Lecithin:cholesterol acyltransferase (LCAT) mass; its relationship to LCAT activity and cholesterol esterification rate

John J. Albers, Ching Hong Chen, and Janet L. Adolphson

Department of Medicine, Northwest Lipid Research Clinic, Harborview Medical Center, University of Washington School of Medicine, Seattle, WA

Abstract The relationship between plasma lecithin:cholesterol acyltransferase mass and enzyme activity and between mass and plasma cholesterol esterification rate was determined in 25 adult volunteers without overt disease (14 normolipidemic and 11 hyperlipidemic). Furthermore, the relationship of lecithin:cholesterol acyltransferase mass and cholesterol esterification rate to lipids, apoproteins, age, and ideal body weight was assessed. Lecithin:cholesterol acyltransferase mass determined by radioimmunoassay was highly correlated with enzyme activity assayed using a heated plasma substrate ($r = 0.636$) and with the molar cholesterol esterification rate determined either by radioassay ($r = 0.809$) or by measurement of the decrease of unesterified cholesterol ($r = 0.621$). Lecithin:cholesterol acyltransferase mass was also positively correlated with total cholesterol ($r = 0.608$), unesterified cholesterol ($r = 0.562$), age ($r = 0.544$), and percent ideal body weight ($r = 0.619$), but was not significantly correlated with log triglyceride, high density lipoprotein cholesterol, or apolipoproteins A-I, A-II, or D. Plasma cholesterol esterification rate by both methods was highly positively correlated with total cholesterol, unesterified cholesterol, log triglyceride, and age, but was inversely correlated with high density lipoprotein cholesterol. Upon partial correlation analysis with lecithin:cholesterol acyltransferase mass kept constant the cholesterol esterification rate remained significantly positively related to total cholesterol, unesterified cholesterol, and log triglyceride and inversely related to high density lipoprotein cholesterol. Two subjects had normal lecithin:cholesterol acyltransferase but approximately half normal molar cholesterol esterification rate. Measurement of lecithin:cholesterol acyltransferase mass and activity along with plasma cholesterol esterification rate will permit differentiation of abnormalities of enzyme from qualitative or quantitative substrate or cofactor abnormalities. Also, the finding that the regression line between LCAT mass and the plasma esterification rate by direct determination of unesterified cholesterol passes through the origin suggests that all immunodetectable LCAT in plasma is active in normal subjects.—**Albers, J. J., C-H. Chen, and J. L. Adolphson.** Lecithin:cholesterol acyltransferase (LCAT) mass; its relationship to LCAT activity and cholesterol esterification rate. *J. Lipid Res.* 1981. **22:** 1206–1213.

Supplementary key words radioimmunoassay · enzyme · lipids · lipoproteins

The enzyme lecithin:cholesterol acyltransferase (LCAT) is responsible for the formation of most of the cholesteryl esters found in human plasma (1). The enzyme has been estimated by a variety of chemical and radiochemical techniques which measure either enzyme activity on a common substrate or the cholesterol esterification rate. LCAT activity is usually determined by the radioassay of Glomset and Wright (2) in which plasma containing active enzyme is added to an excess of substrate obtained by equilibrating radioactive cholesterol with heat-inactivated serum. This method is not ideal, because heated serum is a much less efficient substrate than unheated serum and the heated serum substrate cannot be readily standardized. The plasma cholesterol esterification rate which measures both enzyme activity and substrate and cofactor effects is often measured by the radiochemical assay of Stokke and Norum (3). This assay requires preincubation with a reversible LCAT inhibitor and equilibration with radioactive cholesterol. The preincubation and equilibration phases may alter the substrate properties of the plasma and the labeled exogenous cholesterol may not be in complete equilibrium with endogenous cholesterol. This approach may not measure the true initial esterification rate and therefore may not accurately reflect the turnover of cholesterol. A direct determination of the initial rate of the LCAT reaction is obtained by measurement of changes in unesterified cholesterol after incubation at 37°C by sensitive enzymatic (4, 5) or gas–liquid chromatographic methods (6). While these latter assays may reflect the initial cholesterol esterification rate, they do not readily distinguish the influence of altered enzyme or of changes in enzyme levels from

Abbreviations: LCAT, lecithin:cholesterol acyltransferase; HSA, human serum albumin; DTNB, dithiobis nitrobenzoic acid; HDL, high density lipoprotein.

Downloaded from www.jlr.org by guest, on June 19, 2012

the effects of substrate, cofactors, inhibitors, or products.

Hitherto, it has not been possible to measure LCAT mass. We recently reported the development of a sensitive and specific double-antibody radioimmunoassay for human plasma LCAT (7). Assuming that activity of the enzyme at least, in part, reflects LCAT mass, then a measurement of LCAT mass coupled with the measurement of cholesterol esterification rate would permit differentiation of abnormalities of the enzyme from qualitative or quantitative substrate or cofactor abnormalities. However, the relationship between LCAT mass and LCAT activity is yet to be reported. A lack of association between immunoreactive LCAT levels and enzyme activity would suggest the presence of inactive or nonfunctional enzyme.

