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Low-density lipoprotein cholesterol and risk of coronary heart disease among Japanese men and women: The Circulatory Risk in Communities Study (CIRCS)

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ABSTRACT

Objective. The objective of this study was to assess the association between serum LDL-cholesterol levels and risk of coronary heart disease (CHD) among Japanese who have lower means of LDL-cholesterol than Western populations.

Methods. The predictive power of estimated serum LDL-cholesterol levels in casual blood samples for risk of CHD was evaluated among residents from four Japanese communities participating in the Circulatory Risk in Communities Study (CIRCS). A total of 8131 men and women, aged 40 to 69 years with no history of stroke or CHD, completed baseline risk factor surveys between 1975 and 1987. By 2003, 155 cases of incident CHD (myocardial infarction, angina pectoris and sudden cardiac death) had been identified.

Results. Mean LDL-cholesterol values were 99.4 mg/dL for men and 109.4 mg/dL for women. The crude incidence rate (per 100,000 person-years) of CHD was 152.0 for men and 51.9 for women. The respective multivariable hazard ratios for ≥140 mg/dL versus <80 mg/dL LDL-cholesterol were 2.80 (95% confidence interval: 1.59 to 4.92) for total CHD, 3.83 (1.78–8.23) for myocardial infarction, 4.07 (2.02–8.20) for non-fatal CHD, and 1.24 (0.44–3.47) for fatal CHD.

Conclusion. Serum LDL-cholesterol levels ranging from around 80 mg/dL to 200 mg/dL were positively associated with risk of CHD in a Japanese population.

Introduction

Low-density lipoprotein cholesterol (LDL-cholesterol) is one of the major atherogenic lipoproteins and has been identified by the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (NCEP-ATPIII) (2002) as a primary target for prevention of coronary heart disease (CHD).

Associations of high concentrations of LDL-cholesterol with increased risk of CHD have been examined mainly in high cholesterol populations, but lower cholesterol populations have been the subject of only a few studies (Chien et al., 2007; Law et al., 2003; Liu et al., 2006; NCEP-ATPIII, 2002). It has therefore remained a matter of debate whether serum LDL-cholesterol levels are associated with risk of CHD for populations with low to moderate mean LDL-cholesterol levels.

A review article (O'Keefe et al., 2004) showed that the optimal levels of LDL-cholesterol are 50 to 70 mg/dL, because the range of LDL-cholesterol was estimated to be 50 to 70 mg/dL among hunter–gatherer humans as well as wild primates and mammals, none of whom has atherosclerosis, and because atherosclerosis progression and CHD events were minimal among participants in a cholesterol lowering trial whose LDL-cholesterol level were less than 70 mg/dL. However, the NCEP-ATPIII (2002) has recommended clinical management and dietary therapy even for low-risk persons with ≥160 mg/dL of LDL-cholesterol, because few studies have examined whether the aforementioned associations are also observed when LDL-cholesterol levels are in the lower ranges.

The Seven Countries Study demonstrated that the association between total cholesterol and mortality from CHD holds only for high cholesterol populations such as Americans, but not for low cholesterol populations such as the Japanese (Verschuren et al., 1995). On the other

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hand, a recent increase in CHD incidence rates detected among urban Japanese men shows the need for controlling an upward shift of population distributions of serum cholesterol levels (Iso et al., 1999). It is therefore of the utmost importance to determine whether LDL-cholesterol levels are associated with risk of CHD among Japanese.

To this purpose, we conducted a population-based cohort study of Japanese men and women with lower means of LDL-cholesterol levels than seen in Western populations (Verschuren et al., 1995).

Methods

Study cohort

The participants were recruited from population-based samples obtained under the Circulatory Risk in Communities Study (CIRCS) (Imano et al., 2009). They were aged 40 to 69 years, living in four Japanese communities and participated in cardiovascular risk surveys between 1975 and 1987, from which we obtained data related to lipid profiles and confounding variables. The participation rate in the study presented here was 77% for a total of census population.

