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Abstract

Data bank project for acute stroke patients

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There is only a few evidence for stroke management has been reported from Japan. It is necessary to make a data bank of acute stroke patients as infrastructure to make evidence for standardization of stroke management. We made Japan standard stroke registry study (JSSRS) supported by ministry of health and welfare from 1999 to 2002. We completed computerized registry system and accumulated about 8,000 acute stroke cases from 45 stroke center hospitals. This system is also functioning as a stroke database for each hospital. From the analysis of the distribution of stroke subtype, the incidence of atherothrombotic infarction and cardiogenic embolism was similar to lacunar infarction as shown in Figure 1. Furthermore, the 38% of ischemic stroke patients admitted within 3 hours. Thrombolytic therapy was performed in 15% of the patients who admitted within 3 hours and their initial severity were NIHSS 6-29. The outcome of the patients treated with thrombolytic therapy was significantly better than those without it. These data indicate that the stroke data bank should be useful tool to make verification of the guideline and planning a clinical trial for EBM in near future.

(Jpn J Stroke 24 : 255—259, 2002)

Key words: acute stroke data bank, computerized database, Japan standard stroke registry study (JSSRS), stroke scale

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Lipids, Lipoproteins, and Cardiovascular Risk Factors

Highly Sensitive Cholesterol Assay with Enzymatic Cycling Applied to Measurement of Remnant Lipoprotein-Cholesterol in Serum

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Background: **Remnant lipoprotein-cholesterol (RLP-C) concentrations in sera of healthy individuals are very low (0.080–0.437 mmol/L), making conventional cholesterol methods poorly suited to this purpose. We have developed a highly sensitive cholesterol assay (CD method) and applied it to the RLP-C assay.**

Methods: **The CD shuttled cholesterol reversibly between reduced and oxidized forms in the presence of thio-NAD and NADH. The production rate of thio-NADH correlated with the cholesterol concentration and was measured by the absorbance at 404/500 nm. This CD method was combined with an immunoaffinity separation procedure with specific monoclonal antibodies to apolipoprotein (apo) A1 and apo B-100 and used for RLP-C assay. Results were compared with a RLP-C method that uses cholesterol oxidase, peroxidase, and chromogenic substrate.**

Results: **The CD method could detect 0.10×10^{-3} mmol/L cholesterol and was at least 5 times more sensitive than the conventional enzymatic method. Within- and between-day imprecision (as CVs) of the RLP-C assay with the CD method was <4%. Regression analysis of RLP-C assays with the new (y) and conventional (x) cholesterol methods yielded: $y = 1.02x - 0.008$ mmol/L ($S_{y|x} = 0.0065$ mmol/L; $r = 0.997$; n = 297).**

Conclusions: **Serum RLP-C can be measured by the CD method. The CD method may be useful for other assays that require sensitive cholesterol measurements in biological materials.**

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Cholesterol oxidase-peroxidase methods *(1, 2)* are widely used to measure serum cholesterol, LDL-cholesterol, and HDL-cholesterol. These assays do not require quantification below 0.026 mmol/L (1 mg/dL). In contrast, assays for remnant lipoprotein-cholesterol (RLP-C)[4] *(3–5)* require lower detection limits. In the RLP-C assay developed by Nakajima and coworkers *(3, 4)* and evaluated by Leary et al. *(5)*, a detection limit <0.003 mmol/L (0.12 mg/dL) is needed for cholesterol because serum samples are diluted 61-fold with affinity gel solution for the separation of remnant lipoproteins. The conventional methods for cholesterol are not sufficiently sensitive; moreover, some are not linear at low cholesterol concentrations, thus requiring multiple calibrations.

We have developed an enzymatic cycling method *(6)*, using cholesterol dehydrogenase (CD; EC no. not certified) *(7)*, that can detect 0.10×10^{-3} mmol/L cholesterol with one-point calibration. Using the CD method, we determined RLP-C concentrations in sera from healthy controls.

Materials and Methods

REAGENTS

We used thio-NAD, oxidized form (Oriental Yeast Co. Ltd.), NADH (Oriental Yeast), recombinant CD *(8–10)* (Amano Enzyme Inc.), and cholesterol ester hydrolase (CEH; stearyl-ester acylhydrolase; EC 3.1.1.13) derived from *Pseudomonas* species *(11)* (Asahi Chemical Industry Co., Ltd.). Synthetic conjugated bilirubin (Porphyrin

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[4] Nonstandard abbreviations: RLP-C, remnant lipoprotein-cholesterol; CD, cholesterol dehydrogenase; CEH, cholesterol ester hydrolase; and PO, peroxidase.

Products Inc.), free bilirubin (Sigma), and hemoglobin prepared from hemolyzed human erythrocytes were used to test for interference. Other reagents were purchased from Wako Chemical Industries, Ltd.

PROCEDURES

Principle of the CD method. CEH and CD were used in the CD method (Fig. 1). The cholesterol esters were hydrolyzed to free cholesterol and fatty acids by CEH. Free cholesterol changed to the oxidized form reversibly, and this cycling reaction was repeated several times in the presence of thio-NAD and NADH. Thio-NADH (reduced form) produced by the CD method was measured by the change in absorbance per minute at 404/500 nm as a measure of the cholesterol concentration.

Reagents for the CD method and application to an automated analyzer. The first reagent contained, per liter, 8000 U of CEH, 1500 U of CD, 0.8 mmol thio-NADH, 2.5 g of sodium cholate, 1 mL of Triton X-100, and 10 mmol HEPES buffer *(12)*. The second reagent contained 2.4 mmol/L NADH in 0.3 mol/L diethanolamine buffer (pH 10).

The assay was performed on a TBA-20R automatic analyzer (Toshiba) optimized for a rate assay measuring absorbance at 404/500 nm for 5 min at 37 °C with 10 μL of sample, 270 μL of the first reagent, and 90 μL of the second reagent.

Procedure for RLP-C. Lipoproteins other than RLP in serum were precipitated with an immunoaffinity separation procedure using monoclonal antibodies to apolipoprotein A1 and B-100 *(3, 4)*; the cholesterol in the supernatant was measured by the CD method as RLP-C. The calibration solution (International Reagents Corp.) contained 0.789 mmol/L (30.5 mg/dL) cholesterol.

The conventional RLP-C assay using the peroxidase (PO; donor:hydrogen peroxide oxidoreductase; EC 1.11.1.7) method (RLP-Cholesterol JIMRO II; Japan Immunoresearch Laboratory Co. Ltd.) was used as the comparison method *(4, 5)*. Five calibration points were used to construct the calibration curve in the PO method.

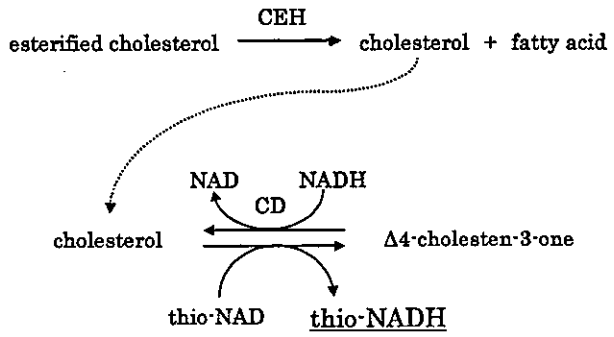

Fig. 1. Reaction scheme for the enzymatic cycling method (CD method).

SAMPLES

Fresh serum samples were randomly collected without anticoagulant as part of a mass examination at Kochi prefecture in cooperation with Osaka Medical Center for Cancer and Cardiovascular Diseases. This study was approved by the Committee for Ethical Standards of Osaka Medical Center for Cancer and Cardiovascular Disease. The 297 individuals studied in this research had no history of cardiovascular disease and were apparently healthy.

STATISTICAL ANALYSIS

Linear regression, reference intervals, and cutoff values were calculated by the least-squares method, the nonparametric method, and the 75th percentile of the population in this study group, respectively.

Results

OPTIMIZATION OF CD METHOD

The optimal pH for the CD method at 37 °C was assessed between pH 7.0 and 9.5 with 0.026 mmol/L cholesterol solution. The highest absorbance was between pH 8.5 and 9.5 (Fig. 2A). This optimal pH was attained by mixing the first reagent (pH 6.5) and the second reagent (pH 10.0).