This report concerns the relationship between LCAT mass and LCAT activity and between LCAT mass and plasma cholesterol esterification rate. The relationship of LCAT mass and plasma cholesterol esterification rate to lipids, apoproteins, age, and ideal body weight is also reported.

MATERIALS AND METHODS

Radioimmunoassay of LCAT

Total plasma LCAT mass was measured by a sensitive double-antibody radioimmunoassay as described (7). All samples were measured in triplicate. Three quality control frozen plasma pools were included in each assay. LCAT mass was measured with a coefficient of variation (CV) of 7 to 9%.

Plasma LCAT activity

Plasma LCAT activity was determined by the procedure based on that of Glomset and Wright (2). For all assays, the heated plasma–[^{14}C]cholesterol substrate was prepared as follows: 0.5 ml of acetone solution containing 108 μCi of [4-^{14}C]cholesterol (sp act 54 mCi/mmol, New England Nuclear Co.) was slowly injected below the surface of 5 ml of a 5% human serum albumin (HSA) in saline solution with constant stirring, and acetone was carefully evaporated under a stream of nitrogen. The HSA-stabilized [^{14}C]cholesterol emulsion was mixed rapidly with 395 ml of heat-inactivated plasma which was prepared by heating fresh plasma at 57°C for 50 min and centrifuging at 2,000 rpm for 20 min to remove precipitated protein. The plasma–[^{14}C]cholesterol mixture was incubated in a 37°C shaking waterbath for 4 hr to allow equilibration of the tracer cholesterol with the unlabeled lipoprotein cholesterol pool. This substrate was subdivided into 20-ml portions and stored frozen at −20°C. For LCAT activity assay, 1 ml of the freshly thawed substrate, which contained 0.25 μCi (5 nmol) of [^{14}C]cholesterol, was pipetted into six identical screw-capped culture tubes. After a preincubation period of 30 min at 37°C, 0.1 ml of 100 mM 2-mercaptoethanol in saline solution and 0.1 ml of fresh plasma were added to each tube. The mixture was incubated at 37°C for 1 hr and the LCAT reaction was stopped by adding 2 ml of ethanol and extracted with 8 ml of hexane as described (8). The hexane extract was then evaporated to dryness and dissolved in 0.5 ml of chloroform. Twenty-five μl of this chloroform solution was spotted onto a 0.5 mm layer of silica gel H and cholesterol and cholesteryl ester were separated with a solvent system of petroleum ether and ethyl acetate (9). The radioactivity in each lipid region was determined in a toluene PPO-POPOP scintillation mixture, using a Beckman LS 7000 scintillation counter. Control reactions, in six tubes containing 0.15 M saline rather than fresh plasma, were performed simultaneously to correct for nonenzymatic reaction. For the calculation of the molar LCAT activity, the fractional LCAT activity (% conversion of [^{14}C]cholesterol/hr) was multiplied by the number of nanomoles of unesterified cholesterol/ml in the heated-plasma substrate to give the number of nanomoles of cholesterol per hr per milliliter of plasma. Unesterified cholesterol of the heated plasma substrate was determined by an enzymic cholesterol procedure (4). The within-assay coefficient of variation of the LCAT activity determinations for the 25 subjects was 6.5 ± 2.1% (mean ± SD), range 3.1 to 10.9%.

Plasma cholesterol esterification rate

Plasma cholesterol esterification rate was assayed according to Stokke and Norum (3) with a slight modification. One hundred microliters of fresh plasma was pipetted into six screw-capped culture tubes. To each tube 20 μl of 5 mM 5,5′-dithiobis nitrobenzoic acid (DTNB) in 100 mM phosphate buffer (pH 7.4) was added, and the mixtures were preincubated at 37°C for 30 min to inhibit the LCAT reaction. Then, 25 μl of a 5% HSA solution containing 0.25 μCi (5 nmol) of [4-^{14}C]cholesterol was added to each tube, and the mixtures were incubated at 37°C for 4 hr to allow equilibrium of the tracer cholesterol with unlabeled plasma lipoproteins. For every 40 assays, the HSA–[^{14}C]cholesterol mixture was prepared by injecting 0.2 ml of an acetone solution containing 10.8 μCi (200 nmol) of [4-^{14}C]cholesterol (sp act 54 mCi/mmol, New England Nuclear Co.) into 1 ml of 5% HSA in 100 mM phosphate buffer solution (pH 7.4) with continuous stirring. The acetone was carefully evaporated under a stream of nitrogen. To reverse the inhibition of

