Table 3. Parameter estimates of WBC count in the background model

Males:

model $WBC = \alpha_m + \beta_{m1}*(age) + \beta_{m2}*(age)^2 + \beta_{m3}*(age)^3 + \beta_{m4}*(smk) + \beta_{m5}*(bmi) + \beta_{m6}*(inf)$

	α_m	β_{m1}	β_{m2}	β_{m3}	β_{m4}	β_{m5}	β_{m6}
estimate	1.95	0.21	–0.004	2.3E-5	0.71	0.053	0.18
(95% CI)	(0.42,3.45)	(0.12,0.30)	(–0.006, –0.003)	(1.2E-5, 3.4E-5)	(0.49,0.93)	(0.03,0.07)	(0.05,0.31)

Females:

model $WBC = \alpha_f + \beta_{f1}*(age) + \beta_{f2}*(age)^2 + \beta_{f3}*(city) + \beta_{f4}*(smk) + \beta_{f5}*(bmi)$

	α_f	β_{f1}	β_{f2}	β_{f3}	β_{f4}	β_{f5}
estimate	6.47	–0.07	0.0005	–0.15	0.67	0.072
(95% CI)	(6.07,6.87)	(–0.09, –0.06)	(0.0004, 0.0006)	(–0.26, –0.04)	(0.52,0.81)	(0.06,0.08)

Variable definitions

WBC = WBC count (1,000/mm^3)
age = age at time of health examination
smk = indicator of ever smoker (1 or 0)
bmi = body mass index (kg/m^2)
inf = indicator of infectious diseases (1 or 0)
city = indicator of Nagasaki AHS subject (1 or 0).

at baseline.

Background Model

AICs with various models for age and variance-covariance specifications are presented in Table 2. We selected the unstructured covariance specification for this study[b]. For males, a third-order (linear, quadratic, and cubic) model in age had the lowest AIC value. However, for females, the cubic term was not significant and a linear-quadratic model was selected.

The best-fitting background model indicated that WBC and differential WBC counts declined with age (Fig. 1). The temporal pattern for males was best depicted by a linear-quadratic-cubic function in age, whereas the trend for females appeared to be linear-quadratic in age. The WBC count for males appeared to reach a plateau by age 45 and subsequently declined gradually, while the trend for female WBC count decreased throughout adulthood. Most of the differential WBC counts had declining trends with increasing age, except that lymphocyte counts in females seemed to remain stable with little decline over time.

Among all risk factors considered in this study, smoking caused the largest elevations in WBC and differential WBC counts over time (Table 3). The WBC counts of ever smokers were on average 710/mm^3 higher for males and 670/mm^3 higher for females than never smokers ($p < 0.001$). However, the impact of smoking varied by type of differential WBC count, independent of their original proportion in the sense that although lymphocytes occupy a small proportion of baseline WBCs they constituted a proportion nearly the same as that of neutrophils among the increased WBC. The increase in lymphocytes caused by smoking accounted for 47% of the total WBC change for males and 38% of the total WBC change for females, respectively. In other words, smoking caused a larger proportion of lymphocyte increase relative to that for neutrophils or monocytes. BMI was also a significant risk factor for increased WBC and differential WBC counts for both males and females, but was not a significant risk factor for neutrophil count in males (data not shown). For an increase of one kg/m^2 in BMI, the WBC counts increased by 53/mm^3 ($p < 0.001$) and 72/mm^3 ($p < 0.001$) for males and for females, respectively. A city difference was observed among females but not among males.

Full Model for detecting radiation effect

In the full model including all subjects, we added radiation dose and an indicator of age at exposure (age at exposure < 20) in order to investigate the association of WBC and radiation dose, as well as possible effect modification by birth cohort and other risk factors. Initial assessment of the shape of dose-response compared linear, pure quadratic, and linear quadratic models. For both men and women, the data showed positive and possibly non-linear associations of WBC with radiation dose. The results did not clearly distinguish the precise form of the dose-response based on AIC, although the pure quadratic model had a slightly smaller

[b] A compound symmetry covariance matrix was used in estimating neutrophil, lymphocyte, and monocyte trends due to the problems of converging. The predicted values should not be too different from unstructured covariance assumption.

AIC value for men (Table 4). We also examined the possibility of a threshold. By comparing the deviance values under different assumed thresholds, a value of 1.40 Gy for men was indicated but only marginally significant (95% CI 0 to 2.6). On the other hand, for women a threshold at 0.7 was indicated but not statistically significant (95% CI 0 to 2.5). Based on the baseline WBC measurements in Table 1, there was indisputable evidence pointing to an effect of radiation at high doses. When a categorical dose-response model was estimated, no significant risk below 2 Gy was found. Using an indicator for dose > 2 Gy, the AIC value was reduced for men but remained unchanged for women. The model with an indicator of dose > 2 Gy was the preferred model in the final analysis. Figure 2 shows the different dose-response models with dose-group point estimates.

Even after adjusting for other risk factors, radiation exposure remained to be a significant risk factor resulting in increased WBC and differential WBC counts over time (Table 5, Fig. 3). There was a significant increase in WBC counts of 240/mm^3 for males (p = 0.004) and 176/mm^3 for females (p = 0.003) who were exposed to > 2 Gy of radiation. Smoking significantly elevated WBC counts for both

Table 4. Values of Akaike's Information Criterion for dose-response model selection

Dose-response Model	AIC	
	Men	Women
Linear	97,699	174,844
Pure quadratic	97,698	174,844
Linear quadratic	97,700	174,846
Threshold at 1.4 Gy	97,696	–
Threshold at 0.7 Gy	–	174,843
Categorical (5 dose category)	97,697	174,849
Categorical (> 2 Gy indicator)	97,694	174,844

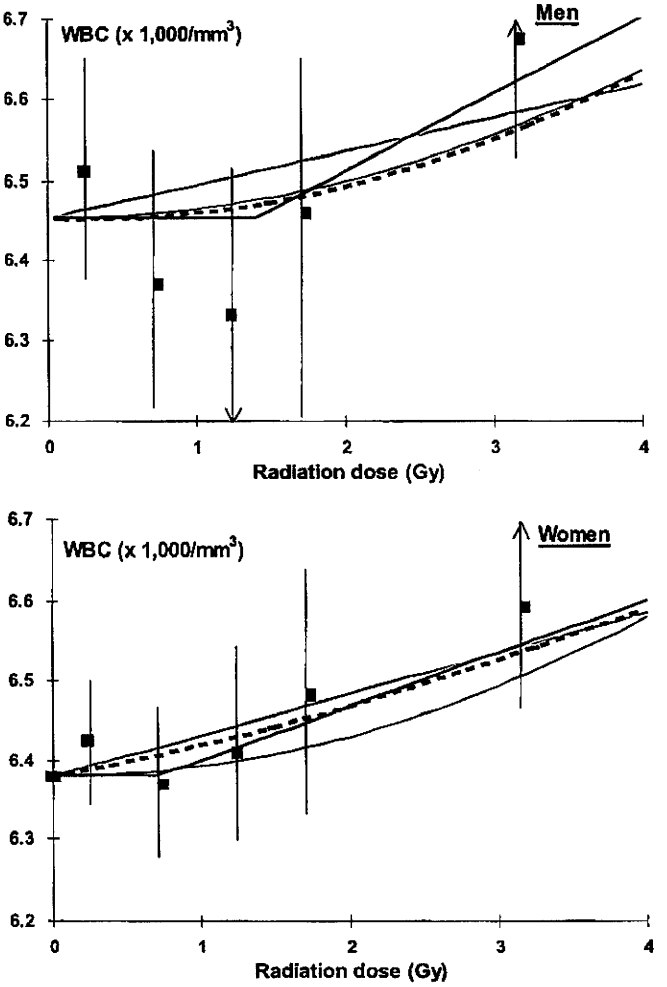

Fig. 2. Comparison of linear, quadratic, linear quadratic, and threshold dose-response models in association with increased WBC count by gender. The thick solid gray lines are linear dose-response models, the thin solid lines are pure quadratic dose-response models, the dotted lines are linear-quadratic dose-response models, and the thick solid line with knots at 1.4 Gy (for men) and 0.7 Gy (for women) are threshold models. Both plots assumed age at 30, never smoker, and BMI 21 for men and 22 for women.

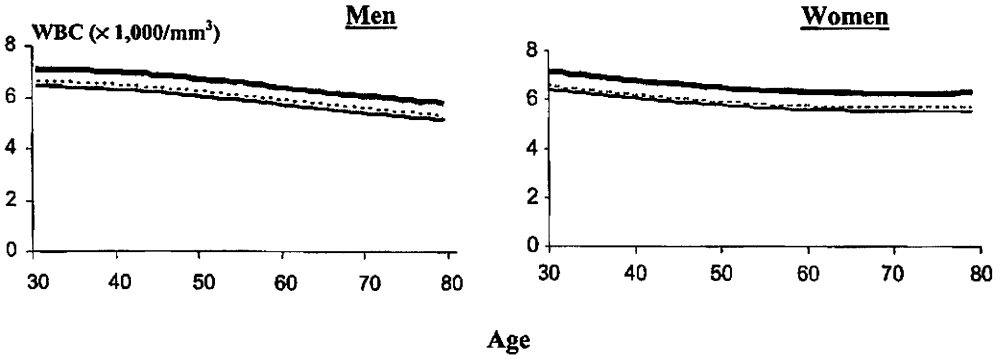

Fig. 3. Different effects of smoking and radiation exposure on WBC count by gender. The solid line indicates the WBC trend for never smokers who were exposed to ≤ 5 mGy. The dotted line and gray line are never smokers with radiation > 2 Gy and ever smokers with radiation > 2 Gy, respectively. The models were adjusted for city, body mass index, and an indicator of infectious disease.

Table 5. Effects of radiation and other risk factors on WBC counts in males and females

	Males		Females	
	Estimate (SD)	*P* value	Estimate (SD)	*P* value
WBC				
Radiation > 2 Gy	0.240 (0.084)	0.004	0.176 (0.059)	0.003
Ever smoker	0.644 (0.069)	< 0.001	0.590 (0.043)	< 0.001
BMI	0.059 (0.006)	< 0.001	0.078 (0.003)	< 0.001
Age at bombing < 20	–0.107 (0.056)	0.053	–0.158 (0.036)	< 0.001
Neutrophils				
Radiation > 2 Gy	0.158 (0.060)	0.009	0.122 (0.043)	0.004
Ever smoker	0.312 (0.050)	< 0.001	0.300 (0.031)	< 0.001
BMI	0.007 (0.004)	0.084	0.026 (0.002)	< 0.001
Age at bombing < 20	–0.090 (0.038)	0.019	–0.126 (0.025)	< 0.001
Lymphocytes				
Radiation > 2 Gy	0.072 (0.035)	0.044	0.050 (0.024)	0.038
Ever smoker	0.306 (0.029)	< 0.001	0.257 (0.018)	< 0.001
BMI	0.048 (0.002)	< 0.001	0.043 (0.001)	< 0.001
Age at bombing < 20	0.037 (0.022)	0.097	–0.013 (0.014)	0.358
Monocytes				
Radiation > 2 Gy	0.029 (0.008)	< 0.001	0.013 (0.005)	0.020
Ever smoker	0.042 (0.007)	< 0.001	0.034 (0.004)	< 0.001
BMI	0.006 (0.001)	< 0.001	0.004 (0.0003)	< 0.001
Age at bombing < 20	–0.034 (0.005)	< 0.001	–0.042 (0.003)	< 0.001

The analysis was adjusted for city, birth cohort, BMI, and an indicator of inflammation-related disease.

males and females ($p < 0.001$). The effects of smoking on WBC counts were relatively larger than those of radiation for both males and females. BMI remained a significant risk factor for WBC count in both males and females ($p < 0.001$), but the estimated change per 1 kg/m^2 increase was small. The effects of smoking and BMI on differential WBC counts were similar to the results in the background models, except the effect of BMI on neutrophils became marginally significant in males (P = 0.084). WBC and differential WBC—except lymphocyte—counts were lower among those survivors whose ages were less than 20 at the time of bombing.

