Increased Serum Cholesterol Esterification Rates Predict Coronary Heart Disease and Sudden Death in a General Population

Shin-ichiro Tanaka, Tomoyuki Yasuda, Tatsuro Ishida, Yoshio Fujioka, Takeshi Tsujino, Tetsuo Miki, Ken-ichi Hirata

Objective—Lecithin:cholesterol acyltransferase (LCAT) is thought to be important in reverse cholesterol transport. However, its association with coronary heart disease (CHD) and sudden death is controversial.

Approach and Results—We prospectively studied 1927 individuals from the general population. Serum concentrations of apolipoprotein A-I, A-II, B, C-II, C-III, E, and LCAT activity measured as a serum cholesterol esterification rate were evaluated. We documented 61 events of CHD and sudden death during 10.9 years of follow-up. After adjustment for age and sex, LCAT activity was significantly associated with the risk of CHD and sudden death (hazard ratio, 3.02; 95% confidence interval, 1.49–6.12; P=0.002). In multivariate analysis adjusted for age, sex, current smoking status, history of diabetes mellitus, body mass index, systolic blood pressure, serum total cholesterol, and serum high-density lipoprotein cholesterol concentrations, the hazard ratio of LCAT activity for the risk of CHD and sudden death remained significant (hazard ratio, 3.07; 95% confidence interval, 1.35–7.01; P=0.008). However, when it was analyzed for men and women separately, this association remained significant only in women.

Conclusions—Increased LCAT activity measured as a serum cholesterol esterification rate was a risk for CHD and sudden death in a Japanese general population. (***Arterioscler Thromb Vasc Biol***. 2013;33:1098-1104.)

Key Words: cohort study ■ coronary heart disease ■ lecithin:cholesterol acyltransferase ■ sex differences ■ sudden death

High-density lipoproteins (HDL) are thought to play a central role in reverse cholesterol transport (RCT), which is the uptake of cholesterol from peripheral tissues and its transport to the liver,[1] and many epidemiological studies have shown that a decreased concentration of HDL-cholesterol (HDL-C) is associated with an increased risk of coronary heart disease (CHD).[2]

Lecithin:cholesterol acyltransferase (LCAT) associates with HDL, and to a lesser extent low-density lipoproteins.[3] LCAT hydrolyzes the 2-acyl group of phosphatidylcholine (lecithin), and then transfers and esterifies the fatty acids to the free 3-hydroxy group of cholesterol with apolipoprotein (apo) A-I as a cofactor, and generates cholesterol ester and lysophosphatidylcholine.[1] The mature HDL can then be taken up by the liver and are eventually excreted as cholesterol in the bile. Therefore, esterification of cholesterol by LCAT has been regarded as a key step in RCT. However, the precise role of LCAT in the pathogenesis of CHD is not yet understood.

Experiments with LCAT overexpression models[4–8] and LCAT knockout mice[9–11] have demonstrated both proatherogenic and antiatherogenic effects of LCAT, and these discrepancies have not yet been clearly explained.[12,13] Patients with LCAT deficiency attributable to mutations in the LCAT gene develop severe HDL deficiency. However, they do not seem to be particularly prone to premature CHD,[14] and there are also conflicting results when evaluating atherosclerosis as carotid intima-media thickness in patients with LCAT deficiency.[15,16] In clinical studies, LCAT activity was shown to be elevated in patients with metabolic syndrome and was also associated with increased carotid atherosclerosis,[17] whereas other studies reported decreased LCAT activity in patients with CHD.[18,19] However, all these studies lacked a prospective design.

Recently, 2 prospective case–control studies of general populations have been reported. In a case–control analysis nested in the European Prospective Investigation of Cancer-Norfolk population study, Holleboom et al reported that plasma concentrations of LCAT mass did not differ between CHD cases and controls.[20] In contrast, in a community-based nested case–control study of men, Dullaart et al reported that the age-adjusted incidence of cardiovascular disease was significantly increased in subjects with higher LCAT activity

measured by the exogenous substrate method. However, this relationship was not significant after adjustment for lipids.[21] Thus, the number of prospective studies in humans is still limited, and the results are controversial.[12] Here, we report that increased LCAT activity measured as a serum cholesterol esterification rate by the endogenous substrate method is significantly associated with a future risk of CHD and sudden death in a community-based cohort study.

Materials and Methods

Materials and Methods are available in the online-only Supplement.

Results

Baseline characteristics of the study cohort are presented in Table 1. Women had higher concentrations of total cholesterol (TC), HDL-C, and non–HDL-C than men (all tests for trend, $P<0.001$). Men had higher proportions of current smokers ($P<0.001$) and patients with a history of diabetes mellitus ($P=0.03$), a higher serum concentration of triglycerides (TG) ($P<0.001$), and a higher level of serum thiobarbituric acid reactive substances (TBARS) ($P=0.001$), than Women.

During the 19 866 person-years of follow-up (median follow-up period, 10.9 years), we documented 19 cases of myocardial infarction (15 in men and 4 in women); 12 cases of angina pectoris, coronary artery bypass, and angioplasty procedures (10 in men and 2 in women); and 30 cases of sudden deaths (13 in men and 17 in women). Thus, the total number of events was 61.

Table 2 shows the baseline measurements for each variable stratified by LCAT activity tertiles. Body mass index, waist

circumference, systolic blood pressure, diastolic blood pressure, serum hemoglobin A1c levels, serum TC, non–HDL-C, TG, apoA-I, apoA-II, apoB, apoC-II, apoC-III, and apoE concentrations were all significantly increased in subjects with high LCAT activity (all tests for trend, $P<0.001$), whereas serum HDL-C and HDL$_2$-C concentrations were significantly decreased in subjects with high LCAT activity ($P<0.001$ and $P<0.001$, respectively).

In age- and sex-adjusted analysis, increased levels of waist circumference, hemoglobin A1c, serum apoC-II, and serum LCAT activity were significantly associated with the increasing risk of CHD and sudden death. The hazard ratios (HRs) in the highest tertile as compared with the lowest tertile were as follows: 2.70 for waist circumference (95% confidence interval [CI], 1.25−5.87; $P=0.01$), 2.23 for apoC-II level (95% CI, 1.09−4.58; $P=0.03$), and 3.02 for LCAT activity (95% CI, 1.49−6.12; $P=0.002$). A history of diabetes mellitus and a history of hypertension were also associated with the risk of CHD and sudden death (HR, 2.27; 95% CI, 1.08−4.80; $P=0.03$ and HR, 1.94; 95% CI, 1.15−3.27; $P=0.01$, respectively). We could not find associations between other lipid markers and events.