Downloaded from www.jlr.org by guest, on June 19, 2012

LCAT by DTNB, 20 μl of 100 mM 2-mercaptoethanol in 100 mM phosphate buffer (pH 7.4) was added to each tube, mixed, and incubated at 37°C for 40 min. The assay mixture contained 0.1 ml of fresh plasma, 5 nmol [4-^{14}C]cholesterol, 0.6 mmol DTNB, and 12.1 mmol 2-mercaptoethanol in a final volume of 165 μl. The LCAT reaction was stopped, and the mixture was extracted, separated, and counted as described above. Control reactions, in which the enzyme was not reactivated, were run simultaneously in the same manner, except that 20 μl of 100 mM phosphate buffer (pH 7.4) was added to each tube instead of mercaptoethanol. For the calculation of LCAT activity, the fractional LCAT activity (% conversion of labeled cholesterol/hr) was multiplied by the number of nmol of unesterified cholesterol present in the assay mixture to give the amount nmol cholesterol esterified/hr per ml of plasma. The within-assay coefficient of variation for the molar esterification rate by the radioassay procedure was 4.1 ± 1.4%, range 1.9 to 7.2%.

The initial rate of cholesterol esterification in plasma was also assessed by quantifying the decrease in unesterified cholesterol according to a modification of the procedure of Nagasaki and Akanuma (4). Fresh plasma (50 μl) was pipetted into test tubes in sextuplicate for both control and test samples. At zero time, 20 μl of 150 mM iodoacetate in 50 mM phosphate buffer (pH 7.4) was added to each control sample to inhibit the LCAT reaction, whereas 20 μl of 50 mM phosphate buffer (pH 7.4) was added to each test sample. All samples were incubated at 37°C for 40 min. At the end of this incubation, 20 μl of 150 mM iodoacetate in 50 mM phosphate buffer (pH 7.4) was added to each test sample and 20 μl of phosphate buffer was added to each control sample. Then, 1.5 ml each of color reagent was added to all samples and the mixtures were incubated for 15 min at 37°C. Each 1.5 ml of color reagent contained 0.04 U of cholesterol oxidase, 24 U of peroxidase, 0.8 mg (0.004 mmol) of 4-aminoantipyrine, 2 mg (0.021 mmol) of phenol, and 0.2% Triton X-100 in 50 mM phosphate buffer, pH 7.0. The absorbance of the assay mixtures was measured spectrophotometrically at a wavelength of 500 nm. Unesterified cholesterol in each sample was determined by comparison against the color of cholesterol standard solution (Boehringer Mannheim) containing 1 to 60 μg of unesterified cholesterol in which color was developed in the same manner as in the sample solution. The rate of cholesterol esterification was obtained by subtraction of the amount of cholesterol in the test samples from that in the control samples. The within-assay coefficient of variation for the molar esterification rate by the measurement of the decrease of cholesterol mass for the 25 subjects was 7.5 ± 2.2%, range 4.8 to 11.7%.

Subjects and plasma samples

Blood was obtained from adult volunteers without overt disease or from subjects referred to the Northwest Lipid Research Clinic for hyperlipidemia. Eleven of the 25 subjects were hyperlipoproteinemic and 20 were males (**Table 1**). Blood was drawn into tubes containing EDTA according to standard conditions from subjects after an overnight fast of 12–14 hr (10).

Analytical methods

Plasma cholesterol and triglyceride and HDL cholesterol assays were performed according to standard methods of the Lipid Research Clinic Program (10). The HDL cholesterol was determined on the supernatant fraction after precipitation of plasma with heparin–Mn^{2+} (10, 11). Apolipoproteins A-I, A-II, and D were measured by radial immunodiffusion (12–14).

Calculations and statistics

Estimation of percent ideal body weight was obtained by using the Metropolitan Life Insurance tables. In order to convert plasma esterification rate from nmol/hr per ml to nmol/hr per kg, it was assumed that the plasma volume in ml was equal to 4.5% of body weight in kg and a correction factor was added for overweight (15).

Pearson's correlation coefficient r was used to show the degree of linear association between the different variables. The significance of r was found from the t distribution (16). Partial correlation coefficients were calculated as described (16).

RESULTS

LCAT mass was highly correlated with the molar cholesterol esterification rate as determined either by the radioassay procedure or by measurement of the decrease in the mass of unesterified cholesterol (**Table 2, Fig. 1**). The linear relationship of LCAT mass to cholesterol esterification rate determined by the direct chemical method can be expressed by the equation: cholesterol esterification rate (nmol/hr per ml) = 18.0 LCAT mass (μg) − 0.34 (nmol/hr per ml). (see Fig. 1). Therefore, as the plasma esterification rate approaches zero, so does the plasma LCAT mass. This suggests that all immunodetectable LCAT is active in "normal" plasma. On the other hand, the linear relationship of LCAT mass to cholesterol esterification rate deter-