In order to assess radiation risk modification, interactions of radiation and other risk factors were estimated. Because the precise shape of the dose response could not be determined and the assessment of interaction can be sensitive to a possibly incorrect form of the dose-response model, risk modification was assessed using the more common, simple linear dose-response model. The likelihood-ratio test and backward stepwise elimination were used for testing significance of effect modification. For men, only the interaction of dose and indicator of birth cohort (age at exposure < 20) was significant (P = 0.006), however, the overall dose response became insignificant (P = 0.40). In other words, among men, only the subjects in the younger birth cohort had a significant dose-response with an increased WBC of 130/mm^3 (95% CI 38 to 222) per Gy. There was no significant modification of the dose response for women.

DISCUSSION

Although previous AHS analyses have shown a positive association between WBC count and radiation dose based on cross-sectional data,[1-3] this is the first study to investigate the shape of the dose-response and show the longitudinal trends of total WBC and differential WBC (neutrophil, lymphocyte, and monocyte) counts among A-bomb survivors. Being based on longitudinal methods, it is also the first study to convincingly demonstrate that elevated WBC count in

radiation-exposed survivors persists over time.

Different dose-response functions (linear, pure quadratic, linear quadratic, threshold, and categorical) were examined. The model with an indicator of greater than 2 Gy was deemed preferable based on its lower AIC statistic. In the cross-sectional study by Neriishi *et al.*,[3] mean WBC count was estimated to increase by 70/mm^3 per Gy in a linear dose-response model. Although our final model shows that only a high dose of radiation resulted in significant long-term increased levels of WBC and differential WBC counts, the estimated increase was comparable to the findings by Nériishi *et al.* and the increase of WBC count was about 130/mm^3 (95% CI 38 to 222) per Gy among males who were exposed to radiation less than age 20[c]. In this study, there is clear evidence for a radiation effect on WBC and differential WBC counts at high doses. Elevated WBC count might reflect persist inflammation among A-bomb survivors over time as Hayashi *et al.*[28,29] showed that plasma levels of inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α) and IL-6, increase with radiation dose. Effects on WBC count of low radiation dose, especially under 1 Gy, remain unclear.

Regarding the effect of radiation on differential WBC count, there are few studies and inconsistent results. We found that there were significant and positive associations between radiation dose and neutrophil, lymphocyte, and monocyte counts. In a previous cross-sectional analysis of differential WBC counts collected between 1968 and 1980, increased WBC count in the group with high radiation exposure was apparently due to an increased proportion of neutrophils,[2] but direct comparison with our study is difficult. A significant relationship of neutrophil count with radiation dose was observed only in non-smokers in a cross-sectional study conducted in 2001.[3] Increased lymphocyte and monocyte counts among those with radiation exposure have not been reported in previous studies.[1,2]

Comparing the AIC values of different models, a linear-quadratic-cubic function of age best explains the trends of WBC and differential WBC counts for males. On the other hand, a linear-quadratic pattern best fits the trend for females. Despite different curvature for males and females, the WBC and differential WBC counts show a declining overall trend with a strictly linear model. This downward trend in WBC count by age is consistent with what was reported in the Baltimore Longitudinal Study of Aging.[30] In that study, a secular downward trend of WBC count was observed in both genders (linear, quadratic, and cubic effects of date) in the period from 1958 to 2002, and higher WBC count observed in the participants enrolled at age 40 to 59.9 years. Similarly, our study found a higher WBC level around age 45 for men.

It is well known that smoking affects WBC count.[14–19] In our study, cigarette smoking is associated with the largest and most significant increase in WBC count. As with previous studies, BMI is another risk factor related to increased WBC and differential WBC counts,[20–22] except for neutrophils in men. However, the effect of BMI was small compared to that of smoking or radiation.

There are several limitations of this study. Smoking status reflects smoking habits at baseline. Therefore, changes in WBC level due to changes in either smoking status or amount smoked could not be completely captured. There are reports of studies where the impact of smoking cessation on levels of WBC count was examined; these show that WBC levels decrease or return to levels similar to those in never-smokers years after quitting.[16,17,19] Therefore, by combining current and former smokers into one category, we might have underestimated the true effect of smoking on WBC and differential WBC counts. It is also known that within-individual variation in WBC count depends on many other factors such as infection and medical therapy (chemotherapy and radiation therapy). Although we adjusted for acute inflammatory diseases (pneumonia etc.), information on chemotherapy and radiation therapy, which are known to reduce WBC count, was not available. However, because most medical therapy is administered over a short term, the effect should have little impact on long-term longitudinal analysis.

In summary, we found that radiation exposure at high dose is related to elevated WBC and differential WBC counts. Our findings support the view that inflammation may be an intermediate step on the way to occurrence of CVD, cancer and other late effects resulting from radiation exposure. Elevated WBC due to radiation exposure appears to persist decades after exposure.

[c] The mean age in the study by Neriishi *et al.* (2001) is 63.4. In other words, the mean age is about 18.4 at the time of bombing.

ACKNOWLEGEMENT

This paper was based on research performed at the Radiation Effects Research Foundation (RERF), Hiroshima and Nagasaki, Japan. RERF is a private, non-profit foundation funded equally by the Japanese Ministry of Health, Labour and Welfare (MHLW) and the US Department of Energy (DOE), the latter in part through the National Academy of Sciences. This publication was supported by RERF Research Protocols RP # 2-75 and RP # 3-05.

REFERENCES

1. Belsky JL, Tachikawa K and Jablon S (1973) ABCC-JNIH Adult Health Study, Report 5. Results of the first five examination cycles, Hiroshima-Nagasaki 1958–68. Yale J Biol Med 46: 284–296.
2. Sawada H, *et al* (1987) Adult health study report 6. Results of six examination cycle, 1968–1980. RERF Technical Report

3–86.
3. Neriishi K, Nakashima E and Delongchamp RR (2001) Persistent subclinical inflammation among A-bomb survivors. Int J Radiat Biol **77**: 475–482.
4. Tatsukawa Y, *et al* (2008) White blood cell count, especially neutrophil count, as a predictor of hypertension in a Japanese population. Hypertens Res **31**: 1391–1397.
5. Shankar A, Klein BE and Klein R (2004) Relationship between white blood cell count and incident hypertension. Am J Hypertens **17**: 233–239.
6. Brown DW, *et al* (2004) Associations between white blood cell count and risk for cerebrovascular disease mortality: NHANES II Mortality Study, 1976–1992. Ann Epidemiol **14**: 425–430.
7. Lee D, *et al* (2001) White blood cell count and incidence of coronary heart disease and ischemic stroke and mortality from cardiovascular disease in African-American and white men and women. Am J Epidemiol **154**: 758–764.
8. Margolis KL, *et al* (2005) Leukocyte count as a predictor of cardiovascular events and mortality in postmenopausal women: the Women's Health Initiative Observational Study. Arch Intern Med **165**: 500–508.
9. Zalokar JB, Richard JL and Claude JR (1981) Leukocyte count, smoking, and myocardial infarction. N Engl J Med **304**: 465–468.
10. Jee SH, *et al* (2005) White blood cell count and risk for all-cause, cardiovascular, and cancer mortality in a cohort of Koreans. Am J Epidemiol **162**: 1062–1069.
11. Erlinger TP, Muntner P and Helzlsouer KJ (2004) WBC count and the risk of cancer mortality in a national sample of U.S. adults: Results from the Second National Health and Nutrition Examination Survey Mortality Study. Cancer Epidemiol Biomarkers Prev **13**: 1052–1056.
12. Macarthur M, Hold GL and El-Omar EM (2004) Inflammation and cancer II. Role of chronic inflammation and cytokine gene polymorphisms in the pathogenesis of gastrointestinal malignancy. Am J Physiol Gastrointest Liver Physiol **286**: G515–G520.
13. Shankar A, *et al* (2006) Association between circulating white blood cell count and cancer mortality: a population-base cohort study. Arch Intern Med **166**: 188–194.
14. Corre F, Leilouch J and Schwartz D (1971) Smoking and leukocyte-counts: results of an epidemiological survey. *Lancet* **2**: 632–634.
15. Sunyer J, *et al* (1996) Longitudinal relation between smoking and white blood cells. Am J Epidemiol **144**: 734–741.
16. Abel GA, *et al* (2005) Effects of biochemically confirmed smoking cessation on white blood cell count. Mayo Clin Proc **80**: 1022–1028.
17. Bakhru A and Erlinger TP (2005) Smoking cessation and cardiovascular disease risk factors: results from the third National Health and Nutrition Examination Survey. PLoS Med **2**: 528–536.
18. Smith MR, *et al* (2003) Smoking status and differential white cell count in men and women in the EPIC-Norfolk population. Atherosclerosis **169**: 331–337.
19. Kawada T (2004) Smoking-induced leukocytosis can persist after cessation of smoking. Arch Med Res **35**: 246–250.
20. Saito I, Yonemasu K and Inami F (2003) Association of body mass index, body fat, and weight gain with inflammation markers among rural residents in Japan. Circ J **67**: 323–329.
21. Nagasawa N, *et al* (2004) Association of white blood cell count and clustered components of metabolic syndrome in Japanese men. Circ J **68**: 892–897.
22. Ford ES (2003) The metabolic syndrome and C-reactive protein, fibrinogen, and leukocyte count: findings from the Third National Health and Nutrition Examination Survey. Atherosclerosis **168**: 351–358.
23. Yamada M, *et al* (2004) Noncancer disease incidence in atomic bomb survivors, 1958–1998. Radiat Res **161**: 622–632.
24. International Statistical Classification of Diseases and related Health Problems (ICD-10) (1992) World Health Organization, Geneva.
25. Cullings HM, *et al* (2006) Dose estimation for atomic bomb survivor studies: its evolution and present status. Radiat Res **166**: 219–254.
26. Laird NM and Ware JH (1982) Random-effects models for longitudinal data. Biometrics **38**: 963–974.
27. Singer DJ (1998) Using SAS PROC MIXED to fit multilevel models, hierarchical models, and individual growth models. J of Edu and Behav Stat **24**: 323–355.
28. Hayashi T, *et al* (2003) Radiation dose-dependent increases in inflammatory response markers in A-bomb survivors. Int J Radiat Biol **79**: 129–136.
29. Hayashi T, *et al* (2005) Long-term effects of radiation dose on inflammatory markers in atomic bomb survivors. Am J Med **118**: 83–86.
30. Ruggiero C, *et al* (2007) White blood cell count and mortality in the Baltimore Longitudinal Study of Aging. J Am Coll Cardiol **49**: 1841–1850.