From the 8157 participants (3201men and 4956 women), we excluded 26 persons with a history of CHD and/or stroke at the time of the baseline inquiry, so that a total of 8131 persons (3178 men and 4953 women) were enrolled in the analysis.

Informed consent was obtained for conducting this epidemiological study, which was based on the guidelines of the Council for International Organizations of Medical Science (1991). The Ethics Committee of Osaka Medical Center for Health Science and Promotion approved this study.

Measurement of risk factors

Serum total cholesterol was measured with the Liebermann–Burchard direct method using Autoanalyzer II (Technicon, Tarrytown, NY) for 1975–1979 and Autoanalyzer SMA-6/60 (Technicon) for 1979–1986, and with the enzymatic method using Autoanalyzer SMAC (Technicon) since 1986. Serum triglycerides were measured with the fluorometric method using Autoanalyzer II for 1975–1986, and with the enzymatic method using Autoanalyzer SMAC since 1986. After precipitation by heparin–manganese, serum high-density lipoprotein cholesterol (HDL-cholesterol) was measured with the Liebermann–Burchard method using Autoanalyzer II. These measurements were performed at the laboratory of the Osaka Medical Center for Health Science and Promotion, an international member of the US National Cholesterol Reference Method Laboratory Network (Nakamura et al., 2003).

LDL-cholesterol was calculated with the Friedewald formula as follows: LDL-cholesterol (mg/dL) = total cholesterol (mg/dL) − HDL-cholesterol (mg/dL) − 0.2 × triglycerides (mg/dL) (Friedewald et al., 1972). A previous study showed no bias related to LDL-cholesterol levels among persons with <781 mg/dL of triglycerides in fasting blood samples (Tremblay et al., 2004). Since 77% of the subjects enrolled in the present study, were not fasting, we compared LDL-cholesterol estimated with the Friedewald formula and values measured by direct method as the gold standard in 14,072 men and 10,479 women aged 40–69 years who participated in health check-ups by the Osaka Medical Center for Health Science and Promotion between 2001 and 2009. We found that the LDL-cholesterol values determined with those two methods were comparable when triglycerides were <781 mg/dL in both fasting and non-fasting blood samples. The Spearman's rank correlation coefficients for estimated and directly measured LDL-cholesterol values were 0.96 (0.96 for men and 0.97 for women) for fasting and 0.94 (0.93 and 0.95, respectively) for non-fasting subjects. Mean values ± standard deviations for estimated and directly measured LDL-cholesterol were 129 ± 33 mg/dL and 130 ± 32 mg/dL, respectively, for fasting subjects, and 125 ± 33 mg/dL and 129 ± 32 mg/dL, respectively, for non-fasting subjects.

The details of other baseline examinations have been described in a previous report of ours (Iso, et al. 2001). Mild hypertension was defined as systolic blood pressure 140–159 mm Hg or diastolic blood pressure 90–99 mm Hg; the corresponding values for moderate hypertension were 160–179 mm Hg or 100–109 mm Hg and ≥180 mm Hg or ≥110 mm Hg for severe hypertension, regardless of antihypertensive medication use. Diabetes was defined as a serum glucose level ≥126 mg/dL in the fasting or ≥200 mg/dL in the non-fasting state, or as use of medication for diabetes. Borderline diabetes was defined as serum glucose level 110–125 mg/dL in the fasting or 140–199 mg/dL in the non-fasting state, and as no use of medication for diabetes. An interview was conducted to ascertain smoking status, number of cigarettes smoked per day, and usual alcohol intake per week.

Follow-up study

The details of endpoint determination have been described in a previous report of ours (Shimamoto, et al. 1989). Briefly, to validate the diagnosis, all living patients were telephoned or visited or invited to attend annual cardiovascular risk factor surveys or a medical history was obtained from their families. In addition, relevant medical records at local clinics and hospitals were reviewed. In cases of death, history was obtained from the family and/or attending physician and medical records were reviewed.