Downloaded from www.jlr.org by guest, on June 19, 2012

TABLE 1. Characteristics of study subjects

Subject	Age	Sex	% Ideal Body Wt.	LCAT Mass[a]	Total CH[b]	Total TG[c]	Medication[d]	LP Classification
A	55	M	103.0	3.17	193	72	NAD	N
B	29	M	102.8	4.07	155	44	None	N
C	61	M	114.8	4.57	234	259	NAD	N
D	32	F	103.0	4.71	183	141	None	N
E	51	M	115.5	5.11	238	564	None	IV
F	42	M	116.8	5.23	161	65	None	N
G	29	F	125.2	5.40	232	545	NAD	IIb
H	55	F	105.9	5.51	258	199	A/P	N
I	28	M	113.4	5.51	189	72	None	N
J	61	M	105.3	5.65	214	424	None	IV
K	60	M	135.4	5.83	204	91	NAD	N
L	33	M	122.8	5.97	196	27	None	N
M	63	M	136.4	6.06	262	101	A/P	N
N	29	F	125.1	6.07	232	545	None	IIb
O	60	M	127.6	6.13	300	250	None	IIa
P	53	M	112.5	6.25	229	156	None	N
Q	58	M	132.4	6.26	255	63	NAD	N
R	62	M	136.0	6.45	190	320	None	IV
S	68	M	141.0	6.73	310	2448	None	V
T	60	M	120.0	6.86	224	352	NAD	IV
U	42	M	117.0	6.87	277	244	None	IIa
V	60	M	133.3	7.11	242	152	A/P	N
W	61	F	130.7	7.13	261	284	NAD	IV
X	55	M	116.4	7.19	228	121	NAD	N
Y	58	M	123.7	7.51	315	67	None	IIa

[a] Lecithin:cholesterol acyltransferase, μg/ml.
[b] Cholesterol, mg/dl.
[c] Triglyceride, mg/dl.
[d] NAD, nicotinic acid derivatives; None, no medications likely to affect lipoproteins; A/P, Atromid, Premarin.

mined by the radioassay method can be expressed by the equation: cholesterol esterification rate (nmol/hr per ml) = 13.2 LCAT mass (μg) − 16.9 (nmol/hr per ml). LCAT mass was also significantly correlated with LCAT activity (Table 2, Fig. 1).

Two subjects had normal LCAT levels but had plasma esterification rates approximately half normal. Subject F (Table 1), a 42-year-old normolipidemic male who had not been taking any medication, had 5.23 μg/ml of LCAT and a cholesterol esterification rate of 34.3 nmol/hr per ml by the radioassay method, or only 57% of the average value obtained in this study, and an esterification rate of 47.9 nmol/hr per ml by the assay of cholesterol mass by the enzymic method, or 45% of the average value. The fractional esterification rate (% cholesterol esterified/hr) was also below average: 2.66% by radioassay and 3.71% by the chemical method compared with average values of 3.24% and 5.62%, respectively. Interestingly, this subject had relatively high levels of HDL cholesterol (61 mg/dl) and apolipoprotein A-I (151 mg/dl) compared with normal levels of 45 and 120 mg/dl, respectively (12). Similarly, subject I (Table 1), a 28-year-old normolipidemic male taking no medication, had an LCAT level of 5.51 μg/ml. Yet, his cholesterol esterification rate was only 38.4 or 60.4 nmol/hr per ml, respectively, approximately half the average rate. The fractional cholesterol esterification rate was approximately two-thirds of the average rate by each

TABLE 2. Correlation of plasma LCAT mass with cholesterol esterification rate, LCAT activity, lipids, HDL-cholesterol, and apolipoproteins, age, and percent ideal body weight

Variable	r	(P)
Molar cholesterol esterification rate[a]	0.809	(<0.001)
Molar cholesterol esterification rate[b]	0.621	(<0.001)
LCAT activity[c]	0.636	(<0.001)
Total cholesterol	0.608	(<0.002)
Cholesteryl ester	0.479	(<0.02)
Unesterified cholesterol	0.562	(<0.005)
Log triglyceride	0.247	(NS)
HDL-cholesterol	−0.134	(NS)
Apolipoprotein A-I	−0.060	(NS)
Apolipoprotein A-II	0.351	(NS)
Apolipoprotein D	−0.034	(NS)
Age	0.544	(<0.005)
Percent ideal body weight	0.619	(<0.001)

[a] Stokke and Norum procedure (3).
[b] Modification of the method of Nagasaki and Akanuma (4).
[c] Glomset and Wright procedure (2).