The concentrations of thio-NAD and NADH in the CD method at 37 °C were assessed using a 0.026 mmol/L cholesterol solution. The reaction was increased by addition of the coenzymes (Fig. 2, B and C). To achieve the necessary sensitivity and the absorbance range needed for measurement of cholesterol in RLP-C (0–2.587 mmol/L as RLP-C), we chose 0.6 mmol/L for both thio-NAD and NADH.

The optimal CD activity at 37 °C was assessed using a 0.026 mmol/L cholesterol solution. The sensitivity was increased in proportion to the amount of CD (Fig. 2D). To achieve the necessary sensitivity and the absorbance range for the RLP-C assay, we used a CD activity of 1.13 kU/L.

The optimal CEH activity at 37 °C was assessed using three human sera with total cholesterol concentrations of 3.5, 5.9, and 10.8 mmol/L, respectively, which were diluted 1:401 (1 μL of serum in 400 μL of saline) in saline. The amount of CEH for the hydrolysis of cholesterol esters was fixed at 8 kU/L. This CEH activity hydrolyzed cholesterol esters of high-cholesterol sera (Fig. 2E).

DETECTION LIMIT OF CD METHOD FOR CHOLESTEROL

The detection limit of the CD method, evaluated with a sequentially diluted cholesterol solution (1.29 mmol/L), was 0.10×10^{-3} mmol/L (mean + 3 SD of zero calibrator; n = 10).

PERFORMANCE OF THE NEW RLP-C ASSAY

We measured 0, 0.098, 0.197, 0.336, and 0.789 mmol/L cholesterol solutions determined as RLP-C by the CD and conventional (PO) method (Fig. 3). The CD method had a linear calibration curve (n = 5) that passed through the

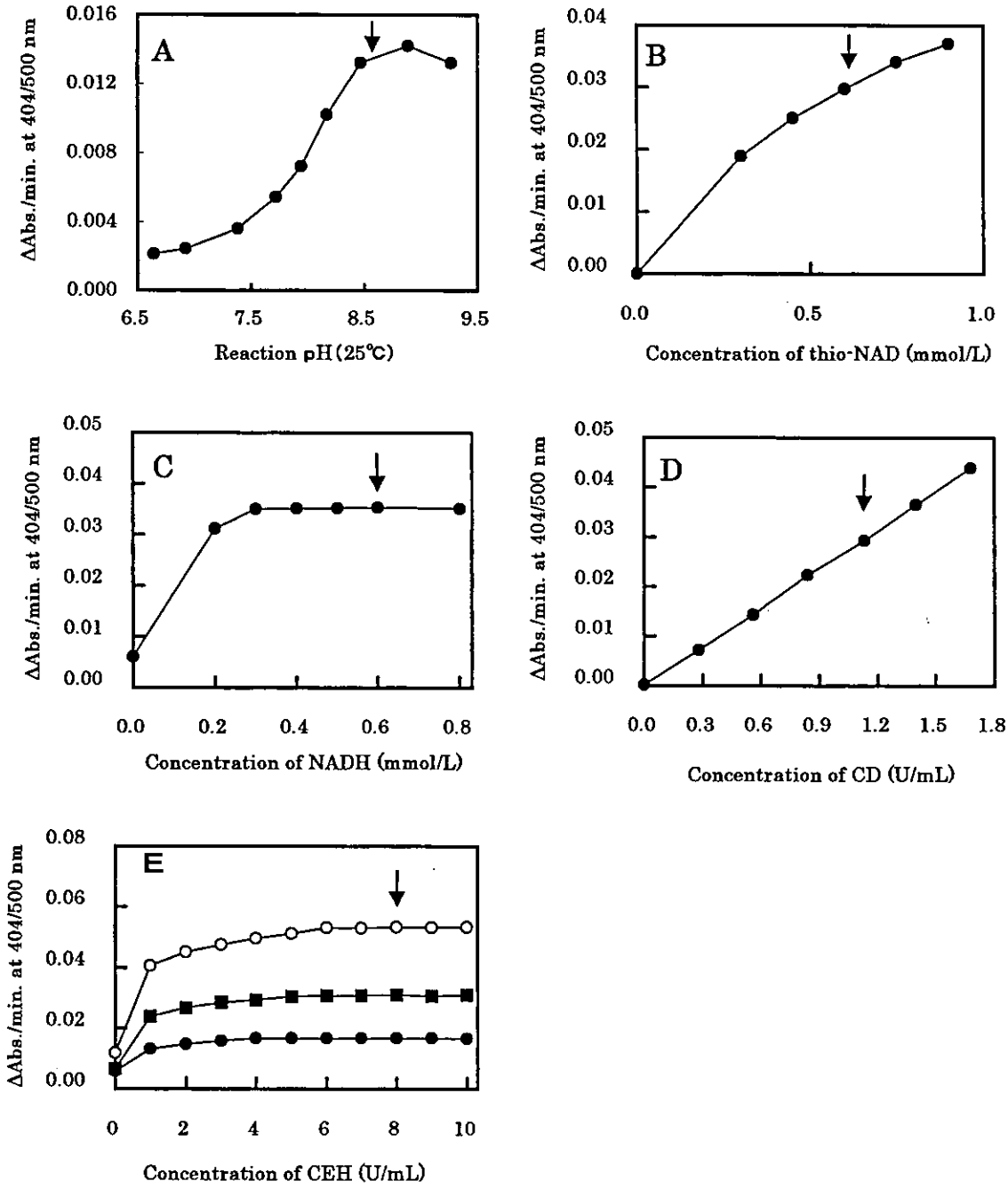

Fig. 2. Optimal conditions for the CD method.

(*A*), effect of pH on the CD method with 0.6 mmol/L thio-NAD, 0.6 mmol/L NADH, and 1.13 kU/L CD. (*B*), effect of thio-NAD concentration on the CD method with 0.6 mmol/L NADH and 1.13 kU/L CD at pH 8.5. (*C*), effect of NADH concentration on the CD method with 0.6 mmol/L thio-NAD and 1.13 kU/L CD at pH 8.5. (*D*), effect of CD activity on the CD method with 0.6 mmol/L thio-NAD and 0.6 mmol/L NADH at pH 8.5. (*E*), effect of CEH activity on the CD method with 0.6 mmol/L thio-NAD, 0.6 mmol/L NADH, and 1.13 kU/L CD at pH 8.5. For the experiment in *E*, we used three different human sera containing 3.54 mmol/L (●), 5.92 mmol/L (■), and 10.81 mmol/L (○) total cholesterol, diluted 1:401 in saline.

origin, with fivefold higher absorbance (sensitivity) than that of the PO method. The PO method had a nonlinear calibration curve (n = 5) that did not pass through the origin, and the increase in absorbance was disproportional with the concentration of cholesterol.

The linearity of the RLP-C assay with the CD method was evaluated by sequential dilution of a high RLP-C control serum with saline. The curve was linear up to 2.587 mmol/L RLP-C in this method.

Within- and between-day precision was determined by

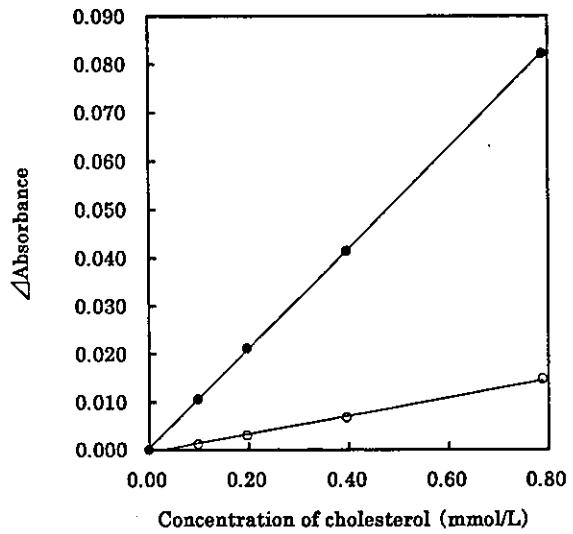

Fig. 3. The linearity of two methods using five calibration points at low cholesterol concentrations.