Revision received on February 19, 2010
Accepted on March 20, 2010
J-STAGE Advance Publication Date: June 11, 2010

Original Article

Association between Non-High-Density Lipoprotein Cholesterol Concentrations and Mortality from Coronary Heart Disease Among Japanese Men and Women: The Ibaraki Prefectural Health Study

Hiroyuki Noda[1,2], Hiroyasu Iso[1], Fujiko Irie[3], Toshimi Sairenchi[4,5], Emiko Ohtaka[5], and Hitoshi Ohta[5]

[1]Public Health, Department of Social and Environmental Medicine, Graduate School of Medicine, Osaka University, Osaka, Japan
[2]Harvard Center for Population and Development Studies, Harvard School of Public Health, Cambridge, USA
[3]Department of Health and Social Services, Ibaraki Prefectural Office, Ibaraki, Japan
[4]Department of Public Health, Dokkyo Medical University School of Medicine, Tochigi, Japan
[5]Ibaraki Prefectural Health Plaza, Ibaraki Health Service Association, Ibaraki, Japan

Aim: The aim of this study was to examine whether non-high-density lipoprotein cholesterol (non-HDL-cholesterol) raises the risk of coronary heart disease in a dose-response fashion in a non-obese population with low total cholesterol levels and high HDL-cholesterol levels, such as Japanese.
Methods: A total of 30,802 men and 60,417 women, aged 40 to 79 years with no history of stroke or coronary heart disease, completed a baseline risk factor survey in 1993 under the auspices of the Ibaraki Prefectural Health Study. Systematic mortality surveillance through 2003 identified 539 coronary heart disease deaths.
Results: The mean values for non-HDL-cholesterol were 140 mg/dL for men and 151 mg/dL for women. The corresponding mean values were 193 mg/dL and 208 mg/dL total cholesterol and 52 mg/dL and 57 mg/dL HDL-cholesterol, respectively. Men with non-HDL-cholesterol ≥180 mg/dL had a two-fold higher age-adjusted risk of mortality from coronary heart disease than did those with non-HDL-cholesterol <100 mg/dL, whereas no such association was found for women. The multivariable hazard ratio for ≥180 mg/dL versus <100 mg/dL of non-HDL-cholesterol was 2.22 (95% confidence interval: 1.37 to 3.62) for men and 0.71 (0.37 to 1.34) for women.
Conclusion: Higher concentrations of non-HDL-cholesterol were associated with an increased risk of mortality from coronary heart disease for men, but not for women.

J Atheroscler Thromb, 2010; 17:30-36.

Key words; Non-high-density lipoprotein cholesterol, Coronary heart disease, Follow-up studies

Introduction

Non-high-density lipoprotein cholesterol (non-HDL-cholesterol) is one of the major atherogenic lipoproteins and has been identified by the National Cholesterol Educational Program (NCEP) Expert Panel as one of the secondary targets for the prevention of coronary heart disease[1,2].

Address for correspondence: Hiroyasu Iso, Public Health, Department of Social and Environmental Medicine, Osaka University, Graduate school of Medicine, 2-2 Yamadaoka, Shuita-shi, Osaka 565-0871 Japan
E-mail: iso@pbhel.med.osaka-u.ac.jp
Received: November 4, 2008
Accepted for publication: July 2, 2009

Previous studies[3-10] showed that high concentrations of non-HDL-cholesterol were associated with an increased risk of coronary heart disease, mainly in obese populations with higher concentrations of total cholesterol and lower concentrations of HDL-cholesterol. However, little evidence is available for less obese populations with lower concentrations of total cholesterol and higher concentrations of HDL-cholesterol.

Japanese populations are less obese[11] and have lower concentrations of total cholesterol[12] and higher concentrations of HDL-cholesterol[13] than populations in western countries. Thus, their characteristics may be suitable to examine the association of non-HDL-cholesterol with the risk of coronary heart disease within lower ranges of non-HDL-cholesterol. A

recent prospective cohort study of 4,694 urban Japanese observed a positive association between non-HDL-cholesterol and the risk of myocardial infarction[10]; however, it showed a non-linear association for men and no association for women, possibly due to the small number of myocardial infarction cases.

To examine whether non-HDL-cholesterol raises the risk of coronary heart disease mortality in current Japanese rural populations, we conducted a large cohort study of approximately 91,000 Japanese men and women.

Methods

Study Cohort and Population

In 1993, the Ibaraki Prefectural government initiated a community-based cohort study, known as the Ibaraki Prefectural Health Study, to obtain information on health status for the purpose of health education and policy making[14]. The participants in the cohort were 98,196 individuals (33,414 men and 64,782 women) aged 40-79 years, living in Ibaraki Prefecture, who underwent an annual health check-up in 1993, which included the examination of blood lipids for 96,610 individuals (32,984 men and 63,626 women).

We excluded 5,391 individuals (2,182 men and 3,209 women) from our analysis because of a previous history of stroke and coronary heart disease at the time of baseline inquiry. Thus, 91,219 individuals (30,802 men and 60,417 women) were enrolled in the study presented here.

Informed consent was obtained from the community representatives to conduct an epidemiological study based on the guidelines of the Council for International Organizations of Medical Science[15]. The Ethics Committee of Ibaraki Prefecture approved this study.

Measurement of Risk Factors

Serum total cholesterol and triglycerides were measured with enzymatic methods using an RX-30 device (Nihon Denshi, Tokyo, Japan) and high-density lipoprotein cholesterol (HDL-cholesterol) levels were measured with phosphotungstic acid-magnesium methods using an MTP-32 (Corona Electric, Ibaraki, Japan). These measurements were performed on the premises of the Ibaraki Health Service Association, and were standardized by the Osaka Medical Center for Health Science and Promotion under the aegis of the US National Cholesterol Reference Method Laboratory Network (CRMLN). The laboratory of the Osaka Medical Center for Health Science and Promotion has been standardized since 1975 by the CDC-NHLBI Lipid Standardization Program provided by the Center for Disease Control and Prevention (Atlanta, GA) and has met all the criteria for both precision and accuracy of lipid measurements[16]. Non-HDL-cholesterol was calculated as follows: non-HDL-cholesterol (mg/dL) = total cholesterol (mg/dL) − HDL-cholesterol (mg/dL).

Mild hypertension was defined as systolic blood pressure 140-159 mmHg or diastolic blood pressure 90-99 mmHg, and the corresponding values were 160-179 mmHg or 100-109 mmHg for moderate hypertension and ≥180 mmHg or ≥110 mmHg for severe hypertension. Diabetes was defined as a plasma glucose level of ≥126 mg/dL (≥7.0 mmol/L) during fasting or ≥200 mg/dL (≥11.1 mmol/L) during non-fasting, or the use of medication for diabetes, and impaired glucose tolerance was defined as a plasma glucose level of 110-125 mg/dL (6.1-6.9 mmol/L) at fasting or 140-199 mg/dL (7.8-11.0 mmol/L) at non-fasting, and no use of medication for diabetes. Kidney dysfunction was defined as a serum creatinine level of ≥1.2 mg/dL (≥110 μmol/L) for men or ≥1.0 mg/dL (≥90 μmol/L) for women, and/or a history of kidney disease. Height in stocking feet and weight in light clothing were measured, and body mass index (BMI) was calculated as weight (kg)/height (m)^2. An interview was conducted to ascertain smoking status, the number of cigarettes smoked per day, usual weekly intake of alcohol in go units (a Japanese traditional unit of alcohol intake converted to grams of ethanol per day at 23 g ethanol per go unit), and histories of stroke and heart disease. Current drinkers were defined as occasional and habitual drinkers.

Follow-Up Surveillance

To ascertain deaths in the cohort, the investigators conducted a systematic review of death certificates, which in Japan are all forwarded to the local public health center of every community. It is believed that all deaths that occurred in the cohort were ascertained, except for subjects who died after they had moved from their original community, in which case the subject was treated as a censored case. Mortality data are centralized at the Ministry of Health and Welfare, where the underlying causes of death are coded for National Vital Statistics according to the International Classification of Disease, 9th (1993-1994) and 10th (1995-2004) revisions (410-414 for International Classification of Disease, 9th revision, and code I20 to I25 for 10th revision).

The follow-up inquiry for this study was conducted until the end of 2003, and the median fol-

low-up was 10.3 years. Only 3.2% of the subjects had moved out of their respective communities, and were treated as censored.

Statistical Analysis

Statistical analysis was based on mortality rates from coronary heart disease divided by clinical categories of non-HDL-cholesterol (<100, 100–120, 120–139, 140–159, 160–179, ≥180 mg/dL: <2.59, 2.59–3.09, 3.10–3.61, 3.62–4.13, 4.14–4.64, and ≥4.65 mmol/L). Person-years of follow-up were calculated from the date of the baseline survey to the date of death, exit from the community, or the end of 2003, whichever occurred first.

Sex-specific age-adjusted means and proportions of selected cardiovascular risk factors at baseline were determined in terms of the non-HDL-cholesterols categories. The t-test or chi-square test was used to examine differences in age-adjusted mean values and proportions of baseline characteristics from those of the lowest non-HDL-cholesterol category. Age-adjusted and multivariable hazard ratios (HR) and 95% confidence intervals (95%CI) were calculated with the Cox proportional hazards model after adjustment for age and potential confounding factors. These potential confounding factors included body mass index (sex-specific quintiles), blood pressure categories (normal, mild hypertension, moderate hypertension, or severe hypertension), anti-hypertensive medication use (yes or no) diabetes status (normal, impaired glucose tolerance, or diabetes), gamma-glutamyl transferase (sex-specific quintiles), kidney dysfunction (yes or no), smoking status (never, ex-smoker, or current smokers of 1 to 19 or ≥20 cigarettes per day), alcohol intake category (never, ex-drinkers, occasional drinkers and habitual drinkers consuming <69 g/day or ≥69 g/day of ethanol, respectively), HDL-cholesterol (<40, 40–49, 50–59, 60–69, ≥70 mg/dL or <1.03, 1.03–1.28, 1.29–1.54, 1.55–1.80, ≥1.81 mmol/L) and triglycerides (<100, 100–149, 150–199, 200–249, 250–299, ≥300 mg/dL or <1.12, 1.12–1.68, 1.69–2.24, 2.25–2.81, 2.82–3.37, ≥3.38 mmol/L). We tested the assumption of proportional hazards for non-HDL-cholesterol categories[17] and found no violation of proportionality. Tests for effect modification by sex or other variables were conducted with an interaction term generated by multiplying the continuous variables of non-HDL-cholesterol by sex or other variables. We conducted an additional analysis after the exclusion of individvals with hypertriglyceridemia (triglycerides ≥802 mg/dL) at baseline survey (55 men and 23 women), and those using lipid-lowering medication at the baseline survey (370 men and 1,903 women). We also analyzed the data of the sub-population with fasting serum samples (within 8 hours after last meal) at the baseline survey (5,375 men and 10,268 women).

We further analyzed the Cox proportional hazard model with time-dependent covariates, using the additional data of non-HDL-cholesterol and confounding factors for 80,578 persons (88.3% of the participants) whose blood lipids had been examined more than twice. The median duration between the date of the latest examination and the date of the end of follow-up was 0.7 years.

All statistical tests were two-sided. SAS, version 9.13 (SAS Institute, Inc., Cary, NC, USA) was used for all statistical analyses.

Results

A total of 91,219 persons (30,802 men and 60,417 women) were followed up for a median of 10.3 years, during which time 295 men and 244 women died from coronary heart disease. The mean (standard deviation: SD) non-HDL-cholesterol level was 140.4 (35.3) mg/dL for men and 150.8 (35.6) mg/dL for women. The corresponding mean (SD) was 192.5 (33.9) mg/dL and 207.6 (34.8) mg/dL in total cholesterol and 52.3 (14.9) mg/dL and 56.7 (14.0) mg/dL in HDL-cholesterol. The prevalence of obesity (BMI ≥30.0 kg/m^2) was 1.7% for men and 3.3% for women.

Table 1 shows selected cardiovascular risk factors by non-HDL-cholesterol concentration category. Compared with men who had the lowest levels of non-HDL-cholesterol (<100 mg/dL: <2.59 mmol/L), those who had the highest levels (≥180 mg/dL: ≥4.65 mnom/L) were younger, more likely to use medication for hypertension and lipid abnormality, and less likely to smoke currently or drink heavily. They also tended to have diabetes and kidney dysfunction, a higher mean of systolic and diastolic blood pressure, body mass index, total cholesterol levels and triglyceride levels, and a lower mean of HDL-cholesterol. Similar associations were observed for women, except for age and gamma-glutamyl transferase levels. Compared with women who had the lowest non-HDL-cholesterol levels, women with the highest levels were older and had higher gamma-glutamyl transferase levels.