Downloaded from www.jlr.org by guest, on June 19, 2012

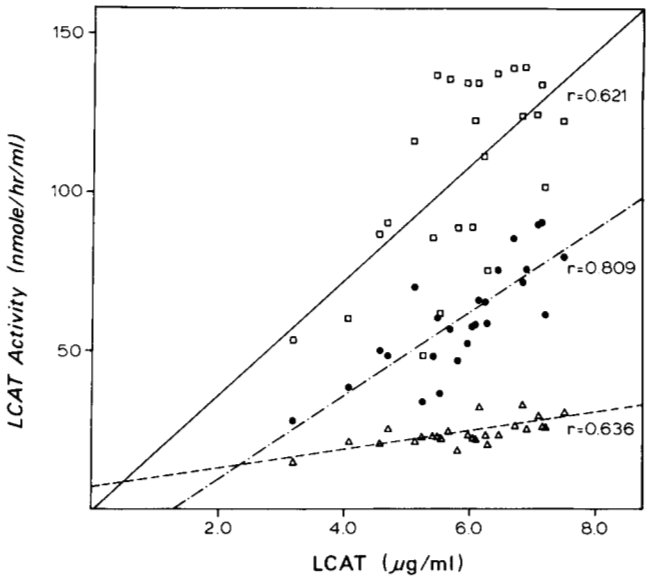

Fig. 1. Correlation of plasma LCAT mass with LCAT activity and molar cholesterol esterification rate. LCAT activity was determined by the method of Glomset and Wright (2) using heated plasma as the LCAT substrate (△). The cholesterol esterification rate was determined by the radioassay method of Stokke and Norum (3, ●) and by measurement of the decrease in unesterified cholesterol with an enzymic method similar to that described by Nagasaki and Akanuma (4, □).

of the esterification methods (2.32%/hr and 3.90%/hr, respectively). Both subjects had LCAT activities consistent with their plasma LCAT levels. Therefore, the reduced esterification rates in these subjects can not be explained by reduced levels of enzyme or altered enzyme, but must be related to quantitative or qualitative alterations in the substrate or cofactors, or to the presence of an LCAT inhibitor.

LCAT mass was also positively correlated with total cholesterol, unesterified cholesterol, and cholesteryl ester (Table 2). It was not significantly correlated with log triglyceride, HDL cholesterol, or apolipoproteins A-I, A-II, and D. However, LCAT mass was significantly positively correlated with age and percent ideal body weight (Table 2). Since ideal body weight is significantly correlated with age ($r = 0.520, P < 0.01$, in this study), the partial correlation coefficient beween LCAT mass and age was not significant if percent ideal body weight was kept constant ($r = 0.311$, NS).

The two methods of estimating the initial plasma cholesterol esterification rate were highly correlated ($r = 0.828, P < 0.001$). However, the cholesterol esterification rate by the Stokke and Norum procedure (3) in the 25 subjects averaged 60.7 ± 17.0 nmol/hr per ml or 2.37 ± 0.60 nmol/hr per kg or 58.5% of that obtained by measurement of the decrease in the mass of unesterified cholesterol (105.6 ± 30.2 nmol/hr per ml or 4.12 ± 1.15 nmol/hr per kg) (see Fig. 1). LCAT activity determined with a common substrate of heated plasma was highly correlated with both methods of cholesterol esterification rate (**Table 3**) but gave values of 24.9 ± 4.9 nmol/hr per ml, significantly lower than both estimates of plasma cholesterol esterification rate (Fig. 1). Both methods of plasma cholesterol esterification rate were highly positively correlated with total cholesterol, unesterified cholesterol, log triglyceride, and age (Table 3). Furthermore, cholesterol esterification rate tended to be inversely correlated with HDL cholesterol. Partial correlation analysis with LCAT mass kept constant indicated that the cholesterol esterification rate was no longer significantly related to LCAT activity, but the cholesterol esterification rate remained significantly positively related to total cholesterol, unesterified cholesterol, and log triglyceride, and inversely related to HDL cholesterol (Table 3).

DISCUSSION

Cholesterol esterification was measured in each of the subjects by two methods. Cholesterol esterification as measured by the decrease in unesterified cholesterol determined by the enzymic method was considerably higher than that measured by radioassay, although the values obtained by the two methods were highly correlated ($r = 0.828$). The mean rate of cholesterol esterification by the direct cholesterol assay was 106 ± 30 nmol/hr per ml compared with only 61 ± 17 nmol/hr per ml by radioassay. The reason for this large difference between the two methods is unclear. The latter values are lower than the values reported by Stokke and Norum (mean 92 nmol/hr per ml) who found that the rate of cholesteryl ester formation by direct cholesterol assay was about 10% higher than values obtained by their radioassay procedure (3). Some workers have found that the cholesterol esterification rate determined by the chemical measurement of unesterified cholesterol is nearly identical to that obtained by the radioassay method (5, 17), while others have reported that the endogenous cholesterol esterification by gas–liquid chromatography was significantly higher (38% on the average) than that obtained by the radioassay technique (18). It is likely that lower values are obtained by the radioassay technique because the labeled cholesterol is not completely equilibrated with the endogenous cholesterol and because the preincubation and equilibration phases of the radioassay procedure may alter the substrate properties of the plasma. If the radioassay technique could be made to approximate more closely the absolute values obtained by the direct measure of the endoge-

Downloaded from www.jlr.org by guest, on June 19, 2012

TABLE 3. Correlation of plasma cholesterol esterification rate with LCAT activity, lipids, HDL cholesterol, apolipoproteins, age, and ideal body weight