The criteria for coronary heart disease were modified from those established by the World Health Organization Expert Committee (1962). Definite myocardial infarction was diagnosed as typical severe chest pain (lasting for ≥30 min) together with the appearance of new abnormal and persistent Q or QS waves, consistent changes in cardiac enzyme levels, or both. Probable myocardial infarction was indicated by typical chest pain, but for which no electrocardiographic findings or findings related to enzyme activity were available. Myocardial infarction was considered present if either definite or probable myocardial infarction was diagnosed. Angina pectoris was defined as repeated episodes of chest pain during effort, especially when walking, usually disappearing rapidly after the cessation of effort or by the use of sublingual nitroglycerin. Sudden cardiac death was defined as death within 1 h of onset, a witnessed cardiac arrest, or abrupt collapse not preceded by ≥1 h of symptoms. CHD was defined as including myocardial infarction, angina pectoris, and sudden cardiac death. We also defined incident CHD death within 28 days as fatal CHD.

For each of the participants, the person-years of follow-up were calculated from the date of completion of the baseline survey to the date of cardiovascular incidence, death, exit from the community, or the end of 2003, whichever occurred first. The participants who moved away from the community (5.8%) were treated as censored.

Statistical analysis

ANCOVA and Mantel–Haenszel chi-square tests were used to examine differences between persons with and those free of incident CHD in terms of sex- and age-adjusted mean values and proportions of baseline characteristics.

The sex- and age-adjusted, sex-specific age-adjusted, and multivariable hazard ratios (HR) and 95% confidence intervals (95% CI) were calculated with the Cox proportional hazards model after adjustment for potentially confounding factors. The reference was <80 mg/dL LDL-cholesterol at baseline. The potentially confounding factors included blood pressure category, antihypertensive medication use, glucose category, body mass index (sex-specific quartiles), smoking status (never, ex- and current smokers of cigarette smoking at <20 and ≥21 cigarettes per day), alcohol intake category (never, ex-drinker, and current drinker of ethanol at 1 to 22, 23 to 45, 46 to 68, and ≥69 g per day), lipid lowering medication use (yes or no), category of HDL-cholesterol (<40, 40–49, 50–59, 60–69, and ≥70 mg/dL) and triglycerides (<100, 100–149, 150–199, 200–249, and ≥250 mg/dL), fasting status (<8 or ≥8 h), year of baseline survey, and study area. We also calculated the HR per 30 mg/dL increment in LDL-cholesterol. We tested the assumption of proportional hazards (Ng'andu, 1997), and found no violation of proportionality.

We also examined, non-parametrically and with restricted cubic splines (Durrleman and Simon, 1989), possible non-linear associations between LDL-cholesterol levels and risk of CHD. Tests for non-linearity used the likelihood ratio test to compare the model with only the linear term to the model with the linear and the cubic spline terms.

All statistical tests were two-sided and a p-value <0.05 was regarded as statistically significant. SAS, version 9.1.3 (SAS Institute, Inc., Cary, NC, USA) was used for all statistical analyses.

Results

Table 1 shows selected cardiovascular risk factors at the baseline survey for persons developing/free of incident CHD. The mean of LDL-cholesterol was 105.5 mg/dL for all participants, 99.4 mg/dL for men and 109.4 mg/dL for women at baseline. Compared with persons free of incident CHD, those who were developing CHD, myocardial infarction or non-fatal CHD were more likely to be male and older. They also tended to have higher means of total and LDL-cholesterol

Table 1
Baseline characteristics of participants developing incident coronary heart disease (CHD) and those remaining free of it.

