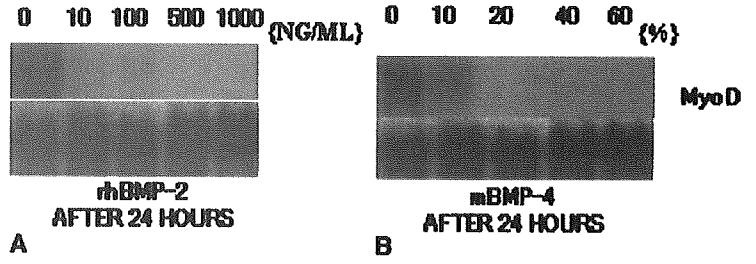

Fig. 9. The expression of MyoD in muscle-derived primary culture cells by Northern blot analyses. G3PDH mRNA levels obtained by Northern blotting were used for normalization. The expression of MyoD mRNA was not detected after BMP-2 or -4 exposure, and the expression was detected only at 0 and 24h, and not after 24h BMP stimulation

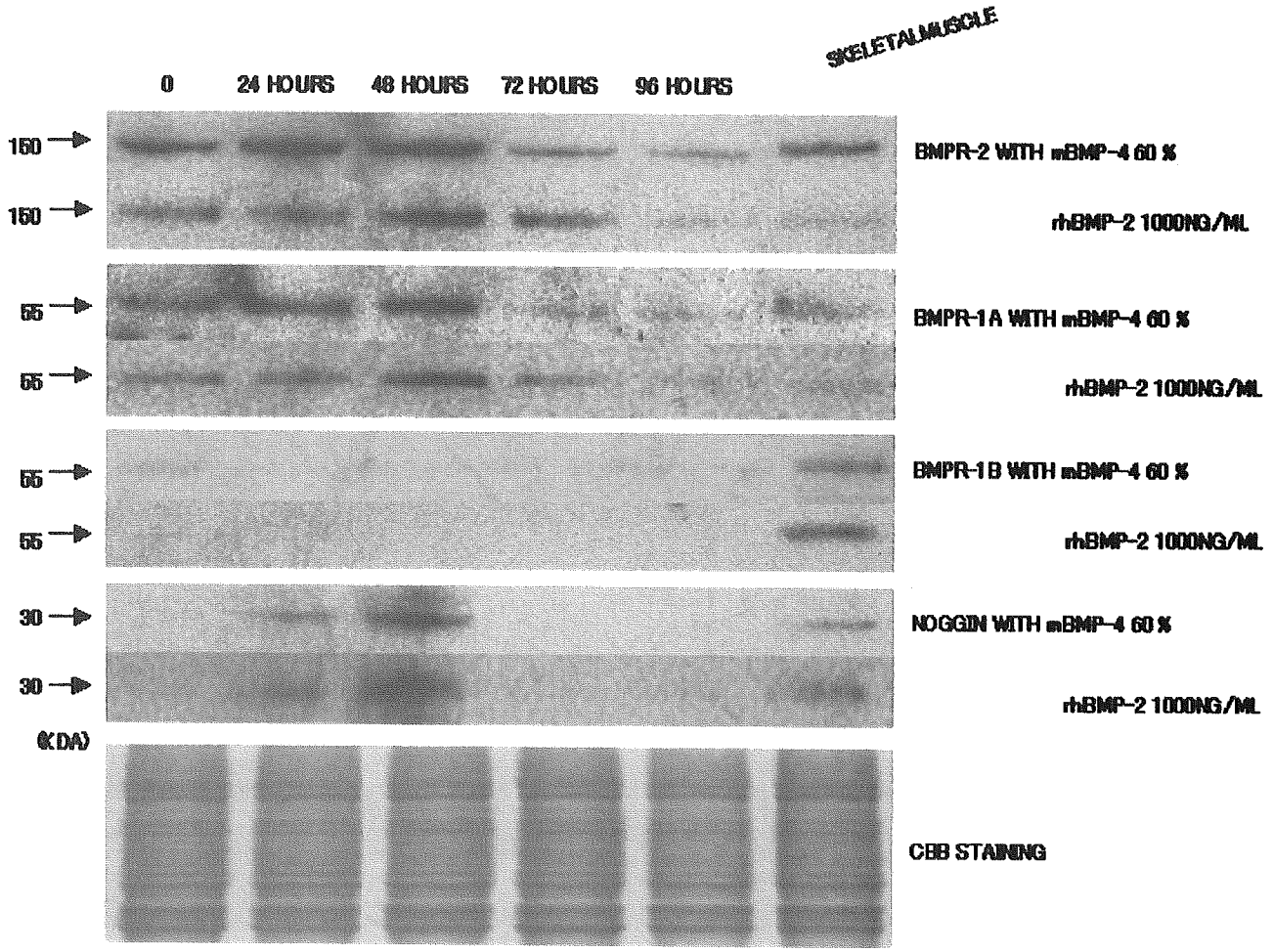

Fig. 10. Western blot analysis of BMPR-1A, -1B, -2, and Noggin after 60% mBMP-4 or 1000ng/ml rhBMP-2 stimulation in muscle-derived primary culture cells. Equivalent loading and integrity of protein were confirmed by Coomassie brilliant blue staining on the gel (*lower panel*). Mouse skeletal muscle proteins were used as positive controls. BMPR-1A and -2 were detected at 0h, induced at 24h, peaked at 48h, and then gradually decreased in both 60% mBMP-4 and 1000ng/ml rhBMP-2 stimulation groups. Expression was greater for BMPR-2 than for BMPR-1A. BMPR-1B was not detectable during any stages in either treatment group. Noggin was not detected at 0h, was up-regulated at 24h, peaked at 48h, and decreased thereafter