Variable	Molar Cholesterol Esterification Rate of Plasma							
	Radioassay Method[a]				Chemical Method[b]			
	r	(P)	r^c	(P)	r	(P)	r^c	(P)
LCAT activity (2)	0.628	(<0.001)	0.251	(NS)	0.509	(<0.01)	0.189	(NS)
Total cholesterol	0.687	(<0.001)	0.481	(<0.02)	0.628	(<0.001)	0.402	(<0.05)
Unesterified cholesterol	0.736	(<0.001)	0.579	(<0.005)	0.708	(<0.001)	0.554	(<0.005)
Log triglyceride	0.504	(<0.01)	0.534	(0.01)	0.972	(<0.001)	1.08	(<0.001)
HDL-cholesterol	−0.390	(<0.05)	−0.483	(<0.02)	−0.596	(<0.001)	−0.660	(<0.001)
Apolipoprotein A-I	0.039	(NS)	0.149	(NS)	−0.092	(NS)	−0.070	(NS)
Apolipoprotein A-II	0.338	(NS)	0.010	(NS)	0.435	(<0.05)	0.296	(NS)
Apolipoprotein D	−0.150	(NS)	−0.209	(NS)	−0.349	(NS)	−0.419	(<0.05)
Age	0.973	(<0.001)	1.08	(<0.001)	0.469	(<0.02)	0.199	(NS)
Percent ideal body weight	0.549	(<0.005)	0.109	(NS)	0.318	(NS)	−0.108	(NS)

[a] Stokke and Norum procedure (3).
[b] Modification of the method of Nagasaki and Akanuma (4).
[c] Partial correlation of molar esterification rate with each variable with LCAT mass kept constant.

nous cholesterol esterification rate, it would be the preferred method because it is subject to less error and is technically less demanding. On the other hand, direct measurement of the decrease of unesterified cholesterol either by gas–liquid chromatography or by an enzymic method requires a large number of replicates because it is obtained by subtraction of two similar numbers.

LCAT activity as measured by the Glomset and Wright procedure (2) was much lower (mean values 25 nmol/hr per ml) than either estimation of the plasma esterification rate. This method gives significantly lower values because heated plasma is a much poorer substrate than fresh plasma and the contribution of the cholesterol of the test sample to the esterification rate is considered negligible. This method is far from ideal because the low values give rise to small differences between individuals and thereby increase the error of the determination. Furthermore, standardization is difficult because the value depends upon the particular plasma or serum used for the assay. Although LCAT mass was linearly related to LCAT activity, as predicted, the strength of this association was lower than expected, presumably because of the errors of the assays, particularly in the estimation of LCAT activity. Use of a common substrate that is well-defined and reproducible, and yet contains substrate properties comparable to fresh plasma is needed. Preliminary investigations with a synthetic substrate containing apolipoprotein A-I, egg lecithin, and cholesterol suggest that preparing a substrate with these properties is possible (19).

We report for the first time that LCAT mass is significantly positively correlated with plasma esterification rate and LCAT activity. The high correlation of the molar cholesterol esterification rate by the radioassay method with LCAT mass ($r = 0.809$) indicates that, on the average, at least 65% of the variance (r^2) of the plasma esterification rate is determined by the amount of enzyme in plasma. The remaining variation in the plasma esterification rate is due, in part, to the errors in the determination, but primarily to differences in substrate, cofactors or inhibitors of the LCAT reaction or to cholesteryl ester transfer activity or to the availability of cholesteryl ester acceptors. The strong relationship between esterification rate and LCAT mass was observed even though many of the subjects were taking hypolipidemic agents. The finding that the regression line between LCAT mass and plasma esterification rate (by direct determination of unesterified cholesterol) passed through the origin suggests that all immunodetectable LCAT is active in the plasma of normal subjects. In contrast, the failure of the regression line of LCAT mass versus esterification rate (determined with the Stokke-Norum assay) to pass through the origin may reflect the limitations of this assay methodology and the high degree of uncertainty of this method at low esterification rates. Because of the uncertainty involved in the extrapolation of regression lines and the practical limitation of the assay methods, additional studies will be required to confirm the suggestion that all immunoreactive LCAT in plasma is active in normal subjects.

Recent evaluation of a large kindred from Sardinia with familial LCAT deficiency indicated that the LCAT-deficient homozygotes have low levels of enzyme mass and activity whereas the heterozygotes have enzyme activity and mass approximately half normal (20, 21). Similarly, in a Canadian family with LCAT deficiency, the two homozygotes had unde-

Downloaded from www.jlr.org by guest, on June 19, 2012

tectable enzyme mass and activity and the heterozygotes had half the normal levels of enzyme mass and activity (22). In contrast to these families, studies of one Japanese and four Norwegian LCAT-deficient families clearly demonstrate that homozygotes in these families have low but variable amounts of a functionally defective enzyme in their plasma.[1]