Age-adjusted mortality from coronary heart disease was twice as high for the highest as for the lowest non-HDL-cholesterol category for men, while there was no such association for women (**Table 2**). Multivariable HR (95%CI) of coronary heart disease mortality for the highest versus lowest concentrations of

Table 1. Gender-specific age-adjusted mean values or prevalence of cardiovascular risk factors according to non-HDL-cholesterol levels

	Men						Women					
	Non-HDL-cholesterol, mg/dL						Non-HDL-cholesterol, mg/dL					
	<100	100-120	120-139	140-159	160-179	180+	<100	100-120	120-139	140-159	160-179	180+
Range, mmol/L	<2.59	2.59-3.09	3.10-3.61	3.62-4.13	4.14-4.64	4.65+	<2.59	2.59-3.09	3.10-3.61	3.62-4.13	4.14-4.64	4.65+
Number of subjects	3,512	5,307	7,065	6,493	4,399	4,026	3,700	8,126	12,200	13,391	10,674	12,326
Age, year	61.8	61.4†	60.6‡	60.1‡	59.3‡	58.1‡	51.9	54.3‡	56.6‡	58.4‡	59.9‡	60.6‡
Systolic blood pressure, mmHg	135.9	135.5	136.0	136.4	137.3‡	137.6‡	128.4	129.4‡	130.7‡	132.2‡	133.1‡	134.5‡
Diastolic blood pressure, mmHg	80.3	80.1	80.5	81.2‡	81.9‡	82.2‡	74.7	75.8‡	76.9‡	78.0‡	78.8‡	79.7‡
Hypertensive medication use, %	19.3	18.1	19.2	20.4	20.5	21.1†	16.1	17.3	18.2‡	19.5‡	21.1‡	22.0‡
Diabetes, %	7.8	6.2‡	7.1	7.4	8.3	9.4‡	3.2	2.9	3.1	3.9	4.4‡	5.6‡
Body mass index, kg/m^2	21.6	22.3‡	23.1‡	23.8‡	24.2‡	24.6‡	21.9	22.5‡	23.2‡	23.7‡	24.2‡	24.6‡
Gamma-glutamyl transferase, U/L	44.9	35.4‡	34.5‡	36.0‡	37.5‡	43.8	14.9	14.4	15.4	16.4‡	17.9‡	20.5‡
Kidney dysfunction, %	8.8	9.4	11.7‡	13.8‡	15.7‡	18.7‡	7.9	8.0	7.9	8.2	8.9	10.1‡
Current smoker, %	61.0	53.9‡	51.6‡	49.3‡	46.7‡	46.7‡	5.5	4.7	4.6†	4.4‡	4.9	5.6
Heavy drinkers, %	11.6	9.2‡	7.4‡	6.2‡	5.1‡	4.6‡	0.2	0.2	0.1	0.1	0.1	0.1
Lipid medication use, %	0.3	0.3	0.9†	1.1‡	2.0‡	3.0‡	1.2	1.3	1.9†	2.6‡	3.8‡	6.2‡
Total cholesterol, mg/dL	146.8	167.0‡	183.0‡	199.4‡	217.0‡	247.9‡	152.0	171.8‡	188.8‡	206.2‡	223.4‡	254.5‡
HDL-cholesterol, mg/dL	61.3	56.5‡	53.2‡	50.2‡	48.2‡	46.5‡	63.3	61.0‡	58.9‡	56.9‡	54.5‡	52.0‡
Triglycerides, mg/dL	92.2	110.7‡	132.4‡	158.1‡	187.0‡	226.2‡	81.4	95.8‡	113.1‡	131.9‡	152.1‡	184.9‡

Test for difference from the lowest category; †$p<0.05$, ‡$p<0.01$

Table 2. Gender-specific age-adjusted and multivariable hazard ratio (HR) and 95% confidence interval (95%CI) of mortality from coronary heart disease and all-causes according to non-HDL-cholesterol levels

	Non-HDL-cholesterol, mg/dL						HR per 30 mg/dL Increment
	<100	100-120	120-139	140-159	160-179	180+	
Men							
Person-years	32,834	51,236	68,900	63,315	43,171	39,293	298,750
Coronary heart disease							
No	33	38	57	63	44	60	295
Age-adjusted HR	1.0	0.74 (0.46-1.17)	0.86 (0.56-1.31)	1.08 (0.71-1.65)	1.18 (0.75-1.86)	1.93 (1.26-2.95)	1.22 (1.11-1.34)
Multivariable HR*	1.0	0.80 (0.50-1.29)	0.97 (0.62-1.52)	1.24 (0.79-1.95)	1.39 (0.85-2.29)	2.22 (1.37-3.62)	1.23 (1.11-1.37)
All-causes							
No	720	776	884	753	439	397	3,969
Age-adjusted HR	1.0	0.69 (0.62-0.76)	0.61 (0.55-0.67)	0.59 (0.54-0.66)	0.54 (0.48-0.61)	0.58 (0.52-0.66)	0.86 (0.83-0.88)
Multivariable HR*	1.0	0.73 (0.66-0.81)	0.67 (0.60-0.74)	0.66 (0.59-0.73)	0.60 (0.53-0.68)	0.64 (0.56-0.73)	0.88 (0.85-0.91)
Women							
Person-years	36,527	80,408	121,528	133,113	106,090	122,066	599,731
Coronary heart disease							
No	13	22	41	48	48	72	244
Age-adjusted HR	1.0	0.60 (0.30-1.20)	0.63 (0.34-1.17)	0.60 (0.32-1.10)	0.69 (0.37-1.27)	0.87 (0.48-1.57)	1.13 (1.02-1.26)
Multivariable HR*	1.0	0.60 (0.30-1.20)	0.61 (0.32-1.16)	0.55 (0.29-1.04)	0.59 (0.31-1.13)	0.71 (0.37-1.34)	1.07 (0.95-1.21)
All-causes							
No	206	399	615	693	586	676	3,175
Age-adjusted HR	1.0	0.70 (0.59-0.83)	0.61 (0.52-0.71)	0.56 (0.48-0.65)	0.55 (0.47-0.64)	0.53 (0.46-0.62)	0.90 (0.88-0.93)
Multivariable HR*	1.0	0.74 (0.62-0.87)	0.65 (0.55-0.76)	0.60 (0.51-0.71)	0.58 (0.49-0.69)	0.55 (0.47-0.66)	0.91 (0.87-0.94)

*HR (95%CI) adjusted for age and potential confounding factors.
Potential confounding factors: blood pressure categories, anti-hypertensive medication use, diabetes mellitus, lipid medication use, body mass index, gamma-glutamyl transferase, smoking status, alcohol consumptions, kidney dysfunction, and categories of HDL-cholesterol and triglycerides.

Table 3. Multivariable hazard ratio (HR)* and 95% confidence interval (95%CI) of coronary heart disease according to non-HDL-cholesterol levels, stratified by gender and other risk factors

	Non-HDL-cholesterol, mg/dL						HR per 30 mg/dL Increment	*p* for interaction
	<100	100–120	120–139	140–159	160–179	180+		
Men	1.0	0.80 (0.50–1.29)	0.97 (0.62–1.52)	1.24 (0.79–1.95)	1.39 (0.85–2.29)	2.22 (1.37–3.62)	1.23 (1.11–1.37)	
Women	1.0	0.60 (0.30–1.20)	0.61 (0.32–1.16)	0.55 (0.29–1.04)	0.59 (0.31–1.13)	0.71 (0.37–1.34)	1.07 (0.95–1.21)	0.13
Aged 40–59 years	1.0	0.75 (0.23–2.49)	0.77 (0.25–2.39)	1.10 (0.37–3.27)	1.18 (0.37–3.72)	1.37 (0.43–4.36)	1.20 (0.96–1.51)	
Aged 60–79 years	1.0	0.76 (0.51–1.15)	0.88 (0.60–1.30)	0.95 (0.64–1.41)	1.04 (0.69–1.58)	1.41 (0.94–2.12)	1.15 (1.05–1.25)	0.48
Non-hypertensive	1.0	0.41 (0.20–0.83)	0.54 (0.29–1.00)	0.68 (0.36–1.27)	0.81 (0.42–1.56)	0.76 (0.38–1.54)	1.09 (0.93–1.29)	
Hypertensive§	1.0	1.02 (0.63–1.64)	1.11 (0.70–1.75)	1.18 (0.74–1.87)	1.26 (0.78–2.05)	1.86 (1.16–3.00)	1.18 (1.08–1.30)	0.28
Normal glucose	1.0	0.73 (0.47–1.13)	0.82 (0.54–1.23)	0.85 (0.56–1.28)	1.00 (0.65–1.53)	1.20 (0.79–1.84)	1.13 (1.03–1.24)	
Impaired glucose tolerance/Diabetic	1.0	0.86 (0.37–1.96)	1.04 (0.49–2.20)	1.30 (0.62–2.70)	1.28 (0.60–2.75)	2.03 (0.98–4.21)	1.20 (1.05–1.37)	0.04
Gamma-glutamyl transferase <45 U/L	1.0	0.73 (0.47–1.13)	0.82 (0.54–1.23)	0.85 (0.56–1.28)	1.00 (0.65–1.53)	1.20 (0.79–1.84)	1.22 (1.12–1.32)	
Gamma-glutamyl transferase ≥45 U/L	1.0	0.86 (0.37–1.96)	1.04 (0.49–2.20)	1.30 (0.62–2.70)	1.28 (0.60–2.75)	2.03 (0.98–4.21)	0.94 (0.75–1.17)	0.01
Non-smoker	1.0	0.80 (0.45–1.42)	0.94 (0.55–1.59)	1.03 (0.61–1.76)	1.10 (0.63–1.90)	1.51 (0.87–2.59)	1.19 (1.07–1.32)	
Current smoker	1.0	0.73 (0.43–1.25)	0.81 (0.49–1.35)	0.91 (0.54–1.55)	1.09 (0.62–1.93)	1.39 (0.78–2.48)	1.11 (0.97–1.27)	0.92
Non-drinker	1.0	0.66 (0.38–1.13)	0.78 (0.48–1.28)	0.73 (0.44–1.21)	0.87 (0.52–1.45)	1.21 (0.73–2.02)	1.16 (1.05–1.28)	
Current drinker	1.0	0.83 (0.47–1.47)	0.83 (0.47–1.46)	1.13 (0.64–2.01)	1.28 (0.68–2.40)	1.65 (0.87–3.12)	1.16 (1.00–1.34)	0.50
BMI <23.3 kg/m²¶	1.0	0.71 (0.45–1.11)	0.79 (0.51–1.22)	0.95 (0.61–1.48)	1.12 (0.70–1.80)	1.23 (0.75–2.02)	1.08 (0.96–1.21)	
BMI ≥23.3 kg/m²¶	1.0	1.14 (0.48–2.71)	1.36 (0.61–3.04)	1.33 (0.59–2.98)	1.35 (0.59–3.08)	2.14 (0.95–4.82)	1.24 (1.11–1.39)	0.27
HDL-cholesterol ≥54 mg/dL¶	1.0	0.72 (0.43–1.23)	0.75 (0.45–1.25)	1.12 (0.68–1.84)	1.13 (0.65–1.97)	1.27 (0.72–2.24)	1.11 (0.97–1.26)	
HDL-cholesterol <54 mg/dL¶	1.0	0.78 (0.44–1.39)	0.96 (0.56–1.63)	0.86 (0.50–1.48)	1.02 (0.58–1.78)	1.49 (0.86–2.58)	1.19 (1.07–1.31)	0.94
Triglycerides <118 mg/dL¶	1.0	0.70 (0.46–1.08)	0.68 (0.44–1.03)	0.85 (0.55–1.32)	1.01 (0.62–1.66)	1.24 (0.73–2.11)	1.08 (0.95–1.23)	
Triglycerides ≥118 mg/dL¶	1.0	0.86 (0.33–2.24)	1.35 (0.57–3.19)	1.33 (0.57–3.12)	1.44 (0.61–3.40)	2.04 (0.88–4.76)	1.23 (1.11–1.36)	0.36
Fasting (≥8 hours after last meal)	1.0	0.79 (0.22–2.83)	0.98 (0.31–3.08)	1.37 (0.44–4.25)	1.22 (0.37–4.03)	1.69 (0.52–5.52)	1.15 (0.91–1.44)	
Non-fasting (<8 hours after last meal)	1.0	0.76 (0.51–1.15)	0.87 (0.59–1.28)	0.94 (0.63–1.39)	1.07 (0.70–1.61)	1.43 (0.95–2.16)	1.17 (1.07–1.27)	0.33