	Participants free of incident CHD	Participants developing incident CHD	Participants developing myocardial infarction	Participants developing non-fatal CHD	Participants developing fatal CHD
Total (men and women)					
Number of persons	7976	155	91	115	40
Men, %	38.6	63.9‡	69.2‡	67.8‡	52.5
Age, year	51.7	55.5‡	55.1‡	54.4‡	58.4‡
Total cholesterol, mg/dL	188.3	200.6‡	203.2‡	202.3‡	195.7
LDL-cholesterol, mg/dL	104.2	114.2‡	114.0‡	117.3‡	105.3
HDL-cholesterol, mg/dL	56.6	54.7	56.1	53.6†	57.9
Triglycerides, mg/dL	137.3	158.2‡	165.5‡	156.7†	162.5
Lipid lowering medication use, %	0.1	0.0	0.0	0.0	0.0
Body mass index, kg/m^2	23.2	23.6	23.6	23.5	23.8
Systolic blood pressure, mmHg	134.0	141.6‡	140.1‡	141.6‡	141.7†
Diastolic blood pressure, mmHg	80.9	85.2‡	84.5‡	84.9‡	85.9‡
Antihypertensive medication use, %	10.4	15.2†	13.9	11.9	24.8‡
Diabetes, %	3.3	2.4	4.6	2.7	1.5
Current smoker, %	35.1	39.9	44.4†	41.4	35.6
Current drinkers, %	45.2	42.8	44.5	44.4	36.3
Fasting blood samples, %	22.5	27.5	26.8	28.2	25.6
LDL- to total cholesterol ratio	0.55	0.56	0.55	0.57	0.53
Men					
Number of persons	3079	99	63	78	21
Age, year	52.0	53.9‡	53.3	52.9	57.5†
Total cholesterol, mg/dL	183.6	195.9†	197.6†	196.9†	192.1
LDL-cholesterol, mg/dL	99.1	109.7†	107.2	111.3†	103.8
HDL-cholesterol, mg/dL	56.0	54.1	55.6	53.5	56.2
Triglycerides, mg/dL	142.2	160.5	173.9†	160.4	161.1
Lipid lowering medication use, %	0.1	0.0	0.0	0.0	0.0
Body mass index, kg/m^2	22.9	23.4	23.4	23.5	23.3
Systolic blood pressure, mmHg	135.5	142.1†	140.8‡	143.1†	138.1
Diastolic blood pressure, mmHg	82.4	86.1†	85.5‡	86.6†	84.0
Antihypertensive medication use, %	10.3	17.0‡	15.0	16.1	20.1
Diabetes, %	4.4	2.9	4.6	3.8	0.0
Current smoker, %	64.4	66.5	71.8	70.7	50.6
Current drinkers, %	79.9	77.1	78.5	78.4	71.8
Fasting blood samples, %	22.0	26.9	22.0	28.0	22.8
Women					
Number of persons	4897	56	28	37	19
Age, year	51.5	58.3†	59.2†	57.7†	59.4†
Total cholesterol, mg/dL	193.0	204.2‡	208.0‡	206.4‡	199.9
LDL-cholesterol, mg/dL	109.3	117.6	121.5	123.0‡	107.0
HDL-cholesterol, mg/dL	57.2	55.5	56.8	53.3	59.7
Triglycerides, mg/dL	132.3	155.7‡	148.7	150.5	166.0
Lipid lowering medication use, %	0.1	0.0	0.0	0.0	0.0
Body mass index, kg/m^2	23.5	23.6	23.6	23.2	24.5
Systolic blood pressure, mmHg	132.5	141.9†	140.1‡	139.8‡	146.0†
Diastolic blood pressure, mmHg	79.4	84.6†	84.1‡	82.7	88.2†
Antihypertensive medication use, %	10.6	12.0	11.2	2.8	30.0†
Diabetes, %	2.2	2.5	5.9	1.7	4.0
Current smoker, %	5.8	15.4†	19.3†	11.8	22.3†
Current drinkers, %	10.5	9.6	15.6	12.0	1.1
Fasting blood samples, %	22.9	27.8	36.4	27.4	28.6