Data points indicate change in absorbance per minute (Δ*Absorbance*) relative to cholesterol concentrations in calibrators by the CD method (●) and by the conventional method (○). Each point represents the mean of five determinations. The linear regression of the CD method (y) and cholesterol concentrations (x) yielded: $y = 0.0027x + 0.0004$ ($y = 0.993x + 0.0039$ mmol/L). On the other hand, the linear regression of the conventional (PO) method (y) and cholesterol concentrations (x) yielded: $y = 0.0005x + 0.0009$ ($y = 1.053x + 0.046$ mmol/L).

measuring three control sera containing 0.098, 0.341, 1.058 mmol/L cholesterol. Between-day testing was carried out on 10 points over a 2-week period. The assay was calibrated each testing day. The RLP-C assay with the CD method showed good within- and between-day precision [CVs, 0.57–3.1% (n = 20) and 1.0–3.8%, respectively; Table 1]. On the other hand, the RLP-C assay with the conventional (PO) method had within- and between-day CVs of 1.8–6.0% and 2.0–7.5%, respectively.

Table 1. Precision of RLP-C method.

	CD method			Conventional method		
	Low[a]	Middle	High	Low	Middle	High
Within day						
n	20	20	20	20	20	20
Mean, mmol/L	0.095	0.342	1.057	0.090	0.354	1.051
SD, mmol/L	0.0028	0.0052	0.0059	0.0054	0.0132	0.0191
CV, %	3.1	1.5	0.57	6.0	3.7	1.8
Between day						
No. days	10	10	10	10	10	10
Mean, mmol/L	0.099	0.362	1.073	0.092	0.347	1.067
SD, mmol/L	0.0036	0.0085	0.0111	0.0070	0.0122	0.0209
CV, %	3.8	2.3	1.0	7.5	3.5	2.0

[a] Low, middle, and high indicate RLP-C controls containing 0.098, 0.341, and 1.058 mmol/L cholesterol, respectively.

The RLP-C assay with the CD method was not affected by hemoglobin (up to 5.0 g/L), bilirubin (up to 0.35 mmol/L), conjugated bilirubin (up to 0.28 mmol/L), or ascorbic acid (up to 2.84 mmol/L; data not shown).

SERUM RLP-C CONCENTRATIONS IN APPARENTLY HEALTHY PEOPLE

The observed RLP-C concentrations in this study group (n = 297) with the CD method were 0.080–0.437 mmol/L, whereas the results obtained by the conventional (PO) RLP-C assay were 0.085–0.419 mmol/L. The 75th percentile was 0.217 mmol/L with the CD method and 0.220 mmol/L by the conventional (PO) assay.

The correlation by linear regression of RLP-C assay with the CD method (y) and conventional RLP-C assay (x) was: $y = 1.02x - 0.008$ mmol/L (Fig. 4). The mean (SD) of x was 0.188 (0.088) mmol/L, and the mean (SD) of y was 0.184 (0.090) mmol/L. The mean difference between methods was 0.004 mmol/L, and the SD of residuals ($S_{y/x}$) was 0.0065 mmol/L. Samples with <0.13 mmol/L RLP-C were 40–60% higher by the new method than by the conventional RLP-C assay.

Discussion

CD from *Nocardia* species (*13*) has high substrate specificity for cholesterol (*7*) and allows a substrate cycling reaction between the thio-NAD and NADH redox reaction. The number of cycles occurring between oxidized substrate (Δ-4-cholesten-3-one) and reduced substrate (cholesterol) was estimated to be 10/min from the molar absorptivity. This CD method showed good linearity even at very low cholesterol concentrations, and the calibration curve passed through the origin of the coordinated axes.

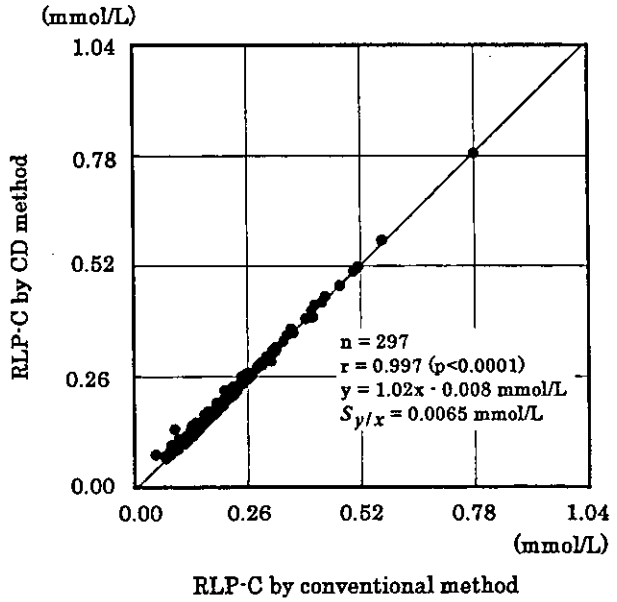

Fig. 4. Correlation between the RLP-C assay with the CD method (y) and the conventional (PO) method (x).

The detection limit of the CD method (10^{-4} mmol/L) was approximately one-tenth that of a PO method reported, for example, by Leary et al. *(5)* as 0.0013 mmol/L (0.08 mmol/L as serum RLP-C). The change in absorbance (sensitivity) of the CD method was at least 5 times higher than that of the PO method. If the sample volume for the cholesterol assay is increased from 10 μL, further improvement of sensitivity appears possible.

The CD method meets the needs for an RLP-C assay. Serum RLP-C >0.194 mmol/L is a cardiovascular risk factor *(4, 5)*. When serum samples with RLP-C of 0.194 mmol/L were diluted 61-fold with the immunoaffinity gel solution for the separation of RLP, the final cholesterol concentration in the supernatants was <0.003 mmol/L (0.194 divided by 61). The RLP-C assay with the CD method required only one calibrator, had suitable precision and linearity, and was not affected by the potential interferences tested.

In conclusion, the CD method is well suited for accurate RLP-C assays and is expected to provide an easy method for the measurement of very low concentrations of cholesterol. The CD method as a highly sensitive cholesterol assay method also appears suitable for measurement of very low cholesterol in other biological materials.

We thank Dr. Tetsunori Akiba (Amano Enzyme Inc., Nagoya, Japan) for kindly supplying recombinant CD.

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Trends in the Incidence of Coronary Heart Disease and Stroke and the Prevalence of Cardiovascular Risk Factors among Japanese Men from 1963 to 1994

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PURPOSE: To determine trends in the incidence of cardiovascular disease in Japan, we examined observational data on coronary heart disease, stroke, and cardiovascular risk factors among urban Japanese working men.

SUBJECTS AND METHODS: The surveyed population included all male employees aged 40 to 59 years who worked for eight industrial companies in Osaka, the second largest metropolitan city in Japan. Surveillance for cardiovascular disease and risk factors was conducted from 1963 to 1994.

RESULTS: The age-adjusted incidence of coronary heart disease increased from 0.4 per 1000 person-years during 1963 to 1970, to 1.5 per 1000 person-years during 1979 to 1986, and then plateaued until 1987 to 1994 (P for trend = 0.002), whereas the incidence of stroke declined from 1.2 per 1,000 person-years during 1971 to 1978, to 0.6 per 1,000 person-years in 1987 to 1994 (P for trend = 0.02). The age-adjusted mean (± SD) total cholesterol level, which was 4.87 ± 2.88 mmol/L during 1963 to 1966, increased to 5.11 ± 0.62 mmol/L during 1982 to 1983 (P <0.001), and 5.09 ± 0.75 mmol/L during 1990 to 1991. Both mean systolic and diastolic blood pressures increased by 1 mm Hg between the periods of 1966 to 1967 and 1982 to 1983, and declined below the 1966 to 1967 levels during 1990 to 1991. The prevalence of smoking declined from 72% during 1975 to 1976, to 58% during 1990 to 1991 (P for trend <0.001).

CONCLUSION: Although these findings were limited to urban middle-aged men, the increase in serum cholesterol is likely to attenuate the reduction in future rates of coronary heart disease in Japan that would have been expected to result from the declining prevalence of smoking. **Am J Med. 2002;112: 104–109.** ©2002 by Excerpta Medica, Inc.

Rates of coronary heart disease have declined since the mid 1960s in the United States and northern European countries (1–9). Trends in Asia are less clear, however (9,10). Japan had the highest mortality from stroke and the lowest mortality from coronary heart disease among developed countries in the 1960s (11), but the age-adjusted mortality from stroke declined approximately 75% in Japan from 1970 to 1997, with a 44% decline in coronary heart disease (12).