Table 3 shows the correlations between baseline LCAT activity and other variables. Body mass index ($R=0.418$), waist circumference ($R=0.365$), TC ($R=0.466$), non–HDL-C ($R=0.369$), TG ($R=0.600$), apoA-II ($R=0.399$), apoB ($R=0.578$), apoC-II ($R=0.556$), apoC-III ($R=0.629$), and apoE ($R=0.457$) levels were strongly correlated with LCAT activity. In contrast, HDL-C, particularly HDL$_2$-C concentrations

Table 1.   Baseline Characteristics of the Study Cohort

Characteristics	Total	Women	Men	P Value*
Number	1927	1136	791	
Age, years	58.4±14.8	58.3±15.0	58.5±14.6	0.75
History of diabetes mellitus, %	5.2	4.3	6.6	0.03
History of hypertension, %	32.6	34.2	30.4	0.09
Antihypertensive medication, %	15.6	16.9	13.7	0.05
Current smoker, %	22.8	2.4	52.2	<0.001
BMI, kg/m^2	22.5±3.0	22.5±3.0	22.4±2.9	0.63
Waist circumference, cm	72.8±8.3	70.1±7.3	76.9±8.0	<0.001
SBP, mm Hg	133±21	133±21	135±21	0.06
DBP, mm Hg	77±12	76±12	79±12	<0.001
HbA1c, %	4.9±0.8	4.9±0.7	5.0±0.8	0.001
TC, mmol/L	5.15±0.93	5.33±0.93	4.86±0.88	<0.001
HDL-C, mmol/L	1.53±0.39	1.55±0.36	1.47±0.39	<0.001
HDL$_2$-C, mmol/L	1.01±0.36	1.05±0.36	0.97±0.36	<0.001
HDL$_3$-C, mmol/L	0.50±0.10	0.51±0.09	0.49±0.11	<0.001
Non–HDL-C, mmol/L	3.62±1.01	3.75±0.98	3.41±0.98	<0.001
Triglycerides, mmol/L	1.00 (0.75)	0.97 (0.67)	1.10 (0.87)	<0.001
Phospholipids, mg/dL	219±33	223±32	213±34	<0.001
LCAT activity, nmol/mL per h	76.6±22.1	75.7±21.4	77.8±23.1	0.04
TBARS, μmol/L	5.0±1.9	4.9±1.7	5.2±2.1	0.001

Values are expressed as mean±SD except triglycerides, which are given as the median (interquartile range). BMI indicates body mass index; DBP, diastolic blood pressure; HbA1c, glycosylated hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; LCAT, lecithin:cholesterol acyltransferase activity; SBP, systolic blood pressure; TBARS, thiobarbituric acid reactive substances; and TC, total cholesterol.

*$P<0.05$ is considered to indicate statistically significant differences between men and women.

Table 2. Baseline Characteristics and Risk Factors According to LCAT Activity Levels

	Tertile 1	Tertile 2	Tertile 3	P Value
LCAT activity, nmol/mL per h	<66	66–84	>84	
Number	645	645	637	
Age, years	59.9±7.0	58.2±14.1	57.2±13.0	0.003
Male sex, %	39.4	36.9	46.9	0.001
History of diabetes mellitus, %	3.6	4.3	7.8	0.001
History of hypertension, %	27.1	32.8	38.0	<0.001
Current smoker, %	22.5	20.3	25.8	0.063
BMI, kg/m^2	21.1±2.6	22.4±2.7	24.0±2.9	<0.001
Waist circumference, cm	69.6±7.2	72.3±7.9	76.6±8.2	<0.001
SBP, mm Hg	130±21	133±21	137±20	<0.001
DBP, mm Hg	75±12	77±12	80±11	<0.001
HbA1c, %	4.8±0.5	4.9±0.7	5.1±0.9	<0.001
TC, mmol/L	4.65±0.88	5.15±0.80	5.61±0.88	<0.001
HDL-C, mmol/L	1.60±0.39	1.55±0.36	1.40±0.36	<0.001
HDL$_2$-C, mmol/L	1.13±0.37	1.05±0.35	0.87±0.32	<0.001
HDL$_3$-C, mmol/L	0.48±0.10	0.51±0.10	0.52±0.11	<0.001
Non–HDL-C, mmol/L	3.03±0.88	3.59±0.83	4.22±0.91	<0.001
Triglycerides, mmol/L	0.73 (0.38)	0.99 (0.56)	1.53 (1.05)	<0.001
ApoA-I, mg/dL	119±26	125±25	127±27	<0.001
ApoA-II, mg/dL	32±8	37±9	41±10	<0.001
ApoB, mg/dL	76±22	92±23	112±25	<0.001
ApoC-II, mg/dL	2.9 (1.2)	3.3 (1.3)	4.7 (2.3)	<0.001
ApoC-III, mg/dL	6.4 (3.5)	8.4 (3.8)	11.9 (5.7)	<0.001
ApoE, mg/dL	3.8 (1.2)	4.2 (1.3)	5.0 (1.6)	<0.001
TBARS, μmol/L	4.6±1.7	4.8±1.4	5.6±2.2	<0.001

Values are expressed as mean±SD except triglycerides, apo-CII, apoC-III, and apoE, which are given as the median (interquartile range). Apo indicates apolipoprotein; BMI, body mass index; DBP, diastolic blood pressure; HbA1c, glycosylated hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; LCAT, lecithin:cholesterol acyltransferase activity; SBP, systolic blood pressure; TBARS, thiobarbituric acid reactive substances; and TC, total cholesterol.

were inversely correlated with LCAT activity (R=–0.278 and R=–0.326, respectively). Systolic blood pressure, diastolic blood pressure, hemoglobin A1c, HDL$_3$-C, apoA-I, and TBARS levels were positively correlated with LCAT activity, but to a lesser extent. These results indicate that LCAT activity is closely correlated with dyslipidemia, particularly with metabolic syndrome that involves increased TG-rich apoB-containing lipoprotein and low HDL-C concentrations.

Table 4 shows the age- and sex-adjusted LCAT activity tertile analysis for the incidence of CHD and sudden death. Serum LCAT activity was significantly associated with the risk of CHD and sudden death in women (HR, 5.41; 95% CI, 1.42–20.63; P=0.01), but not in men (HR, 1.86; 95% CI, 0.77–4.49; P=0.17). When we include both sexes in the analysis, the association remained significant (HR, 3.22; 95% CI, 1.34–7.73; P=0.009). After adjustment for established coronary risk factors, including age, sex, current smoking status, history of diabetes mellitus, body mass index, systolic blood pressure, TC, and HDL-C, the results were almost the same (HR, 4.70; 95% CI, 1.10–20.07; P=0.04 for women; HR, 2.16; 95% CI, 0.73–6.36; P=0.16 for men; and HR, 3.07; 95% CI, 1.35–7.01; P=0.008 for the entire cohort) as shown in Table 4, model 1. Furthermore, ApoB and TG concentrations were highly correlated with LCAT activity; both

have previously been reported to be associated with a risk of CHD.[31] We therefore performed multivariate analysis, adjusting for the same variables as model 1, but substituting apoB and TG for TC and HDL-C (Table 4, model 2). The associations remained nearly unchanged as compared with model 1 (HR, 6.08; 95% CI, 1.29–28.72; P=0.02 for women; HR, 1.85; 95% CI, 0.60–5.71; P=0.29 for men; and HR, 3.22; 95% CI, 1.34–7.73; P=0.009 for the entire cohort).