*HR (95%CI) adjusted for gender, age and potential confounding factors.
§Hypertensive was defined as systolic blood pressure ≥140 and/or diastolic blood pressure ≥90 and/or as use of medication for hypertension.
¶Median value was used as the cut-off point.

non-HDL-cholesterol was 2.22 (1.37–3.62), $p=0.001$ for men and 0.71 (0.37–1.34), $p=0.29$ for women. The corresponding multivariable HR (95%CI) associated with a 30 mg/dL increment in non-HDL-cholesterol was 1.23 (1.11–1.37), $p=0.0001$, and 1.07 (0.95–1.21), $p=0.28$ (p for interaction=0.13). Adjustment for potential confounding factors did not alter these associations materially.

The HR (95%CI) of coronary heart disease mortality for the highest versus lowest non-HDL-cholesterol levels was 2.24 (1.37–3.64), $p=0.001$ for men and 0.71 (0.37–1.34), $p=0.29$ for women, after excluding individuals with hypertriglyceridemia, 2.34 (1.43–3.80), $p=0.0007$ for men and 0.66 (0.35–1.26), $p=0.21$ for women after excluding individuals using lipid-lowering medication, and 1.82 (1.12–2.97), $p=0.02$ for men and 0.94 (0.50–1.77), $p=0.85$ for women when we used the Cox proportional hazard model with time-dependent covariates.

On the other hand, higher levels of non-HDL-cholesterol were associated with a reduced risk of all-cause mortality for both men and women (**Table 2**). Multivariable HR (95%CI) of all-cause mortality for the highest versus lowest non-HDL-cholesterol levels was 0.64 (0.56–0.73), $p<0.0001$ for men, and 0.55 (0.47–0.66), $p<0.0001$ for women.

We observed no interaction of the fasting/non-fasting status in the association between non-HDL-cholesterol and mortality from coronary heart disease (**Table 3**). When we stratified the data on the sub-population by fasting status, the associations did not differ substantially. No statistically significant interaction of the association between non-HDL-cholesterol and mortality from coronary heart disease was observed for other potential risk factors ($p>0.20$) except for gender difference, diabetes and gamma-glutamyl transferase (**Table 3**).

Discussion

In the large population-based prospective cohort study of Japanese reported here, we observed, in a less obese population, significant positive associations of high non-HDL-cholesterol with an increased risk of mortality from coronary heart disease for men, but not for women. These associations did not alter substantially after adjusting for potential confounding factors, and after excluding non-fasting subjects or those with hypertriglyceridemia, or using of time-dependent covariates.

For this study population, the mean non-HDL-cholesterol level was 140 mg/dL for men and 151 mg/dL for women at baseline. Previous studies involving participants with higher non-HDL-cholesterol levels: for example, the Framingham Heart study (mean non-HDL-cholesterol at baseline: 168 mg/dL for men and 160 mg/dL for women)[3], the Health Professionals follow-up Study (158 mg/dL for men)[4], Women's Health Study (155 mg/dL for women)[5, 6], Lipid Research Clinical Program Follow-up Study (181 mg/dL for men and 172 mg/dL for women)[7], the Chin-Shan Community Cardiovascular Cohort study (150 mg/dL)[8], and a recent meta-analysis (170 mg/dL)[9] showed the relationship between higher concentrations of non-HDL-cholesterol and an increased risk of coronary heart disease. Our findings thus extend the previous evidence to the lower ranges of non-HDL-cholesterol.

We observed a statistically significant association of non-HDL-cholesterol with mortality from coronary heart disease among men, but not women. The possible mechanisms of the gender difference interaction are as follows. First, men often have more atherosclerotic risk factors than women, such as higher blood pressure, higher glucose levels and higher smoking prevalence[18], which may accelerate the atherogenic effect of non-HDL-cholesterol. Second, there may be a gender difference in the cumulative burden from non-HDL-cholesterol during atherosclerosis development due to lag time to an increase in non-HDL-cholesterol levels over the lifespan. Pre-menopausal women have lower total cholesterol levels than men of the same age group[19], which may result in a lower cumulative burden of atherosclerosis development for women than for men.

A limitation of the current study is that we used fasting and non-fasting serums for lipid measurements; however, there was no change in the association between non-HDL-cholesterol and coronary heart disease after stratification of the fasting status. Second, we used mortality data based on death certificate diagnoses, not the incidence data; however, three-fourths of death certificate diagnoses of coronary heart disease have been found to be correctly diagnosed[20, 21].

The strength of the present study is that we used lipid measurement values standardized in a single laboratory, which in turn was standardized by the CDC-NHLBI Lipid Standardized Program[16]. This justifies our assumption that misclassification bias due to errors in lipid measurement had been adequately reduced, and that the resultant accuracy lipid measurements were comparable with the results of previous well-standardized studies.

In conclusion, our large cohort study provides epidemiological evidence that, in a less obese population, higher concentrations of non-HDL-cholesterol are associated with an increased risk of coronary heart disease mortality for men, but not for women.

Acknowledgements

This research was supported by a Grant-in-Aid from the Ministry of Health, Labor and Welfare, Health and Labor Sciences Research Grants, Japan (Comprehensive Research on Cardiovascular and Life-Style Related Diseases: H20-Junkankitou[Seishuu]-Ippan-013). Hiroyuki Noda was also supported by the post-doctoral fellowship program of the Uehara Memorial Foundation (Tokyo, Japan).

References

1) National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III): Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation, 2002; 106: 3143-3421

2) Grundy SM, Cleeman JI, Merz CN, Brewer HB Jr, Clark LT, Hunninghake DB, Pasternak RC, Smith SC Jr, Stone NJ; Coordinating Committee of the National Cholesterol Education Program: Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III guidelines. Arterioscler Thromb Vasc Biol, 2004; 24: e149-161

3) Liu J, Sempos CT, Donahue RP, Dorn J, Trevisan M, Grundy SM: Non-high-density lipoprotein and very-low-density lipoprotein cholesterol and their risk predictive values in coronary heart disease. Am J Cardiol, 2006; 98: 1363-1368

4) Pischon T, Girman CJ, Sacks FM, Rifai N, Stampfer MJ, Rimm EB: Non-high-density lipoprotein cholesterol and apolipoprotein B in the prediction of coronary heart disease in men. Circulation, 2005; 112: 3375-3383

5) Ridker PM, Rifai N, Cook NR, Bradwin G, Buring JE.

Non-HDL cholesterol, apolipoproteins A-I and B100, standard lipid measures, lipid ratios, and CRP as risk factors for cardiovascular disease in women. JAMA, 2005; 294: 326-333
6) Everett BM, Kurth T, Buring JE, Ridker PM: The relative strength of C-reactive protein and lipid levels as determinants of ischemic stroke compared with coronary heart disease in women. J Am Coll Cardiol, 2006; 48: 2235-2242
7) Cui Y, Blumenthal RS, Flaws JA, Whiteman MK, Langenberg P, Bachorik PS, Bush TL. Non-high-density lipoprotein cholesterol level as a predictor of cardiovascular disease mortality. Arch Intern Med, 2001; 161: 1413-1419
8) Chien KL, Hsu HC, Su TC, Chen MF, Lee YT, Hu FB: Apolipoprotein B and non-high density lipoprotein cholesterol and the risk of coronary heart disease in Chinese. J Lipid Res, 2007; 48: 2499-2505
9) Prospective Studies Collaboration. Blood cholesterol and vascular mortality by age, sex and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55 000 vascular deaths. Lancet, 2007; 370: 1829-1839
10) Okamura T, Kokubo Y, Watanabe M, Higashiyama A, Miyamoto Y, Yoshimasa Y, Okayama A: Low-density lipoprotein cholesterol and non-high-density lipoprotein cholesterol and the incidence of cardiovascular disease in an urban Japanese cohort study: The Suita study. Atherosclerosis, 2009; 203: 587-592
11) Kromhout D, Bloemberg B, Seidell JC, Nissinen A, Menotti A: Physical activity and dietary fiber determine population body fat levels: the Seven Countries Study. Int J Obes Relat Metab Disord, 2001; 25: 301-306
12) Verschuren WM, Jacobs DR, Bloemberg BP, Kromhout D, Menotti A, Aravanis C, Blackburn H, Buzina R, Dontas AS, Fidanza F, Karvonen MJ, Nedeljković S, Nissinen A, Toshima H: Serum total cholesterol and long-term coronary heart disease mortality in different cultures. Twenty-five-year follow-up of the seven countries study. JAMA, 1995; 274: 131-136
13) Ueshima H, Iida M, Shimamoto T, Konishi M, Tanigaki M, Nakanishi N, Takayama Y, Ozawa H, Kojima S, Komachi Y: High-density lipoprotein-cholesterol levels in Japan. JAMA, 1982; 247: 1985-1987
14) Irie F, Iso H, Sairenchi T, Fukasawa N, Yamagishi K, Ikehara S, Kanashiki M, Saito Y, Ota H, Nose T: The relationships of proteinuria, serum creatinine, glomerular filtration rate with cardiovascular disease mortality in Japanese general population. Kidney Int, 2006; 69: 1264-1271
15) International guidelines for ethical review of epidemiological studies. Law Med Health Care, 1991; 19: 247-258
16) Nakamura M, Sato S, Shimamoto T: Improvement in Japanese clinical laboratory measurements of total cholesterol and HDL-cholesterol by the US Cholesterol Reference Method Laboratory Network. J Atheroscler Thromb, 2003; 10: 145-153
17) Ng'andu NH: An empirical comparison of statistical tests for assessing the proportional hazards assumption of Cox's model. Stat Med, 1997; 16: 611-626
18) Okumiya N, Tanaka K, Ueda K, Omae T: Coronary atherosclerosis and antecedent risk factors: pathologic and epidemiologic study in Hisayama, Japan. Am J Cardiol, 1985; 56: 62-66
19) Kannel WB: Metabolic risk factors for coronary heart disease in women: perspective from the Framingham Study. Am Heart J, 1987; 114: 413-419
20) Yamashita T, Ozawa H, Aono H, Hosokawa H, Saito I, Ikebe T: Heart disease deaths on death certificates re-evaluated by clinical records in a Japanese city. Jpn Circ J, 1997; 61: 331-338
21) Baba S, Ozawa H, Sakai Y, Terao A, Konishi M, Tatara K: Heart disease deaths in a Japanese urban area evaluated by clinical and police records. Circulation, 1994; 89: 109-115

Original Article | Journal of INTERNAL MEDICINE

doi: 10.1111/j.1365-2796.2009.02183.x

Gender difference of association between LDL cholesterol concentrations and mortality from coronary heart disease amongst Japanese: the Ibaraki Prefectural Health Study

■ H. Noda[1,2], H. Iso[1], F. Irie[3], T. Sairenchi[4,5], E. Ohtaka[5] & H. Ohta[5]

From the [1]Public Health, Department of Social and Environmental Medicine, Graduate School of Medicine, Osaka University, Osaka, Japan, [2]Harvard Center for Population and Development Studies, Harvard School of Public Health, Cambridge, MA, USA, [3]Department of Health and Social Services, Ibaraki Prefectural Office, Ibaraki; [4]Department of Public Health, Dokkyo Medical University School of Medicine, Tochigi; and [5]Ibaraki Prefectural Health Plaza, Ibaraki Health Service Association, Ibaraki, Japan

Abstract. Noda H, Iso H, Irie F, Sairenchi T, Ohtaka E, Ohta H (Osaka University, Osaka, Japan; Harvard School of Public Health, Cambridge, MA, USA; Ibaraki Prefectural Office, Ibaraki; Dokkyo Medical University School of Medicine, Tochigi; Ibaraki Health Service Association, Ibaraki; Japan). Gender difference of association between LDL cholesterol concentrations and mortality from coronary heart disease amongst Japanese: the Ibaraki Prefectural Health Study. *J Intern Med* 2010; **267**: 576–587.