The decrease in coronary heart disease mortality has not been uniform among geographical areas, however; it has been smaller in Tokyo and Osaka (13), the largest metropolitan cities in which Westernized lifestyles are more common. Furthermore, mortality trends may not always parallel incidence trends, particularly in urban areas, owing to advances in medical treatments (14).

We hypothesized that the incidence of coronary heart disease increased in urban Japan because of Westernized lifestyles (15). To examine trends in the incidence of coronary heart disease and stroke, and in the prevalence of cardiovascular factors, we analyzed data from 32 years of systematic surveillance of urban Japanese working men.

SUBJECTS AND METHODS

Study Sample

The sample included all male employees aged 40 to 59 years who worked for eight industrial companies in Osaka, the second largest metropolitan city in Japan. These companies consisted of a broadcasting company, a trading company, a hotel, a bank, and four companies producing chains, air conditioners, and aluminum cans and pans. More than 90% of employees of these companies were men; most retired at 60 years of age. During the study period, about 60% of the employees were white-collar workers. The rest were production workers who were not exposed to any cardiotoxic agents. The unemployment rate in Japan was between 1.4% and 3.0% between 1963 and 1994 (16). Employees were enrolled during four periods (1963 to 1970, 1971 to 1978, 1979 to 1986, and 1987 to 1994), and were observed for 8 years.

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Surveillance for Cardiovascular Diseases

Surveillance for coronary heart disease and stroke was conducted annually from 1963 to 1994. Cardiovascular disease endpoints were ascertained from death certificates, absentee reports owing to sickness, insurance claims to companies, and annual surveys. To confirm the diagnosis, study physicians obtained medical histories and resting electrocardiograms (ECGs) for all living patients. If a subject had died, the medical history was obtained from the family or colleagues. Medical records at company clinics and local hospitals were also reviewed for 121 (73%) of reported coronary heart disease events and 74 (70%) of reported stroke events. The criteria for coronary heart disease were modified from those of a World Health Organization Expert Committee (17). Definite myocardial infarction was indicated by typical severe chest pain (lasting at least 30 minutes and with no definite nonischemic cause) accompanied by new abnormal and persistent Q or QS waves, consistent changes in cardiac enzyme levels, or both. If the ECG and enzyme levels were nondiagnostic or not obtainable, but the patient had typical chest pain, a diagnosis of possible myocardial infarction was made. We regarded painless myocardial infarctions as infarction without typical chest pain, accompanied by the development of new Q waves (the Minnesota code: 1-1 or 1-2) (18) on the annual ECG, and confirmed on at least three sequential ECGs. Sudden cardiac death was defined as death within 1 hour of symptom onset, a witnessed cardiac arrest, or both, or abrupt collapse not proceeded by more than 1 hour of symptoms. Angina pectoris was defined as repeated episodes of chest pain during effort, usually disappearing rapidly after the cessation of effort or upon use of sublingual nitroglycerin. Stroke was defined as a constellation of neurological deficits that were sudden or rapid in onset, and lasted at least 24 hours or until death (19,20). Cerebrovascular disease due to infection, trauma, or malignancy, and transient ischemic attacks were excluded.

Risk Factor Surveys

Cardiovascular risk factors were ascertained during the middle year of each of the four survey periods (1966 to 1967, 1975 to 1976, 1982 to 1983, and 1990 to 1991). Participation rates were 79% during 1966 to 1967, 94% during 1975 to 1976, 87% during 1982 to 1983, and 93% during 1990 to 1991.

Systolic and fifth-phase diastolic blood pressures in the right arm were measured by trained nurses using standard mercury sphygmomanometers in seated participants who had rested for 5 minutes (21). The training procedure was repeated during each survey period to reduce inter- and intraobserver differences. Hypertension was defined as a systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or use of an antihypertensive medication. An ECG was obtained in supine position and coded with the Minnesota Code, second version (18), by trained physician-epidemiologists. Body mass index (kg/m^2) was calculated.

Nonfasting blood was obtained from an antecubital vein, and serum was separated. Serum total cholesterol level was measured by the Fe^{3+}-H_2SO_4 (Zak) method (22) in 1965 and by the Liebermann-Burchard reaction method on an AutoAnalyzer II (Technicon, Tarrytown, New York) (23) in the later periods. Between 1975 and 1994, our laboratory participated in external standardization by the Centers for Disease Control (24,25) and successfully met the criteria of precision and accuracy for the measurement of total and high-density lipoprotein cholesterol levels (26). Lipid values from the first survey in 1974 were compared with those from the later surveys using 51 random samples of blood. The regression line was $Y = 0.924*X + 9.20$, where Y is the Liebermann-Burchard reaction method value, and X is the Zak method value (correlation coefficient = 0.96). Lipid values from the first survey were adjusted using this formula.

During 1963 to 1966, we measured total cholesterol levels in 176 subjects (8% of the total), oversampling executives and undersampling clerical and manual workers to examine differences in cholesterol levels among occupational subgroups (27). Therefore, to estimate the population values for serum cholesterol level in the 1960s, we weighted cholesterol levels by the occupational distribution of the total sample during 1963 to 1966.

The number of cigarettes smoked per day and the usual daily intake of ethanol in units of "go" (a traditional Japanese unit of volume corresponding to 23 g ethanol) were ascertained by an interview during 1975 to 1976, 1982 to 1983, and 1990 to 1991. Men who reported smoking at least one cigarette per day were defined as current smokers, and men who reported consuming ethanol of at least 0.3 go per week were regarded as current drinkers. Both ex-smokers and ex-drinkers were defined as abstainers for at least 3 months.

Trends in Risk Factors from National Surveys

National data on blood pressure, serum total cholesterol levels, and body mass index were extracted from surveys of circulatory disorders (28) and nutrition (29,30) of a representative sample of 10,000 to 15,000 men and women aged ≥30 years. Smoking rates were derived from a survey (31) by Japan Tobacco Inc., Tokyo, of 11,000 to 12,000 men and women aged ≥20 years.

Statistical Analysis

Age-adjusted incidence rates were estimated as the number of new cases per 1000 person-years during 1963 to 1970, 1971 to 1978, 1979 to 1986, and 1987 to 1994. Person-years of observation were estimated by summing the number of employees every April during each 8-year period. The direct method of standardization using the age distribution of all men during the four periods was used.

Table 1. Age-adjusted Incidence per 1000 Person-Years of Coronary Heart Disease and Stroke among Male Employees Aged 40 to 59 Years during Four Survey Periods from 1963–1970 to 1987–1994, Osaka, Japan

		1963–1970	1971–1978	1979–1986	1987–1994	*P* for Trend	Rate Ratio between 1963–1970 and 1987–1994
Number		2535	3077	5332	6460		
Person-years		20 280	24 616	36 612	43 259		
Coronary heart disease	No. of cases	8	28	54	75		
	Age-adjusted	0.4	1.2*	1.5†	1.5†	0.002	3.4
	incidence	(0.2–0.7)	(0.8–1.7)	(1.1–1.9)	(1.1–1.8)		(1.7–4.2)
Myocardial Infarction	No. of cases	3	18	42	51		
	Age-adjusted	0.2	0.8*	1.2†	1.0†	0.001	7.6
	incidence	(0–0.3)	(0.5–1.2)	(0.8–1.5)	(0.7–1.3)		(5.7–10)
Definite Myocardial	No. of cases	3	7	17	20		
Infarction	Age-adjusted	0.2	0.3	0.5	0.4	0.17	3.0
	incidence	(0–0.3)	(0.1–0.6)	(0.3–0.7)	(0.2–0.6)		(1.8–4.6)
Possible Myocardial	No. of cases	0	4	12	9		
Infarction	Age-adjusted	0.0	0.2	0.3	0.2	0.08	—
	incidence	—	(0–0.4)	(0.2–0.5)	(0.1–0.3)		
Painless Myocardial	No. of cases	0	7	13	22		
Infarction	Age-adjusted	0.0	0.3‡	0.4‡	0.4*	0.008	—
	incidence	—	(0.1–0.5)	(0.2–0.5)	(0.2–0.6)		
Sudden Cardiac Death	No. of cases	0	2	0	2		
	Age-adjusted	—	—	—	—	—	—
	incidence§	—	—	—	—		
Angina Pectoris	No. of cases	5	8	12	22		
	Age-adjusted	0.3	0.3	0.3	0.4	0.42	1.4
	incidence	(0.1–0.5)	(0.1–0.6)	(0.2–0.5)	(0.2–0.6)		(0.2–2.2)
Stroke	No. of cases	19	25	34	28		
	Age-adjusted	1.1	1.2	1.0	0.6	0.02	0.5
	incidence	(0.6–1.6)	(0.8–1.7)	(0.7–1.3)	(0.4–0.8)		(0.3–0.7)

Numbers in parentheses are 95% confidence intervals.
* $P < 0.01$, † $P < 0.001$, ‡ $P < 0.05$, for differences from 1963–1970.
§ Age-adjusted incidence of sudden cardiac death was not calculated because of the small numbers.