Discussion

Our population-based cohort study demonstrated that increased serum LCAT activity expressed as a cholesterol esterification rate using the endogenous substrate method is associated with an increased risk of CHD and sudden death. This association is significant even after adjustment for established coronary risk factors, most of which are highly correlated with LCAT activity. Increased LCAT activity may therefore be an independent risk factor for CHD and sudden death in the general population. However, in sex-specific analyses, the association was significant in women, but not in men.

In the nested case–control study from the European Prospective Investigation of Cancer-Norfolk population study, Holleboom et al has reported a higher risk for CHD in women, but not men, with high LCAT mass concentrations.[20] This

Table 3. Correlations Between Baseline LCAT Activity and Other Variables

Variables	R*	P Value
BMI	0.418	<0.001
Waist circumference	0.365	<0.001
SBP	0.132	<0.001
DBP	0.157	<0.001
HbA1c	0.162	<0.001
TC	0.466	<0.001
HDL-C	−0.278	<0.001
HDL$_2$-C	−0.326	<0.001
HDL$_3$-C	0.147	<0.001
Non–HDL-C	0.369	<0.001
Triglycerides	0.600	<0.001
ApoA-I	0.135	<0.001
ApoA-II	0.399	<0.001
ApoB	0.578	<0.001
ApoC-II	0.556	<0.001
ApoC-III	0.629	<0.001
ApoE	0.457	<0.001
TBARS	0.246	<0.001

Correlations between LCAT activity and serum triglycerides, apoC-II, apoC-III, and apoE were calculated with the use of Spearman correlation coefficient, and other variables were calculated with the use of Pearson correlation coefficient. Apo indicates apolipoprotein; BMI, body mass index; DBP, diastolic blood pressure; HbA1c, glycosylated hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; LCAT, lecithin:cholesterol acyltransferase activity; SBP, systolic blood pressure; TBARS, thiobarbituric acid reactive substances; and TC, total cholesterol.

*R denotes the correlation coefficient for the corresponding P value.

finding is in good agreement with our results. In addition, their method of measuring LCAT mass concentration is reported to have an excellent correlation with our method of measuring LCAT activity,[26] further supporting our results. The reason why the association between LCAT and CHD is higher in women than in men is not clear. Li et al recently reported that LCAT-deficient female mice are protected from diet-induced obesity and insulin resistance in a sex-specific manner.[32] They found decreased endoplasmic reticulum stress markers and increased gene expressions which prevent adipogenesis, in hyperlipidemic LCAT-deficient female mice. They also reported that LCAT may cause metabolic disorders in association with hyperlipidemia.[11] Another possible explanation for this sex-specific effect may be because of the higher percentage of men who smoke. Albers et al has reported that higher smoking rates are associated with decreased LCAT mass;[33] thus, smoking may attenuate the effect of increased LCAT activity.

We defined LCAT activity in the present study, as the cholesterol esterification rate in the presence of endogenous plasma lipoproteins. The initial rate of cholesterol esterification has been supposed to represent HDL-C esterification.[3] However, plasma TG-rich lipoproteins such as very low-density lipoproteins (VLDL) have been reported to receive the majority of the cholesterol ester generated by the LCAT reaction,[34] and plasma VLDL and lipid transfer proteins have been known to affect cholesterol esterification in human

plasma.[35,36] Furthermore, previous studies have revealed that cholesterol esterification in incubated plasma is positively associated with increasing TG-rich lipoprotein concentrations using both the endogenous,[36–39] and the exogenous substrate methods,[40] and is negatively associated with increasing HDL concentrations using the endogenous substrate methods.[36,38,39] Moreover, HDL$_2$ has been proposed as a competitive inhibitor of LCAT,[36,37,41,42] whereas HDL$_3$ has been reported to be a favorable substrate for LCAT with the use of endogenous substrate method or the reconstituted model using purified LCAT and isolated lipoproteins.[41–43] It should be noted that genetically deficient LCAT individuals are different from individuals from the general population. The former are not able to produce sufficient LCAT enzymes. Therefore, they have the difficulty in cholesterol esterification reactions, leading to low plasma HDL-C levels.[15] In this situation, plasma HDL-C concentration may be positively correlated with plasma LCAT mass concentration and LCAT activity. However, individuals from the general population have sufficient potential to produce the enzyme, and most of them can also produce appropriate plasma HDL. The product HDL-C, particularly HDL$_2$ subfraction, is supposed to have potential to inhibit LCAT, whereas HDL$_3$ and smaller HDL can serve as substrates which facilitate LCAT reaction.[41,42] These findings are consistent with our results showing that LCAT activity is highly correlated with increased serum concentrations of apoB, apoC-II, apoC-III, and apoE, all of which are components of TG-rich lipoproteins, and inversely correlated with serum HDL-C concentration, particularly HDL$_2$-C concentration. Thus, our results indicate that elevated LCAT activity may reflect a dyslipidemic condition. Taken together, we can suggest a mechanistic explanation for the relationship between increased LCAT activities and CHD as follows: increased plasma TG-rich lipoprotein concentrations have been shown to be associated with low plasma HDL-C concentrations,[44] and both plasma LCAT activities and the cholesterol ester transfer from HDL to VLDL may also be facilitated by increasing TG-rich lipoprotein concentrations,[36] leading to high plasma apoB-containing lipoprotein cholesterol and low HDL-C concentrations. The apoB-containing lipoproteins can be removed by the receptor-mediated uptake in the liver as an alternative RCT pathway. However, as distinct from HDL, they are potentially atherogenic lipoproteins, and therefore may promote atherosclerosis. Thus, increased LCAT activity in association with hyperlipidemia may cause CHD.