Participants developing myocardial infarction constitute a subset of participants developing incident CHD. Participants developing incident CHD are divided into those developing non-fatal and fatal CHD.
Test for difference from participants free of incident coronary heart disease.
To convert values for LDL-cholesterol to millimoles per liter, multiply by 0.02586. The convert values for triglycerides to millimoles per liter, multiply by 0.01129.
† $p<0.01$.
‡ $p<0.05$.

and triglycerides and systolic and diastolic blood pressures, whereas there was no difference in total and LDL-cholesterol and triglycerides for those who were developing fatal CHD.

A total of 8131 persons (3178 men and 4953 women) were followed up for a median of 21.9 years, during which time, we identified 155 incidences of CHD (including 115 non-fatal and 40 fatal CHDs). LDL-cholesterol levels correlated linearly with risk of myocardial infarction for men and with total CHD, myocardial infarction and non-fatal CHD for all participants (Table 2). Adjustment for potentially confounding factors did not alter these associations materially. The multivariable hazard ratios for ≥140 mg/dL versus <80 mg/dL LDL-cholesterol for all participants were 2.80 (95% CI: 1.59–4.92) for total CHD, 3.83 (1.78–8.23) for myocardial infarction, 4.07 (2.02–8.20) for non-fatal CHD, and 1.24 (0.44–3.47) for fatal CHD. The corresponding multivariable hazard ratios (95% CI) associated with a 30 mg/dL increment in LDL-cholesterol were 1.30 (1.14–1.49), 1.33 (1.12–1.59), 1.36 (1.17–1.58), and 1.16 (0.87–1.55). These positive associations were similar for men and women with no sex interaction ($p=0.89$ for total CHD, $p=0.45$ for myocardial infarction, $p=0.78$ for non-fatal CHD, and $p=0.55$ for fatal CHD). We also did not observe any fasting status interaction ($p=0.67$, $p=0.67$, $p=0.91$ and $p=0.67$, respectively).

Further, these associations did not alter substantially after the exclusion of persons with hypertriglyceridemia (triglycerides ≥300 mg/dL, 193 men and 172 women, not shown in the table). The multivariable hazard ratios (95% CI) for LDL-cholesterol ≥140 mg/dL versus <80 mg/dL LDL-cholesterol were 2.81 (1.55–5.07) for total CHD, 3.53 (1.60–7.80) for myocardial infarction, 3.97 (1.93–8.17) for non-fatal CHD, and 1.19 (0.38–3.70) for fatal CHD.

Table 2
Crude incidence rate (per 100,000 person-years), sex- and age-adjusted, and sex-specific age-adjusted, and multivariable hazard ratio (HR) and 95% confidence interval (95% CI) of coronary heart disease (CHD) according to categories of LDL-cholesterol.