We have confirmed the findings in our previous report that LCAT mass is significantly correlated with plasma cholesterol (7). Plasma triglyceride was also positively correlated with LCAT mass as reported earlier (7), but this relationship did not reach significance in our present study presumably because of a narrower range of triglyceride values. LCAT mass was highly correlated with age or percent ideal body weight. LCAT mass was not significantly related to HDL cholesterol or to the HDL apolipoproteins A-I, A-II, and D. Consistent with our present study, earlier studies suggested that plasma cholesterol esterification rate is positively correlated with cholesterol, triglyceride, and percent ideal body weight and negatively correlated with HDL cholesterol (17, 23–25). The inverse relationship observed between HDL cholesterol and esterification rate appears to be primarily related to the inverse relationship between triglyceride and the HDL cholesterol, since partial correlation analysis keeping triglyceride constant indicated that HDL cholesterol is no longer significantly negatively related to the esterification rate ($r = -0.085$). The molar esterification rate has recently been reported to be inversely related to the ratio of HDL_2 cholesterol/HDL_3 cholesterol (26). Since the HDL_2/HDL_3 ratio generally increases with an increase in HDL cholesterol, these results would be consistent with our findings. The seven hypertriglycerdemic subjects had higher cholesterol esterification rates (122.4 ± 18.0 nmol/hr per ml) relative to LCAT mass (6.10 ± 0.76 g/ml) than the normolipidemic subjects not on medication (esterification rate, 84.2 ± 33.7 nmol/hr per ml; mass 5.29 ± 81 μg/ml, n = 6). Thus, the present study confirms the earlier suggestion (27) that both an increase in LCAT mass and changes in the substrate or cofactor properties of plasma are responsible for the higher LCAT activity in hypertriglyceridemia. A decrease in HDL_2 often observed in hypertriglyceridemia could be partly responsible for the higher esterification rate observed in these subjects. On the other hand, if LCAT mass were kept constant, the percent ideal body weight was no longer significantly related to the esterification rate. This indicates that the increase in plasma esterification rate observed with increasing percent ideal body weight is primarily due to an increase in enzyme.

These initial studies on the relationship between LCAT mass and enzyme activity suggest that LCAT mass generally reflects enzyme activity. Examination of the relationship of cholesterol esterification along with LCAT mass should therefore permit identification of substrate or cofactor abnormalities. Among the 25 subjects thus far examined, several appeared to have low levels of esterification rate suggesting a defect in substrate or cofactors or an increase in an LCAT inhibitor. One of these two subjects had a high level of HDL consistent with an increase of HDL_2. HDL_2 does not appear to serve as an LCAT substrate (6, 28). Increased levels of this HDL subclass may decrease the plasma molar esterification rate by binding a significant portion of the total enzyme making it unavailable for the preferred substrate for the LCAT reaction. Reduced level of a cholesteryl ester transfer protein or limited availability of cholesteryl ester acceptors resulting in product inhibition of the LCAT (29) reaction is another possible explanation for the reduced cholesterol esterification rate in some subjects. Additional studies are in progress to identify subjects with these potential abnormalities related to the LCAT reaction.

These studies were supported by Grant HL15263 and contract NIHV 12157A from the Lipid Metabolism Branch, NHLBI and a grant from R. J. Reynolds Industries, Inc. John J. Albers is an Established Investigator of the American Heart Association. The authors wish to thank Drs. Robert Knopp and John D. Brunzell for assistance in providing the samples for this study and G. Russell Warnick and the staff of the Northwest Lipid Research Core Laboratory for performing the lipid and lipoprotein analysis. We also thank Dr. Brunzell for review of the manuscript.

Manuscript received 13 February 1981 and in revised form 8 June 1981.

REFERENCES

1. Glomset, J. A., and K. R. Norum. 1973. The metabolic role of lecithin:cholesterol acyltransferase. *Adv. Lipid Res.* **2:** 1–65.
2. Glomset, J. A., and J. L. Wright. 1964. Some properties of a cholesterol esterifying enzyme in human plasma. *Biochim. Biophys. Acta.* **89:** 266–276.
3. Stokke, K. J., and K. R. Norum. 1971. Determination of lecithin:cholesterol acyltransferase in human blood plasma. *Scand. J. Lab. Invest.* **27:** 21–27.
4. Nagasaki, T., and Y. Akanuma. 1977. A new colorimetric method for the determination of plasma lecithin: cholesterol acyltransferase activity. *Clin. Chim. Acta.* **75:** 371–375.
5. Patsch, W., S. Sailer, and H. Braunsteiner. 1976. An enzymatic method for the determination of the initial

[1] Albers, J. J., E. Gjone, and Y. Akanuma. Unpublished observations.