Nevertheless, the underlying mechanism by which increased LCAT activity leads to the development of CHD and sudden death is not clear. Recently, our group[23] and others[45–47] have reported that increased oxidative stress and subsequent oxidative modification of lipids are risk factors for CHD. Furthermore, recent studies have shown that oxidative injury causes oxidation of not only LDL,[48] but also other lipoproteins,[49,50] all of which contribute to atherosclerosis. LCAT has been reported to have antioxidant effects preventing low-density lipoprotein oxidation.[7,51,52] However, LCAT has also been reported to promote VLDL oxidation.[53] Furthermore, Ng et al has reported that LCAT deficiency may be rather favorable for reducing oxidative

Table 4. Multivariate-Adjusted Analysis of the Risk for CHD* and Sudden Death According to Baseline LCAT Activities

	Total	Men	Women
Age-, sex-adjusted			
HR†	3.22	1.86	5.41
95% CI	1.34–7.73	0.77–4.49	1.42–20.63
P value	0.009	0.17	0.01
Multivariate model 1‡			
HR	3.07	2.16	4.70
95% CI	1.35–7.01	0.73–6.36	1.10–20.07
P value	0.008	0.16	0.04
Multivariate model 2§			
HR	3.22	1.85	6.08
95% CI	1.34–7.73	0.60–5.71	1.29–28.72
P value	0.009	0.29	0.02

CHD indicates coronary heart disease; CI, confidence interval; and LCAT, lecithin:cholesterol acyltransferase activity.

*CHD includes fatal or nonfatal myocardial infarction, angina pectoris, and performance of coronary bypass surgery or angioplasty.

†HR indicates a hazard ratio for the tertile 3 subjects as compared with the tertile 1 subjects.

‡Multivariate model 1 includes covariates of age, sex, current smoking status, history of diabetes mellitus, body mass index, systolic blood pressure, serum total cholesterol, and serum high-density lipoprotein cholesterol levels.

§Multivariate model 2 includes covariates of age, sex, current smoking status, history of diabetes mellitus, body mass index, systolic blood pressure, serum apolipoprotein B, and serum triglyceride levels.

stress in a hyperlipidemic mouse model.[11] We measured the serum level of TBARS as a marker of lipid peroxidation and found that it was significantly correlated with serum LCAT activity. This result may support the hypothesis that increased LCAT activity is related to increased oxidative stress on VLDL, as LCAT activity has been shown to be positively correlated with increasing VLDL concentration.[37,38,40,54] However, both serum LCAT activity and serum TBARS level have also been reported to be correlated with serum lipids,[37,38,40,54–56] making it difficult to conclude that increased TBARS level is a consequence of increased LCAT activity. In addition, lysophosphatidylcholine, generated by LCAT, has been reported to inhibit endothelium-dependent vascular relaxation,[57] promote monocyte recruitment to the vascular wall,[58] and reduce the beneficial effects of LCAT on the RCT pathway.[59] These deleterious effects of lysophosphatidylcholine generated by LCAT reaction may promote the development of CHD and sudden death. However, we did not evaluate serum lysophospholipid concentration in this study. Further studies are therefore needed to clarify the underlying mechanism by which an increased cholesterol esterification rate promotes CHD and sudden death.

LCAT has long been believed to play an important role in RCT.[1] However, recent studies have raised the question of this idea. Calabresi et al demonstrated, using LCAT deficient human serum, that functional LCAT is not required for macrophage cholesterol efflux,[60] or for efficient atheroprotection.[15] Tanigawa et al demonstrated that serum from LCAT-overexpressing mice has a reduced ability to promote cholesterol efflux from ex vivo macrophages via ATP-binding cassette transporter A1.[8] Moreover, Schwartz et al reported that HDL can deliver a considerable amount of unesterified cholesterol directly to the liver without LCAT-mediated conversion into cholesterol ester.[35,61] These results suggest that the LCAT reaction may not be critical for RCT.[62] Increased LCAT activity may be the result of an increase in TG-rich lipoproteins,[36–38,40,54] and may therefore represent potentially impaired RCT.[8] Taken together, patients with metabolic disorders have increased LCAT activity,[17,36,38,40] and may be at risk for impaired RCT, leading to CHD and sudden death.

Recent studies have advanced the concept of RCT,[63,64] and measuring plasma HDL-C concentrations is no longer considered sufficient to estimate RCT in individuals.[65,66] Although LCAT has been considered useful in evaluating RCT, our data clearly show that LCAT activity, measured as the serum cholesterol esterification rate, cannot be a suitable marker for RCT. Thus, to estimate the RCT pathway, we should consider another approach.[66]

Our study has several limitations. First, we have measured initial rates of cholesterol esterification in serum, which is considered to take place in HDL.[3] However, we must consider that cholesterol esterification also takes place in other lipoproteins, such as VLDL and low-density lipoproteins. Second, we could not find any associations between lipid markers, other than apoCII, and the risk of CHD and sudden death. It is likely that lipid-modifying treatments during the follow-up period affected the incidence of CHD. To elucidate this, we mailed questionnaires to the participants whose serum total cholesterol concentrations were ≥220 mg/dL at baseline, asking them whether they had been prescribed lipid-modifying agents during the follow-up period. Most participants with hypercholesterolemia indeed received statin therapy after baseline study, probably attenuating the deleterious effects of hypercholesterolemia on the development of CHD and sudden death in this population (data not shown). In addition, we measured serum lipids and LCAT activities only once at the baseline and the follow-up period was >10 years, which may therefore lead to underestimation of the relationships between these variables and events.[67] Third, we could not evaluate the cause of the sex-specific effects of LCAT activity on CHD and sudden death. Further studies are needed to resolve these problems.

In conclusion, increased LCAT activity was associated with a future risk of CHD and sudden death in the general population. This association was significant in women, but not in men. Measuring the cholesterol esterification rate using the endogenous substrate method as a marker for LCAT activity is useful for predicting future CHD and sudden death.

Disclosures

None.