Objective. The aim of this study was to examine whether LDL cholesterol raises the risk of coronary heart disease in a dose–response fashion in a population with low LDL-cholesterol levels.

Design. Population-based prospective cohort study in Japan.

Subjects and main outcome measures. A total of 30 802 men and 60 417 women, aged 40 to 79 years with no history of stroke or coronary heart disease, completed a baseline risk factor survey in 1993. Systematic mortality surveillance was performed through 2003 and 539 coronary heart disease deaths were identified.

Results. The mean values for LDL-cholesterol were 110.5 mg dL^{-1} (2.86 mmol L^{-1}) for men and 123.9 mg dL^{-1} (3.20 mmol L^{-1}) for women. Men with LDL-cholesterol ≥140 mg dL^{-1} (≥3.62 mmol L^{-1}) had two-fold higher age-adjusted risk of mortality from coronary heart disease than did those with LDL-cholesterol <80 mg dL^{-1} (<2.06 mmol L^{-1}), whereas no such association for women was found. The multivariable hazard ratio for the highest versus lowest categories of LDL-cholesterol was 2.06 (95 percent confidence interval: 1.34 to 3.17) for men and 1.16 (0.64 to 2.12) for women.

Conclusion. Higher concentrations of LDL-cholesterol were associated with an increased risk of mortality from coronary heart disease for men, but not for women, in a low cholesterol population.

Keywords: coronary heart disease, gender, LDL.

Introduction

Low-density lipoprotein cholesterol (LDL-cholesterol) is one of the major atherogenic lipoproteins and has been identified by the National Cholesterol Educational Program (NCEP) Expert Panel as a primary target for prevention of coronary heart disease [1, 2].

Previous studies [3–6] showed that high concentrations of LDL-cholesterol were associated with increased risk of coronary heart disease mainly for obese populations with higher concentrations of LDL-cholesterol, whereas little evidence is available for less obese populations with lower concentrations of LDL-cholesterol. It therefore remains unclear whether a similar association as for obese populations is also observed at lower ranges of LDL-cholesterol levels.

As the metabolism of obese populations is affected by different environmental factors than those affecting less obese population, it is of major importance to examine the effect of LDL-cholesterol on the risk of coronary heart disease for populations with its lower ranges. First, it is difficult to examine the threshold values in the lower ranges of LDL-cholesterol amongst obese populations,

because of their higher concentrations of LDL-cholesterol. Seven countries study confirmed the positive association between total cholesterol and mortality from coronary heart disease for high cholesterol populations, including Americans, but not for Japanese, who had the lowest population mean levels of total cholesterol levels [7]. Previous studies [3–6] of participants with a higher mean level of LDL-cholesterol could not examine the effect of LDL-cholesterol amongst individuals in the lower LDL-cholesterol ranges. Thus, the report of Adults Treatment Panel III (ATP III) could not make any recommendations for further reduction of LDL-cholesterol for populations with low mean LDL-cholesterol levels [2].

Secondly, obese populations were found to be more likely to show a mixture of multiple metabolic abnormalities [8], which may lead to high LDL-cholesterol levels, and thus make it more likely for such populations to be at high risk. In fact, a previous study showed that almost all persons (>95%) enrolled in the Third National Health and Nutrition Examination Survey (NHANES III) had border line or higher levels of coronary risk factors [9].

To examine whether LDL-cholesterol raises the risk of coronary heart disease for a less obese population with low LDL-cholesterol levels, we conducted a population-based cohort study of Japanese men and women, who had lower means of total cholesterol and body mass index in comparison with Western populations [7, 10].

Materials and methods

Study cohort and population

In 1993, the Ibaraki Prefectural government initiated a community-based cohort study, known as the Ibaraki Prefectural Health Study, to obtain information on health status for the purpose of health education and policy making [11–13]. The participants in the cohort were 98 196 individuals (33 414 men and 64 782 women) aged 40–79 years, living in Ibaraki Prefecture, who underwent an annual health check-up in 1993, which included the examination of blood lipids for 96 610 individuals (32 984 men and 63 626 women).

We excluded 5391 persons (2182 men and 3209 women) from our analysis because of a previous history of stroke and coronary heart disease at the time of baseline inquiry. Thus, a total of 91 219 individuals (30 802 men and 60 417 women) were enrolled in the study presented here.

Informed consent was obtained from the community representatives for conducting an epidemiological study based on guidelines of the Council for International Organizations of Medical Science [14]. The Ethics Committee of Ibaraki Prefecture approved this study.

Measurement of risk factors

Serum total cholesterol and triglycerides were measured with enzymatic methods using an RX-30 device (Nihon Denshi, Tokyo, Japan) and HDL cholesterol levels were measured with phosphotungstic acid-magnesium methods using an MTP-32 (Corona Electric, Ibaraki, Japan). These measurements were performed on the premises of the Ibaraki Health Service Association, and were standardized by the Osaka Medical Center for Health Science and Promotion under the aegis of the US National Cholesterol Reference Method Laboratory Network (CRMLN). The laboratory of the Osaka Medical Center for Health Science and Promotion has been standardized since 1975 by the CDC-NHLBI Lipid Standardization Program provided by the Center for Disease Control and Prevention (Atlanta, GA) and has met all the criteria for both precision and accuracy of lipid measurements [15]. LDL-cholesterol was calculated using the Friedewald formula as follows: LDL-cholesterol (mg dL^{-1}) = total cholesterol (mg dL^{-1})–HDL-cholesterol (mg dL^{-1})–0.2*triglycerides (mg dL^{-1}) [16]. A previous study showed no bias related to LDL-cholesterol levels amongst persons with <802 mg dL^{-1} (<8.8 mmol L^{-1}) of triglycerides in fasting blood samples [17]. As 83% of subjects were nonfasting, we compared LDL-cholesterol measured by direct method as golden standard and values estimated from the Friedewald formula amongst serum samples from 15 743 men and 13 143 women aged 40–79 years who participated in health check-ups by Osaka Medical Center for Health Science and Promotion [15]. We found that the values by Friedewald formula were comparable with LDL-cholesterol levels measured by direct method when triglycerides were <802 mg dL^{-1} (<8.8 mmol L^{-1}) in both fasting and nonfasting blood samples. The Spearman's rank correlation coefficients between directly measured and estimated LDL-cholesterol values were 0.96 (0.96 for men and 0.97 for women) in fasting and 0.94 (0.93 for men and 0.95 for women) in nonfasting

subjects. Non-HDL-cholesterol was calculated as follows; Non-HDL-cholesterol (mg dL^{-1}) = total cholesterol (mg dL^{-1})–HDL-cholesterol (mg dL^{-1}).

Mild hypertension was defined as systolic blood pressure 140–159 mmHg or diastolic blood pressure 90–99 mmHg, and the corresponding values were 160–179 mmHg or 100–109 mmHg for moderate hypertension and ≥180 mmHg or ≥110 mmHg for severe hypertension. Diabetes was defined as a plasma glucose level of ≥126 mg dL^{-1} (≥7.0 mmol L^{-1}) during fasting or ≥200 mg dL^{-1} (≥11.1 mmol L^{-1}) during nonfasting, or as use of medication for diabetes, and impaired glucose tolerance was defined as a plasma glucose level of 110–125 mg dL^{-1} (6.1–6.9 mmol L^{-1}) at fasting or 140–199 mg dL^{-1} (7.8–11.0 mmol L^{-1}) at nonfasting and no use of medication for diabetes. Kidney dysfunction was defined as a serum creatinine level of ≥1.2 mg dL^{-1} (≥110 μmol L^{-1}) for men or of ≥1.0 mg dL^{-1} (≥90 μmol L^{-1}) for women and/or as a history of kidney disease. Height in stocking feet and weight in light clothing were measured and body mass index (BMI) was calculated as weight (kg) per height $(m)^2$. An interview was conducted to ascertain smoking status, number of cigarettes smoked per day, usual weekly intake of alcohol in *go* units (a Japanese traditional unit of alcohol intake converted to grams of ethanol per day at 23 g ethanol per *go* unit) and histories of stroke and heart disease. Current drinkers were defined as occasional and habitual drinkers.

Follow-up surveillance

To ascertain deaths in the cohort, the investigators conducted a systematic review of death certificates, which in Japan are all forwarded to the local public health centre of every community. It is believed that all deaths that occurred in the cohort were ascertained, except for subjects who died after they had moved from their original community, in which case the subject was treated as a censored case. Mortality data are centralized at the Ministry of Health and Welfare, where the underlying causes of death are coded for the National Vital Statistics according to the International Classification of Disease, 9th (1993–1994) and 10th (1995–2004) revisions (410–414 for International Classification of Disease, 9th revision and code I20 to I25 for 10th revision).

The follow-up inquiry for this study was conducted until the end of 2003 and the median of follow-up was 10.3 years. Only 3.2% of the subjects had moved out of their respective communities and were treated as censored.

Statistical analysis

Statistical analysis was based on mortality rates from coronary heart disease divided by clinical categories of LDL-cholesterol (<80, 80–99, 100–119, 120–139, ≥140 mg dL^{-1} or <2.06, 2.06–2.57, 2.58–3.09, 3.10–3.61, ≥3.62 mmol L^{-1}). Person-years of follow-up were calculated from the date of the baseline survey to the date of death, exit from the community, or the end of 2003, whichever occurred first.