Range, mg/dL (mmol/L)	LDL-cholesterol					HR per 30 mg/dL increment
	<80 (<2.06)	80–99 (2.06–2.57)	100–119 (2.58–3.09)	120–139 (3.10–3.61)	140+ (3.62+)	
Total (men and women)						
Persons	1774	1899	1949	1302	1207	8131
Person-years	38,175	40,754	41,474	27,513	25,109	173,024
Total CHD						
No	23	29	35	31	37	155
Crude incidence rate	60.2	71.2	84.4	112.7	147.4	89.6
Sex- and age-adjusted HR	1.0	1.22 (0.71–2.12)	1.48 (0.87–2.51)	2.09 (1.22–3.61)	2.76 (1.62–4.70)	1.34 (1.17–1.53)
Multivariable HR[a]	1.0	1.35 (0.77–2.36)	1.66 (0.96–2.86)	2.15 (1.22–3.81)	2.80 (1.59–4.92)	1.30 (1.14–1.49)
Myocardial infarction						
No	12	17	20	21	21	91
Crude incidence rate	31.4	41.7	48.2	76.3	83.6	52.6
Sex- and age-adjusted HR	1.0	1.41 (0.67–2.96)	1.69 (0.82–3.47)	2.89 (1.42–5.91)	3.28 (1.59–6.74)	1.33 (1.12–1.59)
Multivariable HR[a]	1.0	1.67 (0.78–3.55)	2.07 (0.98–4.34)	3.42 (1.62–7.26)	3.83 (1.78–8.23)	1.33 (1.12–1.59)
Non-fatal CHD						
No	13	23	24	26	29	115
Crude incidence rate	34.1	56.4	57.9	94.5	115.5	66.5
Sex- and age-adjusted HR	1.0	1.77 (0.90–3.50)	1.89 (0.96–3.72)	3.30 (1.69–6.45)	4.22 (2.17–8.20)	1.43 (1.23–1.66)
Multivariable HR[a]	1.0	1.95 (0.98–3.90)	2.06 (1.03–4.13)	3.25 (1.61–6.53)	4.07 (2.02–8.20)	1.36 (1.16–1.58)
Fatal CHD						
No	10	6	11	5	8	40
Crude incidence rate	26.2	14.7	26.5	18.2	31.9	23.1
Sex- and age-adjusted HR	1.0	0.52 (0.19–1.43)	0.89 (0.37–2.12)	0.62 (0.21–1.84)	0.98 (0.37–2.57)	1.07 (0.81–1.42)
Multivariable HR[a]	1.0	0.57 (0.20–1.64)	1.04 (0.41–2.60)	0.72 (0.23–2.28)	1.24 (0.44–3.47)	1.16 (0.87–1.55)
Men						
Persons	881	795	728	440	334	3,178
Person-years	18,296	16,490	14,921	8901	6522	65,130
Total CHD						
No	20	23	18	16	22	99
Crude incidence rate	109.3	139.5	120.6	179.8	337.3	152.0
Age-adjusted HR	1.0	1.25 (0.69–2.27)	1.07 (0.57–2.02)	1.68 (0.87–3.24)	3.05 (1.66–5.59)	1.34 (1.13–1.59)
Multivariable HR[a]	1.0	1.34 (0.72–2.49)	1.17 (0.60–2.28)	1.72 (0.85–3.48)	2.90 (1.51–5.57)	1.30 (1.09–1.55)
Myocardial infarction						
No	11	15	14	10	13	63
Crude incidence rate	60.1	91.0	93.8	112.3	199.3	96.7
Age-adjusted HR	1.0	1.49 (0.69–3.25)	1.53 (0.69–3.37)	1.91 (0.81–4.49)	3.30 (1.48–7.37)	1.26 (1.01–1.56)
Multivariable HR[a]	1.0	1.79 (0.80–4.01)	1.83 (0.80–4.20)	2.26 (0.90–5.66)	3.59 (1.51–8.55)	1.24 (1.00–1.53)
Women						
Persons	893	1104	1221	862	873	4953
Person-years	19,878	24,264	26,553	18,612	18,588	107,895
Total CHD						
No	3	6	17	15	15	56
Crude incidence rate	15.1	24.7	64.0	80.6	80.7	51.9
Age-adjusted HR	1.0	1.26 (0.31–5.05)	2.84 (0.83–9.75)	3.20 (0.92–11.18)	2.78 (0.79–9.75)	1.26 (1.01–1.56)
Multivariable HR[a]	1.0	1.21 (0.30–4.95)	3.41 (0.98–11.95)	3.80 (1.06–13.58)	3.05 (0.84–11.07)	1.25 (1.00–1.55)
Myocardial infarction						
No	1	2	6	11	8	28
Crude incidence rate	5.0	8.2	22.6	59.1	43.0	26.0
Age-adjusted HR	1.0	1.22 (0.11–13.50)	2.85 (0.34–23.86)	6.67 (0.85–52.20)	4.11 (0.50–33.44)	1.38 (1.02–1.85)
Multivariable HR[a]	1.0	1.26 (0.11–14.26)	3.69 (0.43–31.83)	8.93 (1.11–71.72)	5.43 (0.64–45.92)	1.42 (1.05–1.91)

Potential confounding factors: blood pressure category, antihypertensive medication use, glucose category, body mass index, smoking status, alcohol intake category, lipid lowering medication use, categories of HDL-cholesterol and triglycerides, fasting status, years at entry and study area.