References

1. Glomset JA. The plasma lecithins:cholesterol acyltransferase reaction. *J Lipid Res*. 1968;9:155–167.
2. Gordon DJ, Rifkind BM. High-density lipoprotein–the clinical implications of recent studies. *N Engl J Med*. 1989;321:1311–1316.
3. Akanuma Y, Glomset J. *In vitro* incorporation of cholesterol-14C into very low density lipoprotein cholesteryl esters. *J Lipid Res*. 1968;9:620–626.
4. Hoeg JM, Santamarina-Fojo S, Bérard AM, Cornhill JF, Herderick EE, Feldman SH, Haudenschild CC, Vaisman BL, Hoyt RF Jr, Demosky SJ Jr, Kauffman RD, Hazel CM, Marcovina SM, Brewer HB Jr. Overexpression of lecithin:cholesterol acyltransferase in transgenic rabbits prevents diet-induced atherosclerosis. *Proc Natl Acad Sci USA*. 1996;93:11448–11453.
5. Bérard AM, Föger B, Remaley A, Shamburek R, Vaisman BL, Talley G, Paigen B, Hoyt RF Jr, Marcovina S, Brewer HB Jr, Santamarina-Fojo S. High plasma HDL concentrations associated with enhanced atherosclerosis in transgenic mice overexpressing lecithin-cholesteryl acyltransferase. *Nat Med*. 1997;3:744–749.
6. Föger B, Chase M, Amar MJ, Vaisman BL, Shamburek RD, Paigen B, Fruchart-Najib J, Paiz JA, Koch CA, Hoyt RF, Brewer HB Jr, Santamarina-Fojo S. Cholesteryl ester transfer protein corrects dysfunctional high density lipoproteins and reduces aortic atherosclerosis in lecithin cholesterol acyltransferase transgenic mice. *J Biol Chem*. 1999;274:36912–36920.
7. Mertens A, Verhamme P, Bielicki JK, Phillips MC, Quarck R, Verreth W, Stengel D, Ninio E, Navab M, Mackness B, Mackness M, Holvoet P. Increased low-density lipoprotein oxidation and impaired high-density lipoprotein antioxidant defense are associated with increased macrophage homing and atherosclerosis in dyslipidemic obese mice: LCAT gene transfer decreases atherosclerosis. *Circulation*. 2003;107:1640–1646.
8. Tanigawa H, Billheimer JT, Tohyama J, Fuki IV, Ng DS, Rothblat GH, Rader DJ. Lecithin: cholesterol acyltransferase expression has minimal effects on macrophage reverse cholesterol transport in vivo. *Circulation*. 2009;120:160–169.
9. Lambert G, Sakai N, Vaisman BL, et al. Analysis of glomerulosclerosis and atherosclerosis in lecithin cholesterol acyltransferase-deficient mice. *J Biol Chem*. 2001;276:15090–15098.
10. Furbee JW Jr, Sawyer JK, Parks JS. Lecithin:cholesterol acyltransferase deficiency increases atherosclerosis in the low density lipoprotein receptor and apolipoprotein E knockout mice. *J Biol Chem*. 2002;277:3511–3519.
11. Ng DS, Maguire GF, Wylie J, Ravandi A, Xuan W, Ahmed Z, Eskandarian M, Kuksis A, Connelly PW. Oxidative stress is markedly elevated in lecithin:cholesterol acyltransferase-deficient mice and is paradoxically reversed in the apolipoprotein E knockout background in association with a reduction in atherosclerosis. *J Biol Chem*. 2002;277:11715–11720.
12. Rousset X, Vaisman B, Amar M, Sethi AA, Remaley AT. Lecithin: cholesterol acyltransferase–from biochemistry to role in cardiovascular disease. *Curr Opin Endocrinol Diabetes Obes*. 2009;16:163–171.
13. Rousset X, Shamburek R, Vaisman B, Amar M, Remaley AT. Lecithin cholesterol acyltransferase: an anti- or pro-atherogenic factor? *Curr Atheroscler Rep*. 2011;13:249–256.
14. Ayyobi AF, McGladdery SH, Chan S, John Mancini GB, Hill JS, Frohlich JJ. Lecithin: cholesterol acyltransferase (LCAT) deficiency and risk of vascular disease: 25 year follow-up. *Atherosclerosis*. 2004;177:361–366.
15. Calabresi L, Baldassarre D, Castelnuovo S, et al. Functional lecithin: cholesterol acyltransferase is not required for efficient atheroprotection in humans. *Circulation*. 2009;120:628–635.
16. Hovingh GK, Hutten BA, Holleboom AG, Petersen W, Rol P, Stalenhoef A, Zwinderman AH, de Groot E, Kastelein JJ, Kuivenhoven JA. Compromised LCAT function is associated with increased atherosclerosis. *Circulation*. 2005;112:879–884.
17. Dullaart RP, Perton F, Sluiter WJ, de Vries R, van Tol A. Plasma lecithin: cholesterol acyltransferase activity is elevated in metabolic syndrome and is an independent marker of increased carotid artery intima media thickness. *J Clin Endocrinol Metab*. 2008;93:4860–4866.
18. Solajić-Bozicević N, Stavljenić-Rukavina A, Sesto M. Lecithin-cholesterol acryltransferase activity in patients with coronary artery disease examined by coronary angiography. *Clin Investig*. 1994;72:951–956.
19. Sethi AA, Sampson M, Warnick R, Muniz N, Vaisman B, Nordestgaard BG, Tybjaerg-Hansen A, Remaley AT. High pre-beta1 HDL concentrations and low lecithin: cholesterol acyltransferase activities are strong positive risk markers for ischemic heart disease and independent of HDL-cholesterol. *Clin Chem*. 2010;56:1128–1137.
20. Holleboom AG, Kuivenhoven JA, Vergeer M, Hovingh GK, van Miert JN, Wareham NJ, Kastelein JJ, Khaw KT, Boekholdt SM. Plasma levels of lecithin:cholesterol acyltransferase and risk of future coronary artery disease in apparently healthy men and women: a prospective case-control analysis nested in the EPIC-Norfolk population study. *J Lipid Res*. 2010;51:416–421.
21. Dullaart RP, Perton F, van der Klauw MM, Hillege HL, Sluiter WJ; PREVEND Study Group. High plasma lecithin:cholesterol acyltransferase activity does not predict low incidence of cardiovascular events: possible attenuation of cardioprotection associated with high HDL cholesterol. *Atherosclerosis*. 2010;208:537–542.
22. Ishikawa Y, Nobusawa A, Fujioka Y, Takahashi A, Taniguchi T, Tanaka S, Miki T, Hirouchi H, Yokoyama M. Diet and cardiovascular disease: The past, the present and the future. In: Pietinen C, Nishida C, Khaltaev N eds. *Proceedings of the 2nd WHO symposium on Health Issues for the 21st Century: Nutrition and Quality of Life*. 1993:157–168
23. Tanaka S, Miki T, Sha S, Hirata K, Ishikawa Y, Yokoyama M. Serum levels of thiobarbituric acid-reactive substances are associated with risk of coronary heart disease. *J Atheroscler Thromb*. 2011;18:584–591.
24. Nagasaki T, Akanuma Y. A new colorimetric method for the determination of plasma lecithin-cholesterol acyltransferase activity. *Clin Chim Acta*. 1977;75:371–375.
25. Bartholomé M, Niedmann D, Wieland H, Seidel D. An optimized method for measuring lecithin: cholesterol acyltransferase activity, independent of the concentration and quality of the physiological substrate. *Biochim Biophys Acta*. 1981;664:327–334.
26. Kobori K, Saito K, Ito S, Kotani K, Manabe M, Kanno T. A new enzyme-linked immunosorbent assay with two monoclonal antibodies to specific epitopes measures human lecithin-cholesterol acyltransferase. *J Lipid Res*. 2002;43:325–334.
27. Zhao Y, Thorngate FE, Weisgraber KH, Williams DL, Parks JS. Apolipoprotein E is the major physiological activator of lecithin-cholesterol acyltransferase (LCAT) on apolipoprotein B lipoproteins. *Biochemistry*. 2005;44:1013–1025.
28. Gidez LI, Miller GJ, Burstein M, Slagle S, Eder HA. Separation and quantitation of subclasses of human plasma high density lipoproteins by a simple precipitation procedure. *J Lipid Res*. 1982;23:1206–1223.
29. Tunstall-Pedoe H, Kuulasmaa K, Amouyel P, Arveiler D, Rajakangas AM, Pajak A. Myocardial infarction and coronary deaths in the World Health Organization MONICA Project. Registration procedures, event rates, and case-fatality rates in 38 populations from 21 countries in four continents. *Circulation*. 1994;90:583–612.
30. Nomenclature and criteria for diagnosis of ischemic heart disease. Report of the joint international society and federation of cardiology/world health organization task force on standardization of clinical nomenclature. *Circulation*. 1979;59:607–609
31. Talmud PJ, Hawe E, Miller GJ, Humphries SE. Nonfasting apolipoprotein B and triglyceride levels as a useful predictor of coronary heart disease risk in middle-aged UK men. *Arterioscler Thromb Vasc Biol*. 2002;22:1918–1923.
32. Li L, Hossain MA, Sadat S, Hager L, Liu L, Tam L, Schroer S, Huogen L, Fantus IG, Connelly PW, Woo M, Ng DS. Lecithin cholesterol acyltransferase null mice are protected from diet-induced obesity and insulin resistance in a gender-specific manner through multiple pathways. *J Biol Chem*. 2011;286:17809–17820.
33. Albers JJ, Bergelin RO, Adolphson JL, Wahl PW. Population-based reference values for lecithin-cholesterol acyltransferase (LCAT). *Atherosclerosis*. 1982;43:369–379.
34. Hopkins GJ, Barter PJ. Role of esterified cholesterol transfers in the regulation of plasma cholesterol esterification. *Atherosclerosis*. 1983;49:177–185.
35. Fielding CJ, Fielding PE. Regulation of human plasma lecithin:cholesterol acyltransferase activity by lipoprotein acceptor cholesteryl ester content. *J Biol Chem*. 1981;256:2102–2104.
36. Murakami T, Michelagnoli S, Longhi R, Gianfranceschi G, Pazzucconi F, Calabresi L, Sirtori CR, Franceschini G. Triglycerides are major determinants of cholesterol esterification/transfer and HDL remodeling in human plasma. *Arterioscler Thromb Vasc Biol*. 1995;15:1819–1828.
37. Marcel YL, Vezina C. Lecithin: Cholesterol acyltransferase of human plasma. *J Biol Chem*. 1973;248:8254–8259
38. Wallentin L. Lecithin: cholesterol acyl transfer rate in plasma and its relation to lipid and lipoprotein concentrations in primary hyperlipidemia. *Atherosclerosis*. 1977;26:233–248.