Sex-specific age-adjusted means and proportions of selected cardiovascular risk factors at baseline were determined in terms of the LDL-cholesterols categories. The *t*-test or chi-squared test was used to examine differences in age-adjusted mean values and proportions of baseline characteristics from those of the lowest LDL-cholesterol category. The age-adjusted and multivariable hazard ratios (HR) and 95% confidence intervals (95% CI) were calculated with the Cox proportional hazards model after adjustment for age and potential confounding factors. These potential confounding factors included body mass index (sex-specific quintiles), blood pressure categories (normal, mild hypertension, moderate hypertension or severe hypertension), anti-hypertensive medication use (yes or no) diabetes status (normal, impaired glucose tolerance or diabetes), gamma-glutamyl transferase (sex-specific quintiles), kidney dysfunction (yes or no), smoking status (never, ex-smoker and current smokers of one to 19 or ≥20 cigarettes per day), alcohol intake category (never or ex-drinkers, occasional drinkers and habitual drinkers consuming <69 g day^{-1} and ≥69 g day^{-1} of ethanol respectively), HDL-cholesterol (<40, 40–49, 50–59, 60–69, ≥70 mg dL^{-1} or <1.03, 1.03–1.28, 1.29–1.54, 1.55–1.80, ≥1.81 mmol L^{-1}) and triglycerides (<100, 100–149, 150–199, 200–249, 250–299, ≥300 mg dL^{-1} or <1.12, 1.12–1.68, 1.69–2.24, 2.25–2.81, 2.82–3.37, ≥3.38 mmol L^{-1}). We also calculated the HR per 1 SD increment of LDL-cholesterol (32.5 mg dL^{-1} or 0.84 mmol L^{-1}). We tested the assumption of proportional hazards for LDL-cholesterol categories [18] and found no violation of proportionality. Tests for effect modification by sex or other variables were conducted with an interaction term generated by multiplying the continuous variables of LDL-cholesterol by sex or other variables. As the Friedewald formula introduces biased data for LDL-cholesterol [17], we conducted an additional analysis after the exclusion of persons with

hypertriglyceridaemia (triglycerides ≥802 mg dL^{-1}) at baseline survey (55 men and 23 women), and after exclusion of persons used lipid lowering medication at baseline survey (370 men and 1903 women). Furthermore, we analysed the data excluding deaths within the first 2 years after the baseline (399 men and 264 women) to examine the potential effect by any existing preclinical disorders.

We further analysed the data with Cox proportional hazard model with the time-dependent covariates, using the additional data of LDL-cholesterol and confounding factors for 80 578 persons (88.3% of the participants) whose blood lipids had been examined additionally more than once during follow-ups. The median duration between the date of the latest examination and the date of the end of the follow-up was 0.7 years.

As the presence of competing risks may lead to biased results, we also analysed using proportional hazard model for the subdistribution of competing risks [19]. We also examined possible effects of cut-offs on the significant associations nonparametrically by using restricted cubic splines method [20]. Tests for nonlinearity were examined by the likelihood ratio test, comparing 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 and a *P*-value 0.05 to 0.10 was regarded as borderline significant. All statistical analyses except for proportional hazard model for the subdistribution of competing risks were conducted using sas, version 9.13 (SAS Institute, Inc., Cary, NC, USA). r version 2.6.1 (R Foundation for Statistical Computing, Vienna, Austria) was used for calculations pertaining to the proportional hazard model for the subdistribution of competing risks.

Results

A total of 91 219 persons (30 802 men and 60 417 women) were followed up for a median of 10.3 years, during which time 295 men and 244 women died from coronary heart disease. The mean (standard deviation: SD) LDL-cholesterol level was 110.5 mg dL^{-1} (31.6) for men and 123.9 mg dL^{-1} (31.9) for women. The prevalence of obesity (BMI≥30.0 kg m^{-2}) was 1.7% for men and 3.3% for women.

Table 1 shows selected cardiovascular risk factors by LDL-cholesterol concentration category. Compared with men who had the lowest levels of LDL-cholesterol (<80 mg dL^{-1}: <2.06 mmol L^{-1}), those who had the highest levels (≥140 mg dL^{-1}: ≥3.62 mmol L^{-1}) were younger, more fasted, more likely to use medication for lipid abnormality and less likely to use medication for hypertension, smoke or drink heavily. They also tended to have kidney dysfunction, higher mean body mass index and total cholesterol level, and lower mean systolic blood pressure, gamma-glutamyl transferase, HDL-cholesterol and triglyceride levels. Except for certain risk factors, similar associations were observed for women. Compared with women who had the lowest LDL-cholesterol levels, women with the highest levels were older, more likely to have diabetes and had higher mean systolic and diastolic blood pressure and gamma-glutamyl transferase level. Compared with women, men had higher means of systolic and diastolic blood pressure, gamma-glutamyl transferase and triglyceride levels, and lower means of body mass index, and total, HDL, non-HDL and LDL-cholesterol levels (not shown in the table). Men were likely to have diabetes mellitus and kidney dysfunction, smoke, drink heavily and use medication for lipid abnormality.

Age-adjusted mortality from coronary heart disease was twice as high for the highest than for the lowest LDL-cholesterol category for men, whilst there was no such association for women (Table 2). Adjustment for potential confounding factors did not alter these associations materially. The multivariable HR (95% CI) of coronary heart disease mortality for the highest versus the lowest concentrations of LDL-cholesterol was 2.06(1.34–3.17), $P = 0.001$, for men and 1.16(0.64–2.12), $P = 0.62$, for women. The corresponding multivariable HR (95% CI) associated with a 1 SD increment in LDL-cholesterol was 1.27(1.13–1.43), $P < 0.0001$ and 1.06(0.93–1.21), $P = 0.36$. There was a borderline significant interaction for gender difference in the association between LDL-cholesterol and mortality from coronary heart disease (*P* for interaction = 0.06).

These associations did not alter substantially after the exclusion of persons with hypertriglyceridaemia, persons who used lipid lowering medication or deaths within the first 2 years, for analysis with the time-dependent covariates Cox proportional hazard model or for analysis with proportional hazard model for the subdistribution of competing risks (not shown in the table). The HR (95% CI) of coronary heart disease mortality for the highest versus lowest LDL-cholesterol levels was 2.05(1.33–3.15), $P = 0.001$ for men and 1.16(0.64–2.10), $P = 0.64$ for women after the

Table 1 *Gender-specific age-adjusted mean values or prevalence of cardiovascular risk factors according to LDL-cholesterol levels*

	Men					Women				
	LDL-cholesterol, mg dL^{-1}					LDL-cholesterol, mg dL^{-1}				
	<80	80–99	100–119	120–139	140+	<80	80–99	100–119	120–139	140+
Range, mmol L^{-1}	<2.06	2.06–2.57	2.58–3.09	3.10–3.61	3.62+	<2.06	2.06–2.57	2.58–3.09	3.10–3.61	3.62+
Number of persons	4685	6918	8112	6030	5057	4103	9858	14 728	14 327	17 401
Age, year	60.1	60.6*	60.5**	60.3	59.4*	54.2	55.5*	57.0*	58.7*	59.9*
Systolic blood pressure, mmHg	138	136*	136*	136*	136*	131	131	131	132*	133*
Diastolic blood pressure, mmHg	81	81*	81*	81	82	76	77	77*	78*	79*
Hypertensive medication use, %	21	20	19**	20	20	19	19	19	20	20
Diabetes, %	9	7*	7*	8	8	4	3**	4	4	5**
Body mass index, kg m^{-2}	22.6	22.9*	23.3*	23.7*	24.0*	22.9	23.0	23.4*	23.7*	24.1*
Gamma-glutamyl transferase, U L^{-1}	56	36*	34*	33*	35*	17	15*	16*	17**	19*
Kidney dysfunction, %	11	11	13**	14*	17*	9	8	8	8	9
Current smoker, %	59	54*	50*	48*	46*	6	5*	5*	4*	5*
Heavy drinkers, %	13.7	8.4*	6.2*	5.2*	3.8*	0.4	0.2*	0.1*	0.1*	0.1*
Lipid medication use, %	0.7	0.7	1.0	1.4*	2.5*	2.0	1.7	2.5	2.8*	5.1*
Total cholesterol, mg dL^{-1}	155	173*	190*	209*	240*	157	175*	194*	213*	246*
HDL-cholesterol, mg dL^{-1}	55	54*	52*	51*	50*	57	58*	58*	57	56*
Triglycerides, mg dL^{-1}	180	142*	141*	145*	151*	162	131*	129*	131*	137*
Fasting (≥8 h after last meal)	10.3	13.2*	16.5*	21.2*	27.0*	9.7	11.8	14.9*	17.6*	22.9*

Test for difference from the lowest category; *$P < 0.01$ **$P < 0.05$.

exclusion of persons with hypertriglyceridaemia, 2.22(1.43–3.46), $P = 0.0004$ for men and 1.09(0.60–1.99), $P = 0.78$ for women after the exclusion of persons who used lipid lowering medication, 2.16(1.35–3.45), $P = 0.001$ for men and 1.29(0.67–2.46), $P = 0.44$ for women after excluding deaths within the first 2 years, 1.55(1.02–2.34), $P = 0.04$ for men and 1.01(0.58–1.76), $P = 0.97$ for women, when we used the Cox proportional hazard model with time-dependent covariates and 1.82(1.20–2.76), $P = 0.005$ for men and 1.11(0.62–1.99), $P = 0.72$ for women when we used proportional hazard model for the subdistribution of competing risks.

To examine a potential effect modification by menopausal status, we conducted age-stratified analysis (aged <50 years vs. aged ≥50 years) for women, because we did not have the data on menopausal status. There was a significant age interaction although the number of cases was only five amongst women aged <50 years; the HR (95% CI) of coronary heart disease mortality for 30 mg dL^{-1} increment of LDL-cholesterol levels was 2.45(1.27–4.75), $P = 0.009$ for women aged <50 years and 1.05(0.92–1.19), $P = 0.49$ for women aged ≥50 years (P for interaction was 0.004).

We confirmed the gender difference of associations between LDL-cholesterol and mortality from coronary heart disease using nonparametric analysis (Fig. 1). The hazard ratios was linearly increased amongst men (P for linearity was $P = 0.0003$), whilst there was no linear association for women (P for linearity was 0.70). However, its graph suggested that the mortality from coronary heart disease may start to increase around 160 mg dL^{-1} of LDL-cholesterol levels (corresponding to 243 mg dL^{-1} of total cholesterol levels) amongst women.

To examine an effect of higher levels of LDL-cholesterol on mortality from coronary heart disease, we divided persons with ≥140 mg dL^{-1} into persons with

Table 2 *Gender-specific age-adjusted and multivariable hazard ratio (HR) and 95% confidence interval (95% CI) of mortality from coronary heart disease and all-causes according to LDL-cholesterol levels*

	LDL-cholesterol, mg dL^{-1}					HR per 1 SD increment
	<80	80–99	100–119	120–139	140+	
Men						
Person-years	44 532	67 098	79 049	58 858	49 213	298 750
Coronary heart disease						
No	35	56	74	62	68	295
Age-adjusted HR	1.0	0.99 (0.65–1.51)	1.11 (0.74–1.66)	1.28 (0.84–1.93)	1.78 (1.18–2.67)	1.24 (1.10–1.39)
Multivariable HR[a]	1.0	1.09 (0.71–1.68)	1.29 (0.85–1.95)	1.47 (0.95–2.26)	2.06 (1.34–3.17)	1.27 (1.13–1.43)
All-causes						
No	801	951	996	671	550	3969
Age-adjusted HR	1.0	0.73 (0.66–0.80)	0.65 (0.59–0.71)	0.60 (0.54–0.67)	0.63 (0.56–0.70)	0.84 (0.81–0.87)
Multivariable HR[a]	1.0	0.78 (0.71–0.86)	0.72 (0.66–0.80)	0.68 (0.61–0.75)	0.71 (0.64–0.80)	0.88 (0.85–0.91)
Women						
Person-years	40 539	97 681	146 571	142 469	172 472	599 731
Coronary heart disease						
No	13	40	56	47	88	244
Age-adjusted HR	1.0	1.15 (0.61–2.14)	0.97 (0.53–1.77)	0.74 (0.40–1.37)	1.10 (0.61–1.96)	1.07 (0.94–1.22)
Multivariable HR[a]	1.0	1.29 (0.69–2.43)	1.10 (0.60–2.03)	0.83 (0.44–1.55)	1.16 (0.64–2.12)	1.06 (0.93–1.21)
All-causes						
No	248	539	731	751	906	3175
Age-adjusted HR	1.0	0.81 (0.70–0.94)	0.67 (0.58–0.77)	0.63 (0.55–0.73)	0.60 (0.52–0.69)	0.88 (0.85–0.92)
Multivariable HR[a]	1.0	0.85 (0.73–0.99)	0.71 (0.61–0.82)	0.68 (0.58–0.78)	0.64 (0.55–0.73)	0.90 (0.86–0.93)

Potential confounding factors: blood pressure categories, anti-hypertensive medication use, diabetes mellitus, lipid medication use, body mass index, gamma-glutamyl transferase, smoking status, alcohol consumptions, kidney dysfunction and categories of HDL-cholesterol and triglycerides. 1SD of LDL-cholesterol was 32.5 mg dL^{-1} (0.84 mmol L^{-1}). [a]HR (95% CI) adjusted for age and potential confounding factors.