[a] HR (95% CI) adjusted for sex, age and potential confounding factors.

The dose–response patterns of total CHD, as well as non-fatal CHD, corroborated the results of the categorical analysis (Fig. 1).

Discussion

In the population-based prospective study of Japanese reported here, we found positive relationships between LDL-cholesterol levels and risks of total CHD and myocardial infarction. The risk of total CHD started to increase when the serum LDL-cholesterol level was above 80 mg/dL (corresponding to 167 mg/dL of total cholesterol levels).

Previous cohort studies of an American population (mean LDL-cholesterol levels: 139 mg/dL for men 138 mg/dL for women at baseline) (Liu et al., 2006), a Chinese population (133 mg/dL and 142 mg/dL, respectively) (Chien et al., 2007) and high-risk patients enrolled in cholesterol lowering clinical trials (162 mg/dL for all participants) (Sacks et al., 2000) showed positive relationships between LDL-cholesterol and risk of CHD. Because the lowest category of LDL-cholesterol levels in these previous studies was ≥100 mg/dL, which represented the middle and higher categories in the current study, it was not clear whether a similar relationship holds true for populations with moderate cholesterol levels, the men and women in our study with means of LDL-cholesterol of 99.4 mg/dL and 109.4 mg/dl, respectively. These values corresponded to the optimal individual levels, addressed by the Japan Atherosclerosis Society Guidelines for Prevention of Atherosclerotic Cardiovascular Diseases in 2007. A previous Japanese population-based cohort study showed a non-linear association: a 1.68 times higher multivariable hazard ratio for persons with 126–150 mg/dL of LDL-cholesterol in comparison with men and women with ≤102 mg/dL and the risk plateaued at less than 125 mg/dL (Imamura et al., 2009). Our findings, however, corroborated the evidence for a linear association between LDL-cholesterol levels and the risk of incident CHD among Japanese with lower ranges of LDL-cholesterol levels.