39. Sutherland WH, Temple WA, Nye ER, Herbison PG. Lecithin:cholesterol acyltransferase activity, plasma and lipoprotein lipids and obesity in men and women. *Atherosclerosis*. 1979;34:319–327.

40. Akanuma Y, Kuzuya T, Hayashi M, Ide T, Kuzuya N. Positive correlation of serum lecithin: cholesterol acyltransferase activity with relative body weight. *Eur J Clin Invest*. 1973;3:136–141.

41. Pinon JC, Bridoux AM, Laudat MH. Initial rate of cholesterol esterification association with high density lipoproteins in human plasma. *J Lipid Res*. 1980;21:406–414.

42. Barter PJ, Hopkins GJ, Gorjatschko L, Jones ME. Competitive inhibition of plasma cholesterol esterification by human high-density lipoprotein-subfraction 2. *Biochim Biophys Acta*. 1984;793:260–268.

43. Fielding CJ, Fielding PE. Purification and substrate specificity of lecithin-cholesterol acyl transferase from human plasma. *FEBS Lett*. 1971;15:355–358.

44. Miller GJ, Miller NE. Plasma-high-density-lipoprotein concentration and development of ischaemic heart-disease. *Lancet*. 1975;1:16–19.

45. Walter MF, Jacob RF, Jeffers B, Ghadanfar MM, Preston GM, Buch J, Mason RP; PREVENT study. Serum levels of thiobarbituric acid reactive substances predict cardiovascular events in patients with stable coronary artery disease: a longitudinal analysis of the PREVENT study. *J Am Coll Cardiol*. 2004;44:1996–2002.

46. Meisinger C, Baumert J, Khuseyinova N, Loewel H, Koenig W. Plasma oxidized low-density lipoprotein, a strong predictor for acute coronary heart disease events in apparently healthy, middle-aged men from the general population. *Circulation*. 2005;112:651–657.

47. Kiechl S, Willeit J, Mayr M, Viehweider B, Oberhollenzer M, Kronenberg F, Wiedermann CJ, Oberthaler S, Xu Q, Witztum JL, Tsimikas S. Oxidized phospholipids, lipoprotein(a), lipoprotein-associated phospholipase A2 activity, and 10-year cardiovascular outcomes: prospective results from the Bruneck study. *Arterioscler Thromb Vasc Biol*. 2007;27:1788–1795.

48. Steinberg D. Lewis A. Conner Memorial Lecture. Oxidative modification of LDL and atherogenesis. *Circulation*. 1997;95:1062–1071.

49. Zheng L, Nukuna B, Brennan ML, Sun M, Goormastic M, Settle M, Schmitt D, Fu X, Thomson L, Fox PL, Ischiropoulos H, Smith JD, Kinter M, Hazen SL. Apolipoprotein A-I is a selective target for myeloperoxidase-catalyzed oxidation and functional impairment in subjects with cardiovascular disease. *J Clin Invest*. 2004;114:529–541.

50. Parthasarathy S, Quinn MT, Schwenke DC, Carew TE, Steinberg D. Oxidative modification of beta-very low density lipoprotein. Potential role in monocyte recruitment and foam cell formation. *Arteriosclerosis*. 1989;9:398–404.

51. Itabe H, Hosoya R, Karasawa K, Jimi S, Saku K, Takebayashi S, Imanaka T, Takano T. Metabolism of oxidized phosphatidylcholines formed in oxidized low density lipoprotein by lecithin-cholesterol acyltransferase. *J Biochem*. 1999;126:153–161.

52. Vohl MC, Neville TA, Kumarathasan R, Braschi S, Sparks DL. A novel lecithin-cholesterol acyltransferase antioxidant activity prevents the formation of oxidized lipids during lipoprotein oxidation. *Biochemistry*. 1999;38:5976–5981.

53. McPherson PA, Young IS, McEneny J. A dual role for lecithin:cholesterol acyltransferase (EC 2.3.1.43) in lipoprotein oxidation. *Free Radic Biol Med*. 2007;43:1484–1493.

54. Rose HG, Juliano J. Regulation of plasma lecithin:cholesterol acyltransferase in man. I. Increased activity in primary hypertriglyceridemia. *J Lab Clin Med*. 1976;88:29–43.