Linear trends in incidence rates were tested using the chi-squared test. Rate ratios and 95% confidence intervals (CI) were calculated (32) to compare 1963 to 1970 with 1987 to 1994. Age-adjusted means were estimated with analysis of covariance; age-adjusted prevalences were estimated with the direct method of standardization. The significance of risk factor trends was examined for continuous variables using regression analysis for repeated measures (33), representing the four periods as 1966.5, 1975.5, 1982.5, and 1990.5, and for discrete variables using the chi-squared test for trend. Differences in means or proportions were tested using analysis of covariance or chi-squared tests.

RESULTS

Trends in the Incidence of Coronary Heart Disease and Stroke

The age-adjusted incidence of coronary heart disease tripled from the period 1963 to 1970 to the period 1979 to 1986, and then plateaued until 1987 to 1994 (*P* for trend = 0.002; Table 1). The rate ratio for the comparison of the period 1963 to 1970 with 1987 to 1994 was 3.4 (95% CI: 1.7 to 4.2). The increase in the incidence of coronary heart disease was primarily due to an increase in myocardial infarction (*P* for trend = 0.001). A similar trend was observed for definite myocardial infarction.

These increases in the incidence of coronary heart disease incidence were seen among men aged 40 to 51 years and among those ≥52 years of age. The age-adjusted incidence for men aged 40 to 51 years was 0.3 per 1000 person-years (n = 5 events) during 1963 to 1970, 1.0 per 1000 person-years (n = 19) during 1971 to 1978, 1.5 per 1000 person-years (n = 40) during 1979 to 1986, and 0.8 per 1000 person-years (n = 23) during 1987 to 1994 (*P* for trend = 0.10). Among men aged 52 years or older, the rates were 0.7 per 1000 person-years (n = 3) during 1963 to 1970, 1.8 per 1000 person-years (n = 9) during 1971 to 1978, 1.6 per 1000 person years (n = 14) during 1979 to 1986, and 3.4 per 1,000 person-years (n = 52) during

Table 2. Characteristics (Age-adjusted) of Male Employees Aged 40 to 59 Years during Four Survey Periods from 1966–1967 to 1990–1991, Osaka, Japan

	1966–1967	1975–1976	1982–1983	1990–1991	*P* for Trend
	Number (%) or Mean ± SD				
Number	1997	2889	4617	5998	
Age (years)	47 ± 5	47 ± 5	47 ± 5	49 ± 5*	<0.001
Aged 50–59 years	658 (33)	821 (28)*	1330 (29)*	2719 (45)*	<0.001
White-collar workers	1223 (61)	1596 (56)*	2764 (60)	3964 (66)*	<0.001
Systolic blood pressure (mm Hg)	125 ± 17	125 ± 17	126 ± 17†	124 ± 17	0.004
Diastolic blood pressure (mm Hg)	78 ± 12	79 ± 12†	79 ± 12	76 ± 13*	<0.001
Hypertension	465 (23)	781 (27)‡	1259 (27)*	1474 (25)	0.86
Antihypertensive medication	107 (5)	187 (6)	242 (5)	356 (6)	0.84
Body mass index (kg/m^2)	21.9 ± 2.7	22.3 ± 2.6*	22.4 ± 2.7*	22.8 ± 2.7*	0.09
Total cholesterol					
Number	176§	1,104	4,019	5,553	
Total cholesterol‖ (mmol/L)	4.87 ± 2.88	4.97 ± 0.97	5.11 ± 0.62*	5.09 ± 0.75*	0.03¶
Lipid-lowering medication	—	—	15 (0.4)	54 (1.0)	0.001
Smoking and alcohol					
Number	Not examined	2,890	4,618	6,004	
Smoking					
Never smoked	—	466 (16)	787 (17)	1052 (18)	0.12
Ex-smokers	—	341 (12)	990 (21)	1473 (25)	<0.001
Current smokers	—	2077 (72)	2841 (62)	3479 (58)	<0.001
Alcohol					
Never drank	—	786 (27)	956 (21)	933 (16)	<0.001
Ex-drinkers	—	91 (3)	151 (3)	162 (3)	0.15
Current drinkers					
1–22 g/d of ethanol	—	611 (21)	1086 (24)	1537 (26)	<0.001
23–68 g/d of ethanol	—	1292 (45)	2059 (45)	3051 (51)	<0.001
≥69 g/d of ethanol	—	110 (4)	367 (8)	320 (5)	0.22

* P <0.001, † P <0.05, ‡ P <0.01 for differences from the 1966–1967 survey.
§ Data in the 1963–66 survey.
‖ The conversion factor from SI units (mmol/L) to conventional units (mg/dL) for cholesterol is 38.7.
¶ The trend for total cholesterol levels was examined between 1975–1976 and 1990–1991.

1987 to 1994 (P for trend <0.001). An increase in the incidence of coronary heart disease was observed in both white-collar (P for trend = 0.008) and blue-collar workers (P for trend = 0.06). The 30-day case fatality rate for coronary heart disease was 10% (17 of 165).

In contrast, the incidence of stroke increased slightly from the period 1963 to 1970 to the period 1971 to 1978, and then declined subsequently (Table 1; P for trend = 0.02). The rate ratio between the periods 1963 to 1970 and 1987 to 1994 was 0.5 (95% CI: 0.3 to 0.7). The incidence of stroke was approximately 2.5 times higher than the incidence of coronary heart disease during 1963 to 1970, but 60% lower during 1987 to 1994.

Trends in Risk Factors in the Sample

Mean systolic blood pressure increased significantly between the periods 1966 to 1967 and 1982 to 1983 (Table 2), and declined thereafter. Mean diastolic blood pressure and the prevalence of hypertension increased between 1966 to 1967 and 1975 to 1976, and declined between 1982 to 1983 and 1990 to 1991. The prevalence of antihypertensive medication use did not change with time. Mean body mass index increased progressively.

Mean total cholesterol levels increased between the periods 1963 to 1966 and 1982 to 1983, and leveled off between 1982 to 1983 and 1990 to 1991 (Table 2). The proportion of current smokers declined progressively from the period 1975 to 1976 to the period 1990 to 1991, accompanied by an increase in the proportion of ex-smokers. The proportion of light-to-moderate drinkers of alcohol increased with time.

Trends in Risk Factors from National Surveys

Mean systolic blood pressure declined by 6 mm Hg from 1961 to 1997 (Table 3), while mean diastolic blood pressure increased by 2 mm Hg between 1961 and 1990, and declined thereafter. Mean total cholesterol level and body mass index increased progressively between 1980 and 1997, while the proportion of current smokers declined.

Table 3. Trends in Selected Risk Factors from National Representative Samples of Japanese Men Between 1961 and 1997

Risk Factor (Unit) (Reference)	1961	1971	1980	1990	1997
Systolic blood pressure (mm Hg) (28,29)	142 ± 25 (n = 9403)	140 ± 24 (n = 5587)	138 ± 21 (n = 4795)	138 ± 20 (n = 3538)	136 ± 17 (n = 2037)
Diastolic blood pressure (mm Hg) (28,29)	82 ± 15	83 ± 14	84 ± 12	84 ± 12	82 ± 12
Total cholesterol (mmol/L)* (28,29)	—	—	4.82 ± 0.85 (n = 4690)	5.14 ± 0.95 (n = 3296)	5.18 ± 0.87 (n = 2258)
Body mass index (kg/m^2) (29,30)	—	—	22.5 ± 2.9 (n = 4641)	23.0 ± 3.0 (n = 3496)	23.2 ± 3.0 (n = 3438)
Current smokers (%) (31)	82	77	70	61	56
	—	—	—	(n = 11 474)	(n = 11 255)

* The conversion factor from SI units (mmol/L) to conventional units (mg/dL) for cholesterol is 38.7.

