increased clearance, the degree of reduction will be accentuated if input rate for apoA-I also is low. A primary reduction in apoA-I synthesis thus could contribute to low levels of HDL cholesterol and apoA-I. Still, in our limited number of patients, specific individuals having low apoA-I concentration entirely from decreased input for apoA-I were not found. This of course does not rule out the possibility that other patients with low HDL levels may have this abnormality.

Low-HDL smoking men

Epidemiologic studies (4, 5) implicate cigarette smoking in causation of low HDL-cholesterol levels. The mechanistic link however remains to be determined. The current cigarette smokers, who were both normolipidemic and nonobese, had apoA-I kinetics similar to those of nonsmokers, i.e., on the average, they showed a decrease in residence time and normal input rates for apoA-I. Again, however, among the low-HDL smokers, input rates for apoA-I correlated highly with the concentrations of apoA-I, whereas residence times did not. Consequently, our primary hypothesis that apoA-I kinetics between smokers and nonsmokers would be different was not borne out by the results. This does not necessarily mean that molecular mechanisms responsible for low apoA-I levels for the two groups are the same, even though they show a similar pattern of apoA-I kinetics. Furthermore, since the smokers were first selected on the basis of having low HDL levels, we cannot assume that cigarette smoking was the only cause of the patients' reduced HDL concentrations.

Conclusions

This investigation examined kinetics of apoA-I in three types of patients having typical low HDL-cholesterol levels. The primary focus was on patients manifesting normal body weight and normal levels of plasma triglycerides, both nonsmokers and smokers. But in addition, normolipidemic obese patients were studied for further comparison. Regardless of associated factors, or lack of factors, the pattern for apoA-I kinetics in all low-HDL patients was essentially the same. All groups had a shortened residence time for apoA-I, and this was a consistent change for almost all individuals within each group.

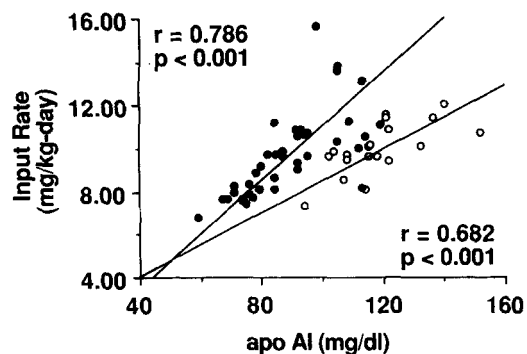


Fig. 5. Correlations between total input rates and plasma concentrations for apoA-I in normal control subjects (O) and low HDL patients (●).

Nonetheless, the investigation does not reveal the precise mechanism for enhanced clearance of apoA-I in the various groups. One common denominator may be an abnormality in the metabolism of plasma triglycerides, but if so, this abnormality was not manifest by definite hypertriglyceridemia; it could, however, be reflected in the patients tendency for high-normal triglyceride levels. For the obese patients, another potential mechanism, i.e., increased uptake of HDL by adipose tissue, cannot be ruled out. Whatever the mechanism, it is apparent that enhanced clearance of apoA-I is an important contributor to low HDL levels under a variety of circumstances, even in the absence of distinct hypertriglyceridemia.

This current study further indicates that a given individual's level of apoA-I is influenced importantly by the input rate for apoA-I. This parameter may be related to the genetic control of apoA-I synthesis, although other factors could affect input rates of apoA-I. The importance of input rate for apoA-I for determining variations in apoA-I concentrations is illustrated in Fig. 5. This figure divides all the subjects in this study into those with normal and low HDL-cholesterol levels. Input rates for apoA-I are plotted against concentrations. The upward displacement of points for low-HDL patients reflects a reduced residence time for apoA-I; this change was characteristic of almost all patients. However, within both groups, input rates also were a major determinant of concentrations of apoA-I. This finding could have therapeutic implications. If a pharmaceutical agent could be developed that would increase production rates of apoA-I, it might offset an increased clearance of apoA-I and thus normalize apoA-I levels. In a word, the role of apoA-I production should not be discounted either in the causation of low apoA-I levels or in the potential for therapeutic modulation of apoA-I concentrations. ■■

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