55. Nishigaki I, Hagihara M, Tsunekawa H, Maseki M, Yagi K. Lipid peroxide levels of serum lipoprotein fractions of diabetic patients. *Biochem Med*. 1981;25:373–378.

56. Ledwozyw A, Michalak J, Stepień A, Kadziołka A. The relationship between plasma triglycerides, cholesterol, total lipids and lipid peroxidation products during human atherosclerosis. *Clin Chim Acta*. 1986;155:275–283.

57. Yokoyama M, Hirata K, Miyake R, Akita H, Ishikawa Y, Fukuzaki H. Lysophosphatidylcholine: essential role in the inhibition of endothelium-dependent vasorelaxation by oxidized low density lipoprotein. *Biochem Biophys Res Commun*. 1990;168:301–308.

58. Quinn MT, Parthasarathy S, Steinberg D. Lysophosphatidylcholine: a chemotactic factor for human monocytes and its potential role in atherogenesis. *Proc Natl Acad Sci USA*. 1988;85:2805–2809.

59. Schmitz G, Ruebsaamen K. Metabolism and atherogenic disease association of lysophosphatidylcholine. *Atherosclerosis*. 2010;208:10–18.

60. Calabresi L, Favari E, Moleri E, Adorni MP, Pedrelli M, Costa S, Jessup W, Gelissen IC, Kovanen PT, Bernini F, Franceschini G. Functional LCAT is not required for macrophage cholesterol efflux to human serum. *Atherosclerosis*. 2009;204:141–146.

61. Schwartz CC, VandenBroek JM, Cooper PS. Lipoprotein cholesteryl ester production, transfer, and output *in vivo* in humans. *J Lipid Res*. 2004;45:1594–1607.

62. Calabresi L, Franceschini G. Lecithin:cholesterol acyltransferase, high-density lipoproteins, and atheroprotection in humans. *Trends Cardiovasc Med*. 2010;20:50–53.

63. Brufau G, Groen AK, Kuipers F. Reverse cholesterol transport revisited. *Arterioscler Thromb Vasc Biol*. 2011;31:1726–1733.

64. Rosenson RS, Brewer HB Jr, Davidson WS, Fayad ZA, Fuster V, Goldstein J, Hellerstein M, Jiang XC, Phillips MC, Rader DJ, Remaley AT, Rothblat GH, Tall AR, Yvan-Charvet L. Cholesterol efflux and atheroprotection: advancing the concept of reverse cholesterol transport. *Circulation*. 2012;125:1905–1919.

65. Frikke-Schmidt R, Nordestgaard BG, Stene MC, Sethi AA, Remaley AT, Schnohr P, Grande P, Tybjaerg-Hansen A. Association of loss-of-function mutations in the ABCA1 gene with high-density lipoprotein cholesterol levels and risk of ischemic heart disease. *JAMA*. 2008;299:2524–2532.

66. Khera AV, Cuchel M, de la Llera-Moya M, Rodrigues A, Burke MF, Jafri K, French BC, Phillips JA, Mucksavage ML, Wilensky RL, Mohler ER, Rothblat GH, Rader DJ. Cholesterol efflux capacity, high-density lipoprotein function, and atherosclerosis. *N Engl J Med*. 2011;364:127–135.

67. MacMahon S, Peto R, Cutler J, Collins R, Sorlie P, Neaton J, Abbott R, Godwin J, Dyer A, Stamler J. Blood pressure, stroke, and coronary heart disease. Part 1, Prolonged differences in blood pressure: prospective observational studies corrected for the regression dilution bias. *Lancet*. 1990;335:765–774.

Significance

We prospectively studied the relationship between baseline serum cholesterol esterification rates as a marker of lecithin:cholesterol acyltransferase (LCAT) activity, a key enzyme involved in cholesterol esterification in plasma, and subsequent coronary heart disease (CHD) and sudden death in the Japanese general population. In this study, we have clearly shown that increased LCAT activity is associated with the future risk of CHD and sudden death. Our study provides new insight into the function of LCAT in reverse cholesterol transport, and contributes to our understanding of plasma cholesterol esterification in relation to atherosclerosis and subsequent CHD. Moreover, we further identified the sex-specific effect of LCAT on the development of CHD and sudden death in this population. Therefore, our study may contribute to prevention of CHD and sudden death in general populations, particularly in women populations.

Materials and Methods

Study Population

The surveyed population was comprised of residents 20 years of age or older, who participated in a cardiovascular risk survey in 1993 in Hidaka town, a typical rural community in Japan.[1, 2] A total of 2,155 individuals participated in this survey. Of these, 155 participants who had a history of cardiovascular disease or cancer were excluded from the study, and 73 participants were lost to follow-up. Therefore, the remaining 1,927 participants (1,136 women and 791 men; mean age ± SD, 58.4±14.8 years) were eligible for this study, which represented 41.6% of the total population of the town. All study protocols were approved by the Medical Ethics Committee of Hidaka Medical Center. Informed consent was obtained from all subjects at both baseline examination and at follow-up survey.

Baseline Examination

The baseline survey was conducted between July 8, 1993 and August 6, 1993. Community nurses interviewed the participants at community centers, and information on current and past health condition, medication, and lifestyle was obtained. Blood pressure was measured with a standard mercury sphygmomanometer on the right arm after the participants had been sitting at rest for at least 3 minutes. Hypertension was defined as a systolic blood pressure of 140 mmHg or higher, and/or a diastolic blood pressure of 90 mmHg or higher or current use of antihypertensive agents. Body mass index was calculated as weight in kilograms divided by the square of height in meters. All participants underwent a physical examination and laboratory blood testing. Participants reported the time since their last meal, which was ≤ 2 hours, 3 - 7 hours, and ≥ 8 hours for 4.3%, 77.5%, and 18.2% of the participants, respectively. Therefore, most blood samples were drawn in a non-fasting state. Serum LCAT activity was determined by a self-substrate method, where the decrease in free cholesterol was measured enzymatically after incubation of the serum with synthetic dipalmitoyl lecithin using a commercially available kit (Nescoat LCAT kit-S, Alfresa Pharma, Osaka, Japan) based on the method by Nagasaki and Akanuma.[3] In brief, 0.5 ml of serum was added to 0.3 ml of 2.5 mg/ml lecithin solution and mixed gently. 0.2 ml of the mixture was taken into a test tube and stored in a refrigerator as a control (A). The remaining mixture was incubated at 37⁰C for 2 hours. 0.2 ml of the mixture was taken into another test tube (B). 3.0 ml of color reagent for cholesterol measurement was added to both (A) and (B), and incubated at 37⁰C for 20 minutes. The difference in absorbance between (A) and (B) at 600 nm was determined, and the decrease of cholesterol concentration was calculated as a LCAT activity (n moles/ml/hr). 1,000 n mole/ml of purified cholesterol was used as a reference for calculating cholesterol esterification rate. The LCAT activities measured by the present method are highly correlated with those measured by the endogenous substrate method using gas-liquid chromatography,[3] with those measured by the exogenous substrate method,[4] and with the LCAT mass concentrations measured by the enzyme-linked immunosorbent assay.[5] We also