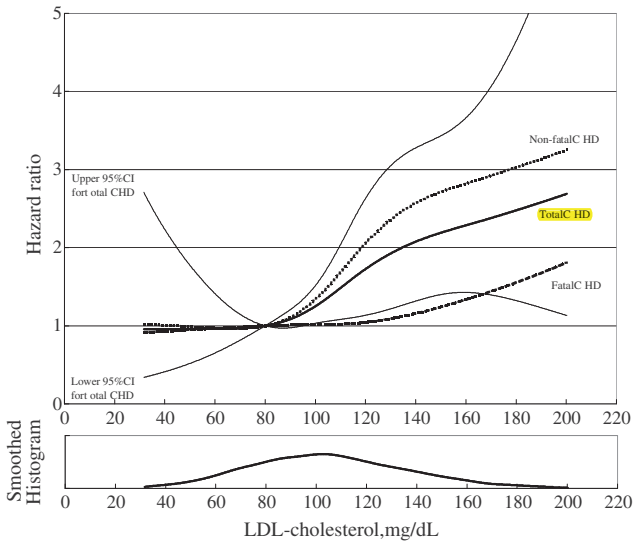

Fig. 1. Multivariable hazard ratios of total, non-fatal and fatal coronary heart diseases (CHD) in relation to LDL-cholesterol levels. 80 mg/dL of LDL-cholesterol was selected as reference level. The values of the 4 knots correspond to LDL-cholesterol levels of 57.0 mg/dL, 91.8 mg/dL, 115.6 mg/dL and 160.0 mg/dL. The smoothed histogram shows the distribution of LDL-cholesterol levels. We did not plot predictions from the top and bottom 1% of the analytical distribution to avoid an undue visual effect of sparse tail data. The p-values for non-linearity were p = 0.0006 for total CHD and p = 0.0002 for non-fatal CHD, whereas the hazard ratio was fairly flat at less than 80 mg/dL of LDL-cholesterol levels. On the other hand, we observed an increase in the risk of fatal CHD only at the upper tail of the distributions of LDL-cholesterol levels. Although the hazard ratios were still fairly flat at less than 80 mg/dL of LDL-cholesterol levels, the graph suggests that the risk of total and non-fatal CHD may start to increase a level of around 80 mg/dL of LDL-cholesterol (corresponding to 167 mg/dL of total cholesterol), whereas the risk of fatal CHD may increase at LDL-cholesterol levels over 120 mg/dL (corresponding to 202 mg/dL of total cholesterol).

It remains debatable what range of LDL-cholesterol levels is optimal for the prevention of CHD. The National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATPIII) (2002) recommended clinical management and dietary therapy for low-risk persons with ≥160 mg/dL of LDL-cholesterol, whereas another review article suggested that the optimal level of LDL-cholesterol may be 50 to 70 mg/dL (Verschuren et al., 1995). Our findings for total and non-fatal CHD support the notion that the threshold of LDL-cholesterol for increased risks of total and non-fatal CHDs may be around 80 mg/dL (corresponding to 167 mg/dL of total cholesterol), although our LDL-cholesterol measurements may have been underestimated as discussed below.

Study limitations

One limitation of the current study is that we estimated LDL-cholesterol levels by using the Friedewald formula, which cannot be used for specific metabolic conditions such as hypertriglyceridemia (Tremblay et al., 2004). However, the association between LDL-cholesterol and risks of total and non-fatal CHDs and myocardial infarction did not alter after the exclusion of persons with hypertriglyceridemia at the baseline survey, and we observed no effect modification resulting from the presence or absence of fasting status. The second limitation is that approximately 77% of participants were non-fasting, which is likely to lead to underestimation of LDL-cholesterol calculation when using the Friedewald formula. According to a validation study conducted by our lipid reference standardization laboratory, LDL-cholesterol levels calculated with the Friedewald formula were underestimated by 4 mg/dL among non-fasting subjects. However, this did not *per se* affect the association we observed, and, as mentioned before, fasting or non-fasting status did not change the association between LDL-cholesterol and risk of CHD. Third, we found weak non-significant association between LDL-cholesterol levels and risk of fatal CHD, which corresponded to the finding from the Seven Countries Study (Verschuren et al., 1995). However, the lack of association could be due to the low statistical power resulting from only 21 fatal CHD cases among men and 19 among women. The statistical power for the association between LDL-cholesterol and risk of fatal CHD was only 15% according to our analysis.

Study strengths

The strength of the current study is the use of a population-based sample from four Japanese communities, so that our findings can probably be generalized to other Japanese populations which also have lower cholesterol levels than Western populations (O'Keefe et al., 2004). Second, the prevalence of lipid lowering medication usage was only 0.1% in the participants and serum lipid level at baseline was nearly equal to the natural state. Third, we used standardized lipid measurement values, which in turn were standardized by the CDC–NHLBI Lipid Standardized Program (Nakamura et al., 2003). This justifies our assumption that misclassification bias due to measurement errors of lipid measurement has been appropriately reduced, and that the resultant accuracy of lipid measurement allows for comparison of our results with those of previous well-standardized studies.

Conclusion

Our population-based cohort study of Japanese showed that serum LDL-cholesterol levels ranging from around 80 mg/dL to 200 mg/dL were positively associated with risk of CHD.

Conflict of interest statement
The authors declare that there are no conflicts of interest.

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