Downloaded from www.jlr.org by guest, on June 19, 2012

rate of cholesterol esterification in human plasma. *J. Lipid Res.* **17:** 182–185.
6. Marcel, Y. L., and C. Vézina. 1973. A method for the determination of the initial rate of reaction of lecithin: cholesterol acyltransferase in human plasma. *Biochim. Biophys. Acta.* **306:** 497–504.
7. Albers, J. J., J. L. Adolphson, and C-H. Chen. 1981. Radioimmunoassay of human plasma lecithin:cholesterol acyltransferase. *J. Clin. Invest.* **67:** 141–148.
8. Chen, C-H., and J. J. Albers. 1981. Further characterization of purified lecithin:cholesterol acyltransferase from human plasma. *Biochem. Med.* In press.
9. Albers, J. J., J. Lin, and G. P. Roberts. 1979. Effect of human plasma apolipoproteins on the activity of purified lecithin:cholesterol acyltransferase. *Artery.* **5:** 61–75.
10. Manual of Laboratory Operations, Lipid Research Clinics Program. Vol. 1. 1974. Lipid and Lipoprotein Analysis. DHEW Publication No. (NIH) 75-628.
11. Albers, J. J., G. R. Warnick, N. Johnson, P. S. Bachorik, R. Muesing, K. Lippel, and O. D. Williams. 1980. Quality control of plasma high-density-lipoprotein cholesterol measurement methods. The Lipid Research Clinics Program Prevalence Study. *Circulation.* **62 Suppl. IV:** 9–16.
12. Albers, J. J., P. W. Wahl, V. G. Cabana, W. R. Hazzard, and J. J. Hoover. 1976. Quantitation of apolipoprotein A-I of human plasma high density lipoprotein. *Metabolism.* **25:** 633–644.
13. Cheung, M. C., and J. J. Albers. 1977. The measurement of apolipoprotein A-I and A-II levels in men and women by immunoassay. *J. Clin. Invest.* **60:** 43–50.
14. Albers, J. J., M. C. Cheung, S. L. Ewens, and J. H. Tollefson. 1981. Characterization and immunoassay of apolipoprotein D. *Atherosclerosis.* **39:** 395–409.
15. Nikkila, E. A., and M. Kekki. 1971. Polymorphism of plasma triglyceride kinetics in normal human adult subjects. *Acta Med. Scand.* **190:** 49–59.
16. Snedecor, G. W., and W. G. Cochran. 1976. Statistical Methods. 6th Ed. Iowa State University Press, Ames, Iowa. 1–593.
17. Dieplinger, H., and G. M. Kostner. 1980. The determination of lecithin:cholesterol acyl transferase in the clinical laboratory: a modified enzymatic procedure. *Clin. Chim. Acta.* **106:** 319–324.
18. Fabien, H. D., J. Davignon, and Y. L. Marcel. 1973. Plasma cholesterol esterification in normal and hyperlipoproteinemic subjects. *Can. J. Biochem.* **51:** 550–555.
19. Chen, C-H., and J. J. Albers. 1981. A new synthetic common substrate for rapid, sensitive, and precise determination of LCAT activity. *Federation Proc.* **40:** 1775 (Abstract).
20. Albers, J. J., and G. Utermann. 1981. Genetic control of lecithin:cholesterol acyltransferase: measurement of LCAT mass in a large kindred with LCAT deficiency. *Am. J. Hum. Genet.* **33:** 702–708.
21. Utermann, G., H. J. Menzel, D. Dieker, K. H. Langer, and G. Fiorelli. 1981. Lecithin:cholesterol acyltransferase deficiency: autosomal recessive transmission in a large kindred. *Clin. Genet.* In press.
22. Albers, J. J., C-H. Chen, and J. L. Adolphson. 1981. Familial lecithin:cholesterol acyltransferase: identification of heterozygotes with half-normal enzyme activity and mass. *Hum. Genet.* In press.
23. Wallentin, L. 1977. Lecithin:cholesterol acyltransferase rate in plasma and its relation to lipid and lipoprotein concentrations in primary hyperlipidemia. *Atherosclerosis.* **26:** 233–248.
24. Wallentin, L., and O. Vikrot. 1975. Lecithin:cholesterol acyltransferase in plasma of normal persons in relation to lipid and lipoprotein concentration. *Scand. J. Clin. Lab. Invest.* **35:** 669–676.
25. Akanuma, Y., T. Kuzuya, M. Hayashi, T. Ide, and N. Kuzuya. 1973. Positive correlation of serum lecithin: cholesterol acyltransferase activity with relative body weight. *Eur. J. Clin. Invest.* **3:** 136–141.
26. Pinon, J-C., A-M. Bridoux, and M-H. Laudat. 1980. Initial rate of cholesterol esterification associated with high density lipoproteins in human plasma. *J. Lipid Res.* **21:** 406–414.
27. Patsch, W., H-J. Lisch, S. Sailer, and H. Braunsteiner. 1978. Initial cholesterol esterification rate in hyperlipoproteinemia: effects of triglyceride-rich lipoproteins. *Eur. J. Clin. Invest.* **8:** 209–215.
28. Fielding, C. J., and P. E. Fielding. 1971. Purification and substrate specificity of lecithin:cholesterol acyltransferase from human plasma. *FEBS Lett.* **15:** 355–358.
29. Chajek, T., A. Lieselotte, and C. J. Fielding. 1980. Interaction of lecithin:cholesterol acyltransferase and cholesteryl ester transfer protein in the transport of cholesteryl ester into sphingomyelin. *Biochemistry.* **19:** 3673–3677.

Downloaded from www.jlr.org by guest, on June 19, 2012