DISCUSSION

We observed a significant increase in the incidence of coronary heart disease in a group of urban Japanese working men between the periods 1963 to 1970 and 1987 to 1994, especially between 1963 to 1970 and 1979 to 1986. The increase in the incidence of coronary heart disease was associated with an increase in the mean total cholesterol level and body mass index, although these values were still low by Western standards. The increased incidence was also associated with a minor rise in systolic blood pressure level and a slight increase in the prevalence of hypertension between 1965 and 1982 to 1983.

The plateau in coronary heart disease incidence between 1979 to 1986 and 1987 to 1994 may be attributable to a decrease in blood pressure levels, a plateau in serum cholesterol levels, a decrease in smoking, and perhaps to an increase in the proportion of light-to-moderate drinkers, all of which would tend to reduce the rates of coronary heart disease.

The incidence of stroke declined between the periods 1971 to 1978 and 1987 to 1994, probably because of a decrease in smoking and a substantial decline in blood pressure levels between 1982 to 1983 and 1990 to 1991. A decrease in stroke incidence was also observed among rural Japanese men during this period (34,35).

Our study had several limitations. First, although we used the same surveillance system for 32 years, the likelihood of diagnosing coronary heart disease may have increased with time. Second, there was more than a twofold increase in the number of employees between 1966 to 1967 and 1990 to 1991. Because our previous study found a greater incidence of coronary heart disease among white-collar workers than among blue-collar workers in the 1960s and the 1970s (36), changes in the proportion of white-collar workers could have biased our results. However, the proportion of white-collar workers did not change substantially between the periods 1966 to 1967 and 1982 to 1983, and even increased thereafter (Table 2). Furthermore, increasing trends in the incidence of coronary heart disease were observed for both white-collar and blue-collar workers. Third, we have no data for female employees or for male employees over 60 years of age. Last, a healthy worker effect may limit the generalizability of our findings. However, national trends in total cholesterol levels, body mass index, and smoking—but not systolic blood pressure—were in general agreement with our findings for urban middle-aged men.

The observed decline in mortality from coronary heart disease in Japan between 1970 and 1997 (12) and the increase in its incidence that we observed in our study suggest that there have been substantial improvements in the treatment of coronary heart disease. Previous studies reported improvements of emergency medical care and a substantial increase in the number of coronary care units since the late 1970s in Japan (14). This inference is supported by the low case fatality rate in our cohort (10%) and in other Japanese studies (15% to 20%) (14,37), compared with the 34% to 83% case fatality rate in other countries (9).

If cholesterol levels and body mass index continue to rise, the incidence of coronary heart disease may increase among Japanese men or at least not decline in response to lower smoking rates. Continuous surveillance is necessary to clarify future trends for risk factors and the rates of cardiovascular disease in Japan.

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Standardization of Laboratory Test in the JPHC Study

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The standardization committee has carried out standardization of 23 laboratories in the cohort area. They participated in the External Quality Control Survey by the Japan Medical Association. Most laboratories got A or B in evaluation criteria for most test items, but the results of AST, ALT and gamma-GTP were unsatisfactory. As for the lipid standardization, accuracy and precision of all 23 laboratories were satisfactory except for one. Close communication and collaborative study with reference laboratory improved the accuracy control.
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CDC, CRMLN, accuracy, precision

INTRODUCTION

Laboratory data which were sent from each study area should be standardized to compare each result. The standardization committee (Chairman: Dr. Iida) has carried out standardization of 23 laboratories in the cohort area. Osaka Medical Center for Cancer and CVD is a member of Cholesterol Reference Method Laboratory Network (CRMLN), and contributed to standardize lipid measurement in Japan for both epidemiology and clinical chemistry.

METHODS OF STANDADIZATION

The subjects for standardization were 10 items that were measured at the health screening program in the local health centers by the Law of Promoting Health for Elderly. These included total protein, glucose, uric acid, creatinine, total cholesterol, AST (GOT), ALT (GPT), gamma-GTP, triacylglycerol, and HDL cholesterol. Accuracy control and standardization among each laboratory in the cohort area were performed by the Division of Mass Screening, Osaka Medical Center for Cancer and CVD.

Basically, External Quality Control Survey by the Japan Medical Association was employed. Accurate reference value was available for total cholesterol, HDL cholesterol and triacylglycerol by the CRMLN Lipid Standardization Program through Osaka Medical Center for Cancer and CVD.

External Quality Control Survey by the Japan Medical Association has been planed in every June, called for participation in July, and 4 samples were sent to laboratories in September. Each participant measured samples and reported the results to the Association in October. The results were evaluated according to the evaluation criteria (Tables 1a-c), and returned to each participant in the next February. We collected copies of these evaluation sheet from each laboratory to the Osaka Medical Center for Cancer and CVD, and evaluated.

Thirty sample sera for lipid analysis were sent to the laboratories in December or at the time of mass screening, according to the CRMLN Lipid Standardization Program by Osaka Medical Center for Cancer and CVD. Each laboratory should randomly select 3 samples for one day, and repeat duplicate measurement for 10 consecutive days. We collected the data and evaluated the results according to the criteria (Table 2). If the results were acceptable, laboratories could receive Certification for Clinical Laboratory issuid by CDC/CRMLN.

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Table 1a. Evaluation criteria for clinical laboratory test by Japan medical association. Total protein, uric acid, creatinine, total cholesterol (4 samples).

Evaluation criteria	Evaluation	Point
Within +/- 1 SD	A	2.5
+/- 1 SD to +/- 2 SD	B	2
+/- 2 SD to +/- 3 SD	C	1
over +/- 3 SD	D*	0
No participation	–	0

Table 1b. Evaluation criteria for clinical laboratory test by Japan medical association. AST, ALT, gamma-GTP (4 samples).

Evaluation criteria	Evaluation	Point
Within +/- 1 SD	A	3 (4)
+/- 1 SD to +/- 2 SD	B	2 (3)
+/- 2 SD to +/- 3 SD	C	1 (1)
over +/- 3 SD	D*	0 (0)
No participation	–	0 (0)

Point for ratio of measured value in parenthesis.

Table 1c. Evaluation criteria for clinical laboratory test by Japan medical association. Triacylglycerol, HDL cholesterol (2 samples).

Evaluation criteria	Evaluation	Point
Within +/- 1 SD	A	5
+/- 1 SD to +/- 2 SD	B	4
+/- 2 SD to +/- 3 SD	C	2
over +/- 3 SD	D*	0
No participation	–	0

Table 2. Criteria for acceptable performance of the CDC-NHLBI lipid standardization program.

Item	Concentration level (mg/dl)	Precision (standard deviation)	Accuracy (bias from RV)
Total cholesterol	100-149	4.00	0.03 RV
	>150	0.03 RV	0.03 RV
LDL cholesterol	50.0-99.9	2.00	2..0
	<40.0	3.00	3.0
HDL cholesterol	<40.0	2.50	0.10 RV
	40-59.9	3.00	0.10 RV
	>=60.0	3.50	0.10 RV
Triacylglycerol	0-88	7	9
	89-176	8	10
	177-220	10	11
	>=221	0.05 RV	0.05

RV; reference value, from Criteria for Acceptable Performance of the CDC-NHLBI Lipid Standardization Program

Table 3. Participation in the external quality control survey by the Japan medical association.

Health center	Lab name	1991 25th	1992 26th	1993 27th	1996 28th
Yokote	Hiraga General Hospital		◎	◎	
	Akita General	◎	◎	◎	
Ninohe	Iwate	◎	◎	◎	◎
	SRL Hachioji Lab	◎	◎	◎	
Kasama	Ibaragi General Health Institute	◎	◎	◎	◎
Katsushika					
Saku	Nagano Health Control Center	◎	◎		◎
	Tsuchiya Enterprise				◎
	Chikuma Hospital				
	Koumi Red Cross Hospital				
Kashiwazaki	Kashiwazaki Medical	◎	◎	◎	◎
	Ojiya General Hospital	◎	◎		◎
Suita	National Cardiovascular Center	◎	◎	◎	◎
	Osaka University Research Institute for Microbial Diseases	◎	◎	◎	◎
Tosayamada	Tosayamada	◎		◎	◎
	Kochi General Health Institute	◎	◎	◎	
Arikawa	Kamigoto Hospital			◎	◎
	Arikawa Hospital			◎	◎
	Narao Hospital			◎	◎
	Odika Clinic			◎	◎
	Fukue Public Health Center			◎	◎
Ishikawa	Okinawa General Health Institute	◎	◎	◎	◎
	Ishikawa Public Health Center				
Miyako	Miyako Public Health Center			◎	◎
	Total:	12	12	17	16

Table 4. Participation in the lipid standardization program.