140–159 mg dL^{-1}, 160–179 mg dL^{-1} and ≥180 mg dL^{-1} (not shown in the table). The multivariable hazard ratio of mortality from coronary heart disease was 1.90(1.18–3.06), $P = 0.008$ (no of persons = 3130, no of events = 40) for 140–159 mg dL^{-1}, 2.32(1.32–4.09), $P = 0.004$ (no of persons = 1316, no of events = 20), for 160–179 mg dL^{-1}, 2.37(1.07–5.22), $P = 0.03$ (no of persons = 611, no of events = 8) for ≥180 mg dL^{-1} amongst men. The respective hazard ratio was 1.04(0.55–1.97), $P = 0.89$, (no of persons = 9695, no of events = 44) for 140–159 mg dL^{-1}, 1.19(0.60–2.36), $P = 0.62$, (no of persons = 4897, no of events = 25), for 160–179 mg dL^{-1}, 1.56(0.76–3.21), $P = 0.23$, (no of persons = 2809, no of events = 19) for ≥180 mg dL^{-1} amongst women.

On the other hand, higher levels of LDL-cholesterol were associated with reduced risk of all-cause mortality for both men and women (Table 2). The multivariable HR (95% CI) of all-cause mortality for the highest versus lowest LDL-cholesterol levels was 0.71(0.64–0.80), $P < 0.0001$ for men and 0.64(0.55–0.73), $P < 0.0001$ for women.

We observed no interaction of fasting/nonfasting status in the association between LDL-cholesterol and mortality from coronary heart disease (Table 3). When we stratified the data on the sub-population by fasting status, the associations did not differ substantially. No statistically significant interaction of the association between LDL-cholesterol and mortality from coronary heart disease was observed for other potential risk factors ($P > 0.20$) except for gender difference (Table 3).

We observed a weaker gender interaction in non-HDL-cholesterol and total cholesterol that that in LDL-cholesterol, although we showed significant

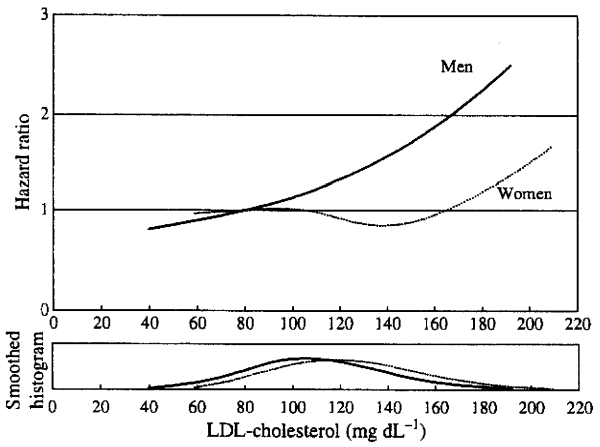

Fig. 1 *Multivariable hazard ratios of mortality from coronary heart disease in relation to LDL-cholesterol levels amongst men (solid line) and women (dotted line). 80 mg dL^{-1} of LDL-cholesterol were selected as reference. The values of the four knots correspond to 64.4 mg dL^{-1}, 98.0 mg dL^{-1}, 120.4 mg dL^{-1} and 161.0 mg dL^{-1} of LDL-cholesterol levels for men, and 78.0 mg dL^{-1}, 110.4 mg dL^{-1}, 134.0 mg dL^{-1} and 175.6 mg dL^{-1} for women. Smoothed histogram showed the distribution of LDL-cholesterol levels. We did not graph predictions from the top and bottom 1% of the analytical distribution to avoid undue visual influence of sparse tail data. P-values for nonlinearity was* P = *0.93 for men and* P = *0.23 and P-values for linearity was* P = *0.0003 for men and* P = *0.70.*

associations for men, but not for women (Table 4). The *P* for interaction was *P* = 0.06 for LDL-cholesterol, *P* = 0.13 for non-HDL-cholesterol and *P* = 0.26 for total cholesterol.

Discussion

In the large population-based prospective study of Japanese reported here, we observed, in a less obese population, significant positive associations of high LDL-cholesterol levels as well as non-HDL-cholesterol and total cholesterol levels, with increased risk of mortality from coronary heart disease for men, but not for women, whereas the gender interaction was more significant for LDL-cholesterol than that for total and non-HDL-cholesterol. These associations did not alter substantially after adjustment for potential confounding factors and after the exclusion of persons with hypertriglyceridaemia or the use of time-dependent covariates.

For this study population, the mean LDL-cholesterol level was 111 mg dL^{-1} for men and 124 mg dL^{-1} for women at baseline. Previous studies involving participants with higher mean LDL-cholesterol levels, showed an association with risk of coronary heart disease for higher LDL-cholesterol ranges. For example, the Framingham study (mean LDL-cholesterol at baseline: 139 mg dL^{-1} for men and 138 mg dL^{-1} for women) [3], the Chin-Shan Community Cardiovascular Cohort study (133 mg dL^{-1} and 142 mg dL^{-1} respectively) [5] and a cholesterol lowering clinical trial of high-risk patients (162 mg dL^{-1}) [21] demonstrated the relationship between higher concentrations of LDL-cholesterol and increased risk of coronary heart disease. The lowest LDL-cholesterol category of these studies comprised persons with over 100 mg dL^{-1} of LDL-cholesterol, who were classified in the middle and higher categories in our study.

A recent Japanese prospective cohort study in urban area [22] showed the relationship between higher concentration of LDL-cholesterol and increased risk of myocardial infarction (the means of LDL-cholesterol was 125 mg dL^{-1} for men and 135 mg dL^{-1} for women) amongst Japanese population, whose LDL-cholesterol levels was higher than that in our study. However, they could not show the relationship amongst women due to small number of case in women (cases of myocardial infarction was 24).

Another previous Japanese cohort study showed a significant association between LDL-cholesterol and incident coronary heart disease amongst men and women [23]. That study showed 1.68 (95% CI 0.99–2.84) times higher multivariable hazard ratio for persons with 125–150 mg dL^{-1} of LDL-cholesterol in comparison with persons with ≤102 mg dL^{-1}, whereas the risk was plateaued under 125 mg dL^{-1}. However, they did not conduct gender-specific analysis, probably due to the small number of cases. Our findings thus extend the previous evidence applying for the lower ranges of LDL-cholesterol.

We observed a gender difference in the associations of LDL-cholesterol with mortality from coronary heart disease. The possible mechanisms of the gender difference interaction are as follows. First, men develop atherosclerosis more often than women [24], which may lead to accelerating the atherogenic effect of LDL-cholesterol. Secondly, there may be a gender difference in the cumulative burden from LDL-cholesterol during atherosclerosis development due to lag time to an increase in LDL-cholesterol levels over a lifespan. Premenopausal women have lower total cholesterol levels than men of the same age group [25], which may result in a lower cumulative burden of atherosclerosis development for women than for

Table 3 *Multivariable hazard ratio (HR)[a] and 95% confidence interval (95% CI) of coronary heart disease according to LDL-cholesterol levels, stratified by gender and other risk factors*

		LDL-cholesterol, mg dL^{-1}					HR per 1 SD	*P* for
		<80	80–99	100–119	120–139	140+	increment	interaction
Men	No	35	56	74	62	68	295	
	Multivariable HR[a]	1.0	1.09 (0.71–1.68)	1.29 (0.85–1.95)	1.47 (0.95–2.26)	2.06 (1.34–3.17)	1.27 (1.13–1.43)	
Women	No	13	40	56	47	88	244	
	Multivariable HR[a]	1.0	1.29 (0.69–2.43)	1.10 (0.60–2.03)	0.83 (0.44–1.55)	1.16 (0.64–2.12)	1.06 (0.93–1.21)	0.06
Aged 40–59 years	No	5	12	13	11	18	59	
	Multivariable HR[a]	1.0	1.81 (0.62–5.27)	1.63 (0.56–4.75)	1.69 (0.56–5.13)	2.43 (0.84–7.08)	1.30 (1.02–1.66)	
Aged 60–79 years	No	43	84	117	98	138	480	
	Multivariable HR[a]	1.0	1.12 (0.77–1.63)	1.21 (0.84–1.73)	1.12 (0.77–1.63)	1.52 (1.05–2.19)	1.16 (1.05–1.27)	0.56
Nonhypertensive	No	13	31	32	24	39	139	
	Multivariable HR[a]	1.0	1.26 (0.66–2.44)	0.97 (0.50–1.88)	0.86 (0.43–1.74)	1.38 (0.71–2.69)	1.10 (0.92–1.31)	
Hypertensive[b]	No	35	65	98	85	117	400	
	Multivariable HR[a]	1.0	1.15 (0.76–1.75)	1.36 (0.91–2.03)	1.32 (0.88–1.99)	1.71 (1.14–2.57)	1.20 (1.09–1.33)	0.40
Normal glucose	No	32	74	92	77	108	383	
	Multivariable HR[a]	1.0	1.28 (0.84–1.95)	1.23 (0.82–1.85)	1.17 (0.76–1.78)	1.61 (1.07–2.44)	1.15 (1.04–1.28)	
Impaired glucose	No	16	22	38	31	47	154	
tolerance/Diabetic	Multivariable HR[a]	1.0	0.86 (0.45–1.64)	1.15 (0.63–2.09)	0.99 (0.53–1.84)	1.29 (0.71–2.34)	1.14 (0.97–1.33)	0.30
Nonsmoker	No	22	53	78	71	112	336	
	Multivariable HR[a]	1.0	1.19 (0.72–1.97)	1.23 (0.76–1.99)	1.16 (0.71–1.90)	1.61 (1.00–2.59)	1.20 (1.07–1.34)	
Current smoker	No	26	43	52	38	44	203	
	Multivariable HR[a]	1.0	1.16 (0.71–1.92)	1.29 (0.79–2.10)	1.19 (0.71–2.01)	1.63 (0.97–2.75)	1.15 (1.00–1.32)	0.77
Nondrinker	No	24	53	84	64	121	346	
	Multivariable HR[a]	1.0	0.97 (0.59–1.57)	1.05 (0.66–1.66)	0.82 (0.51–1.32)	1.33 (0.84–2.10)	1.16 (1.04–1.29)	
Current drinker	No	23	38	40	38	29	168	
	Multivariable HR[a]	1.0	1.34 (0.79–2.27)	1.36 (0.80–2.31)	1.81 (1.05–3.11)	1.78 (1.00–3.18)	1.20 (1.02–1.41)	0.69
BMI<23.3 kg m^{-2}[c]	No	34	55	70	48	73	280	
	Multivariable HR[a]	1.0	0.99 (0.64–1.53)	1.05 (0.68–1.60)	0.86 (0.54–1.36)	1.34 (0.86–2.08)	1.09 (0.96–1.24)	
BMI≥23.3 kg m^{-2}[c]	No	13	40	58	61	80	252	
	Multivariable HR[a]	1.0	1.79 (0.95–3.38)	1.88 (1.02–3.48)	2.06 (1.11–3.82)	2.46 (1.33–4.55)	1.27 (1.12–1.43)	0.50
HDL-cholesterol ≥54 mg dL^{-1}[c]	No	26	37	49	47	60	219	