confirmed a high correlation between the current method and the endogenous substrate method using radio-labeled endogenous substrate (data not shown).[6] Serum concentrations of total cholesterol (TC) and triglycerides (TG) were determined by enzymatic methods. HDL-C concentration was determined by phosphotungstic acid magnesium chloride precipitation method. Serum concentrations of HDL-C subfraction including HDL_2 cholesterol (HDL_2-C) and HDL_3 cholesterol (HDL_3-C) were determined by the selective precipitating method.[7] Serum concentrations of apoA-I, apoA-II, apoB, apoC-II, apoC-III, and apoE were determined by turbidimetric immunoassays using commercially available kits (Sekisui Medical Co. Ltd., Tokyo, Japan). Plasma glycosylated hemoglobin A1c (HbA1c) level was measured by the HPLC method. Serum thiobarbituric acid-reactive substances (TBARS) level as a marker of lipid peroxidation was measured as described previously.[2]

Follow-up

Information on the incidence of CHD was collected by means of self-administered, mailed questionnaires or interviews by telephone from June 2004. Information on deaths and the incidence of diseases was obtained from reviews of vital statistics through the municipal register, hospital records, death certificates, and autopsy reports with the permission of the participants or their relatives.

Ascertainment of CHD Events

The endpoint of the study was the development of CHD and sudden death, including fatal and non-fatal myocardial infarction, angina pectoris, performance of coronary bypass surgery or angioplasty, and sudden death. Myocardial infarction was confirmed if the subject met the World Health Organization criteria for the Monitoring Trends and Determinants of Cardiovascular Disease (MONICA) project.[8] Angina pectoris was diagnosed according to the criteria of the American Heart Association (AHA).[9] In addition to the AHA criteria, angina pectoris was considered present if at least one of the following criteria were met: 1) local abnormality of cardiac wall motion on echocardiography during an attack; or 2) at least 75% stenosis on coronary angiography. Sudden death was defined as death within 24 hours after the onset of acute illness in a patient without any previous restriction of daily activities who was not hospitalized prior to the onset of illness. All coronary events were reviewed and confirmed by three cardiologists who were blinded to baseline examination data.

Statistical Analysis

The t-test, the Mann-Whitney nonparametric test, and one-way analysis of variance followed by Tukey's test or the Kruskal-Wallis test were used to compare continuous variables, and the chi-square test was used to compare categorical variables. Participants were divided into tertiles according to baseline serum LCAT activity and other variables. Data were analyzed continuously, or as tertiles using the lowest tertile as the reference category.

2013/02/09

Hazard ratios (HRs) for future CHD and sudden death according to tertiles of LCAT activities with 95% confidence intervals (CIs) were calculated, adjusted for established coronary risk factors by Cox proportional hazard model. Pearson's or Spearman's correlation coefficients were used to assess the correlations between LCAT activity and other variables. All tests were two-tailed, and a P value of less than 0.05 was considered statistically significant. SPSS 11.01J software for Windows (SPSS, Japan, Tokyo, Japan) was used to perform all statistical analyses.

References

1. Ishikawa Y, Nobusawa A, Fujioka Y, Takahashi A, Taniguchi T, Tanaka S, Miki T, Hirouchi H, Yokoyama M. Diet and cardiovascular disease: The past, the present and the future. *In: Pietinen C, Nishida C, Khaltaev N eds. Proceedings of the 2nd WHO symposium on Health Issues for the 21st Century: Nutrition and Quality of Life*. 1993:157-168

2. Tanaka S, Miki T, Sha S, Hirata K, Ishikawa Y, Yokoyama M. Serum levels of thiobarbituric acid-reactive substances are associated with risk of coronary heart disease. *J Atheroscler Thromb*. 2011;18:584-591

3. Nagasaki T, Akanuma Y. A new colorimetric method for the determination of plasma lecithin-cholesterol acyltransferase activity. *Clin Chim Acta*. 1977;75:371-375

4. Bartholome M, Niedmann D, Wieland H, Seidel D. An optimized method for measuring lecithin : Cholesterol acyltransferase activity, independent of the concentration and quality of the physiological substrate. *Biochim Biophys Acta*. 1981;664:327-334

5. Kobori K, Saito K, Ito S, Kotani K, Manabe M, Kanno T. A new enzyme-linked immunosorbent assay with two monoclonal antibodies to specific epitopes measures human lecithin-cholesterol acyltransferase. *J Lipid Res*. 2002;43:325-334

6. Zhao Y, Thorngate FE, Weisgraber KH, Williams DL, Parks JS. Apolipoprotein E is the major physiological activator of lecithin-cholesterol acyltransferase (LCAT) on apolipoprotein B lipoproteins. *Biochemistry*. 2005;44:1013-1025

7. Gidez LI, Miller GJ, Burstein M, Slagle S, Eder HA. Separation and quantitation of subclasses of human plasma high density lipoproteins by a simple precipitation procedure. *J Lipid Res*. 1982;23:1206-1223

8. Tunstall-Pedoe H, Kuulasmaa K, Amouyel P, Arveiler D, Rajakangas AM, Pajak A. Myocardial infarction and coronary deaths in the world health organization MONICA project. Registration procedures, event rates, and case-fatality rates in 38 populations from 21 countries in four continents. *Circulation*. 1994;90:583-612

9. Nomenclature and criteria for diagnosis of ischemic heart disease. Report of the joint international society and federation of cardiology/world health organization task force on standardization of clinical nomenclature. *Circulation*. 1979;59:607-609

2013/02/09

Increased Serum Cholesterol Esterification Rates Predict Coronary Heart Disease and Sudden Death in a General Population

Shin-ichiro Tanaka, Tomoyuki Yasuda, Tatsuro Ishida, Yoshio Fujioka, Takeshi Tsujino, Tetsuo Miki and Ken-ichi Hirata

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://atvb.ahajournals.org/content/33/5/1098

Data Supplement (unedited) at:
http://atvb.ahajournals.org/content/suppl/2013/02/22/ATVBAHA.113.301297.DC1.html

---

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published
in *Arteriosclerosis, Thrombosis, and Vascular Biology* can be obtained via RightsLink, a service of the
Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for
which permission is being requested is located, click Request Permissions in the middle column of the Web
page under Services. Further information about this process is available in the Permissions and Rights
Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Arteriosclerosis, Thrombosis, and Vascular Biology* is online
at:
http://atvb.ahajournals.org//subscriptions/