Health center	Lab name	1st study 1994 Jan.-Feb.	2nd study 1995 Jan.-Feb.
Yokote	Hiraga General Hospital	◎	◎
	Akita Prefectural General Health Association	◎	◎
Ninohe	Iwate	◎	◎
	SRL Hachioji Laboratories	◎	
Kasama	Ibaraki Health Service Association	◎	◎
Katsushika		◎	◎
Saku	Health Care Center Nagano Prefectural Federation of Agricultural Cooperatives for Health and Welfare	◎	◎
	Tsuchiya Enterprise		◎
	Chikuma Hospital		◎
	Koumi Red Cross Hospital		◎
Kashiwazaki	Kashiwazaki Kariwa Medical Association Kashiwazaki Medical Center	◎	◎
	Ojiya General Hospital	◎	◎
Suita	National Cardiovascular Center	◎	◎
	The Reserch Foundation for Microbial Disease of Osaka University	◎	◎
Tosayamada	Tosayamada Public Health Center	◎	◎
	Kochi Health Service Association	◎	◎
Arikawa	Kamigoto Hospital	◎	◎
	Arikawa Hospital	◎	◎
	Narao Hospital	◎	◎
	Ojika Clinic	◎	◎
	Fukue Public Health Center	◎	◎
Ishikawa	Okinawa General Health Service Association	◎	◎
	Ishikawa Public Health Center	◎	◎
Miyako	Miyako Public Health Center	◎	◎
	Total:	21	23

RESULTS OF STANDADIZATION AMONG LABORATORIES

The participation of the 28th External Quality Control Survey by the Japan Medical Association in 1994 by each laboratory were shown in Table 3. Sixteen of 24 laboratories sent us the results. Participation status to the CRMLN Lipid Standardization Program was shown in Table 4. Twenty-three laboratories participated in the second study which was carried out during January and February in 1995.

Evaluation of 16 laboratories was summarized in Table 5. Most laboratories got A or B in evaluation criteria for most test items, but the results of AST, ALT and gamma-GTP failed to be C or D category in many laboratories. As for the 2nd Lipid Standardization Program, accuracy and precision of 23 laboratories are shown in Figure 1. Accuracy of cholesterol measurement was satisfied in 19 out of 23 and precision satisfied in all laboratories. Triacylglycerol measurement was satisfied in both accuracy and precision by all laboratories except one. The same was for HDL cholesterol measurement.

PROBLEMS OF FUTURE RESOLUTION

From the results of External Quality Control Survey by the Japan Medical Association, AST, ALT and gamma-GTP measurement showed the problem in accuracy control. These should be improved. On the other hand, the results of lipid standardization program were satisfactory. This is the result of our close contact in the cohort study since the project had started. The difficult problem for quality control on the laboratories was that the health screening data were indirectly obtained from laboratories that were nominated by the local city, town or village which should carry out the health screening for the residents by the law. At the beginning of this study, direct communication between reference laboratory and each laboratories was not established, but it has been improved recently, as

Table 5. Evaluation of laboratory test from 16 laboratories.

Test item	Participating laboratories	Criteria	Sample no. 1	(%)	2	(%)	3	(%)	4	(%)
glucose	16	A	11	68.8	14	87.5	13	81.3	13	81.3
		B	5	31.3	2	12.5	3	18.8	2	12.5
		C	0	0.0	0	0.0	0	0.0	1	6.3
		D	0	0.0	0	0.0	0	0.0	0	0.0
uric acid	15	A	10	66.7	13	86.7	13	86.7	10	66.7
		B	4	26.7	2	13.3	2	13.3	3	20.0
		C	1	6.7	0	0.0	0	0.0	2	13.3
		D	0	0.0	0	0.0	0	0.0	0	0.0
creatinine	16	A	13	81.3	12	75.0	14	87.5	12	75.0
		B	2	12.5	4	25.0	1	6.3	2	12.5
		C	0	0.0	0	0.0	0	0.0	1	6.3
		D	1	6.3	0	0.0	1	6.3	1	6.3
Total-cholesterol	16	A	13	81.3	12	75.0	13	81.3	13	81.3
		B	3	18.8	4	25.0	8	18.8	2	12.5
		C	0	0.0	0	0.0	0	0.0	1	6.3
		D	0	0.0	0	0.0	0	0.0	0	0.0
AST	16	A	10	62.5	9	56.3	9	56.3	7	43.8
		B	2	12.5	5	31.3	4	25.0	6	37.5
		C	3	18.8	1	6.3	1	6.3	2	12.5
		D	1	6.3	1	6.3	2	12.5	1	6.3
ALT	16	A	9	56.3	7	43.8	10	62.5	7	43.8
		B	4	25.0	5	31.3	2	12.5	6	37.5
		C	2	12.5	4	25.0	4	25.0	3	18.8
		D	1	6.3	0	0.0	0	0.0	0	0.0
γ -GTP	16	A	10	62.5	8	50.0	9	56.3	8	50.0
		B	4	25.0	4	25.0	5	31.3	5	31.3
		C	0	0.0	3	18.8	0	0.0	2	12.5
		D	1	6.3	0	0.0	1	6.3	0	0.0
Total-protein	14	A	8	57.1	10	71.4				
		B	6	42.9	4	28.6				
		C	0	0.0	0	0.0				
		D	0	0.0	0	0.0				
Triacyl-glycerol	16	A	12	75.0	12	75.0				
		B	4	25.0	3	18.8				
		C	0	0.0	1	6.3				
		D	0	0.0	0	0.0				
HDL-cholesterol	16	A	10	62.5	11	68.8				
		B	5	31.3	5	31.3				
		C	1	6.3	0	0.0				
		D	0	0.0	0	0.0				

shown by the result of lipid standardization. The quality control shall be repeated every year, so the laboratory data should become satisfactorily comparable in the future.

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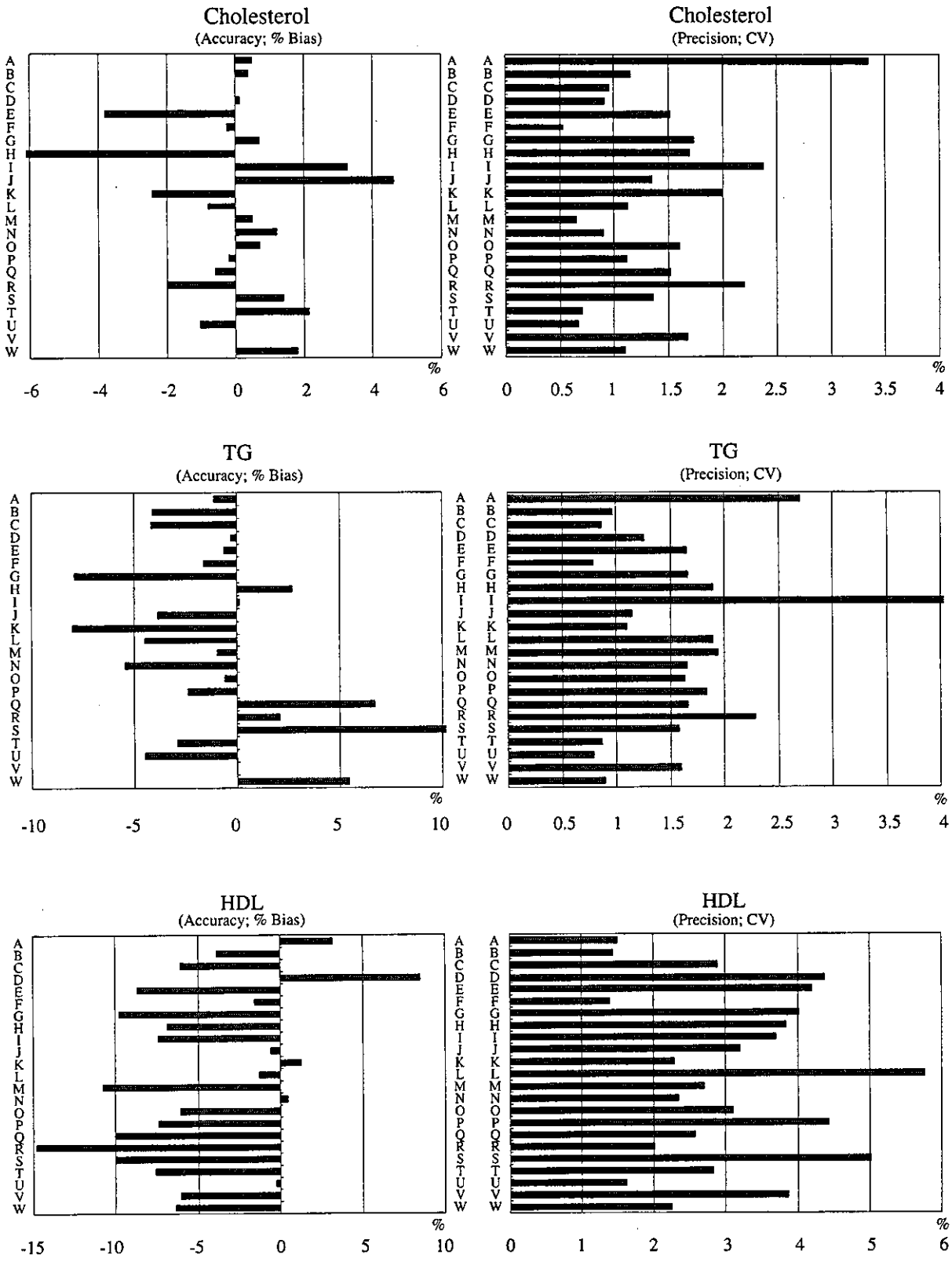

