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 \(\leq 2\) hours, 3 - 7 hours, and \(\geq 8\) 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 37ºC 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 37ºC 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). 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. 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.

**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. Angina pectoris was diagnosed according to the criteria of the American Heart Association (AHA). 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.
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