Figure 1. Accuracy (left column) and precision (right column) of cholesterol, triacylglycerol and HDL cholesterol of 23 laboratories.

資　　料

CDC-NHLBI脂質標準化プログラムの設立の歴史的経緯について

Gerald R. Cooper, PhD, MD*

中村　雅一　　訳**

A Short History of the Lipid Standardization Program : How the CDC-NHLBI Lipid Standardization Program (LSP) was Established

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序　　文

米国臨床化学会(AACC, American Association for Clinical Chemistry)の分科会の一つにLipoproteins and Vascular Diseasesの部門があり，年に4回，The Fats of Lifeと題する機関紙を発行して，リポ蛋白と血管病に関する最新情報を提供しています。その15回記念号として，米国の国立研究機関であるCDC(疾病対策予防センター，Centers for Disease Control and Prevention)の標準化の創設者であるDr. Gerald Cooperが，"A Short History of the Lipid Standardization Program : How the CDC-NHLBI Lipid Standardization Program (LSP) was Established"と題する記事を寄稿しました。

今日，臨床検査の領域におきましては，広く浅い精度管理から，限られた項目ながら深く測定精度を追求する標準化まで，様様なタイプで幅広く利用されております。中でも，標準化は，測定成績を施設間で共有化出来るのではないかという期待を抱かせるだけに，その関心は年々増大しております。ここに取り上げましたCDC-NHLBI脂質標準化プログラムは，標準化の先駆けとなるものであります。この記事では，標準化の成り立ちから今日的な実践活動までの経緯が詳しく解説されています。この点におきまして，今後，わが国が独自の標準化プログラムを開発し，臨床検査領域で適用して行く際のモデルとして，ご参考になるのではないかと考えました。翻訳につきましては，著者のDr. Cooperに直接お会いして，了解を得ました。この記事が，何かのお役に立つことが出来れば，翻訳者の喜びであります。

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【標準化プログラムの発足】

CDC(疾病対策センター，Centers for Disease Control，アトランタ)とNHLBI(国立心・肺・血液研究所，National Heart, Lung, and Blood Institute，ワシントン)の二つの国立研究機関が，共同してCDC-NHLBI Lipid Standardization Program (LSP，脂質標準化プログラム)を正式に立ち上げたのは，

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今から41年前の1961年であった。当時の脂質標準化プログラムの名称は，「Cooperative Cholesterol Standardization Program (CCSP)」である[1]。しかし，LSP(脂質標準化プログラム)の起源は，それよりも10年以上も遡る1946年に始まっている。当時，CDCは“Communicable Disease Center”と呼ばれていたが，その施設の中の“Clinical Pathology Section(臨床病理部)”が手がけたものであった。CDCは，米国南部のジョージア州アトランタ市のシャンブリー地区にあったローソン陸軍病院内に併設されており，臨床病理部門の部長はDr. Emil Mandelであった。1950年にはDr. F. William Sudermanが臨床病理部門の部長に就任したが，一時，この臨床病理部はアトランタ市内のグレディ記念病院に移設され，そこで標準化の仕事が継続された時期がある。

1952年には，臨床病理部は“Hematology and Biochemistry Section(血液・生化学部)”と改称され，再びDr. Mandelが部長を担当することになった。同年6月，Dr. Gerald R. Cooper(著者)が“Laboratory's Physical Chemistry Section(生理・化学分析室)”の室長となり，そこで血液化学検査と細菌学検査の仕事を担当することになった。この生理・化学分析室の本来の業務は，血清中の蛋白やリポ蛋白の電気泳動，尿中の蛋白分析，及び，赤血球中のヘモグロビン異常を検出することにあった。これらの分析法を駆使することによって，生理・化学分析室は，リウマチ熱により鎌状赤血球症に罹患していた子供達の診断に貢献した。この業績により，生理・化学分析室はAmerican Medical Association(米国医学協会)から“Hektoen Medal”を受賞する栄誉を得た。

1956年，Dr. Cooperは血液・生化学部の部長に就任し，そのスタッフの中には生化学担当のDr. Asher，血液学担当のDr. Candler，病理学担当のDr. Hicklinが含まれていた。彼等は，分析法の開発やグルコース測定方法の改良を始めとする多くのプロジェクトや活動に参画した。その活動の中でも，A型肝炎の流行期におけるトランスアミナーゼの測定，リウマチ熱と慢性関節リウマチとの鑑別診断をするためのムコ蛋白の測定，並びに，Dr. Oley Paulを座長とするシカゴ循環器疫学会からの要請，中でもDr. Henry Blackburnからの強い要請を受けたコレステロールの測定法を改良するという仕事を挙げることが出来る。

【心・血管病におけるコレステロールの役割】

心・血管病に対するコレステロールの影響に関する臨床上の関心は古くからあった。しかし，両者の関連性については，1815年にChevreulが初めて胆石からコレステロールを単離して，これをコレステリンと命名するまでは，本格的な研究は殆どなかった。わずかに，1827年にRayerが黄色腫(キサントーマ)の実例を図解で示したり，1856年にVirchowが内皮細胞の損傷によってアテロームが生成されるということを示唆する程度に留まるものであった。それから20年後に，Mallassezが黄色腫(キサントーマ)にはコレステロールが含まれるという報告をした。20世紀の初頭に，Windauはアテローム性動脈には健常動脈の約20倍ものコレステロールが含まれ，しかも，主としてコレステロールはエステル結合として存在しているという事実を明らかにした。第一次世界大戦の最中に，Anitschowはウサギのアテロームは脂質，中でもコレステロールが蓄積して引き起こされることを見出した。DeLangenは，食品や血清中のコレステロールに関する最初の研究を行い，更に1916年にはアテローム性動脈硬化症に関する報告をしている。1919年にはWindauはコレステロールの構造を発表し，Macheboeufは1929年に血清中に存在するリポ蛋白についての報告をしている。それから17年後に，Dockは動脈硬化性病変は特に血管内皮の肥厚部位に認められると報告している。1947年には，Vartiainenは，動脈硬化症は平和時よりも戦時の方が，発生率は低いと報告している。第二次世界大戦後，動脈硬化症のリスクファクターとしてコレステロールがにわかに脚光を浴びるようになった。Wilkinsonは家族性高コレステロール血症について報告し，Duguidは血管中で血栓が形成される血栓症について報告している。1950年，Gofmanは超遠心法で分離されたリポ蛋白と心臓病との関連について報告し，1951年にはBarrは心臓病の患者におけるLDLの重要性について報告している。翌年，Abell等は，検体をアルコール性KOHで加水分解し，次いでヘキサンでコレステロールを抽出して，その後にLiebermann-Burchard反応で発色定量させる方法を発表した。1954年，Gofmanはタイプ III 型の高脂血症に伴う結節性黄色腫の報告をしている。