In our study, BMPs stimulated them to upregulate the expressions of a bone marker (OC) and cartilage markers (type II collagen and aggrecan, data not shown), but not the muscle marker examined previously. However, it is unclear whether bone and cartilage phenotypes were induced by BMPs in separate cells or in a single cell.

To further understand the potential autoregulatory mechanism in response to BMP, further gene expression

studies will be necessary. Ultimately, this knowledge may provide new approaches to the regulation of local and systemic bone formation.

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Effects of Radiation on the Longitudinal Trends of Hemoglobin Levels in the Japanese Atomic Bomb Survivors

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The late effects of radiation on the hematopoietic system have not been fully evaluated. We examined the long-term effects of radiation exposure on hemoglobin levels in the Japanese atomic bomb survivors over a 40-year period from 1958 to 1998. Compared to the unexposed survivors, the mean hemoglobin levels for those exposed to a bone marrow dose of 1 Gy were significantly reduced by 0.10 g/dl (95% CI: 0.04 to 0.16) or 0.67% at 40 years of age ($P < 0.0001$) and by 0.24 g/dl (95% CI: 0.08 to 0.40) or 1.8% at 80 years of age. Radiation effects are greater for smokers than for nonsmokers at age less than 35 years ($P < 0.01$), although cigarette smoking was associated with increased hemoglobin levels. Sex and birth cohort differences in radiation effects were not found after adjusting for smoking. The radiation-induced reduction in hemoglobin levels could not be explained by the presence of certain anemia-associated diseases. © 2005 by Radiation Research Society

INTRODUCTION

Hematopoietic malignancies are well-known late effects of exposure to atomic bomb radiation, of which leukemia is the most notable example (*1, 2*). Late effects of radiation have not been shown so far in the atomic bomb survivors for any of the quantitative aspects of hematopoiesis, including hemoglobin (Hb), hematocrit, red and white blood cell counts, and platelet count. Studies conducted within a few years after the bombings showed an initial slight but statistically significant depression in the hemoglobin level of the exposed group that returned to the control level by the fourth year (*3*). A 1950s anemia study did not show any biologically significant differences in hemoglobin level between the exposed and the unexposed Hiroshima subjects (*4*).

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Since 1958 the Adult Health Study (AHS) program, which was established by the Atomic Bomb Casualty Commission and was succeeded by the Radiation Effects Research Foundation in 1975, has been monitoring about 20,000 atomic bomb survivors on biennial basis for disease occurrences and physiological functions, including hematological measurements. By July 1998, up to 20 serial hemoglobin measurements had been collected per subject. Only a cross-sectional examination of the data from 1968–1980 had been conducted previously, and the results suggested a reduced mean level of hemoglobin with increasing dose (*5*). We apply here the mixed-effects model for the analysis of longitudinal data for repeated measurements to examine the effects of atomic bomb radiation on temporal trends of hemoglobin levels. A previous study of the temporal trends of Hb in the unexposed AHS subjects (*6*) showed that the Hb profile with age varied by sex and birth year, that anemia in the elderly may be due not only to diseases but also to aging, and that the Hb of cigarette smokers was elevated compared to nonsmokers, with a larger increase for female smokers than for male smokers. The current analysis extends that study by including the irradiated subjects to examine whether exposure to atomic bomb radiation further changed the long-term trends of hemoglobin levels during 1958–1998.

MATERIALS AND METHODS

Study Subjects

The subjects are the Adult Health Study cohort members in Hiroshima and Nagasaki. They originally comprised 19,961 subjects, half of whom were within 2,000 m of the hypocenter, a quarter were beyond 3,000 m, and the rest were not in the cities (NIC) at the time of bombing. They were invited to participate in biennial clinical examinations held at the RERF, which included physical examination and history taking as well as general laboratory tests, including total serum cholesterol, blood pressure, and hemoglobin. Examinations were conducted according to established procedures and with the consent of the participants. Between July 1, 1958 and June 30, 1998, 18,243 AHS subjects attended at least one clinical examination with one or more hemoglobin levels measured. Of these, 4,046 NIC subjects whose follow-up terminated after 1974 and 1,874 persons who lacked radiation dose estimates were excluded, leaving a total of 12,323 subjects for this analysis. *In utero* exposed subjects were not included. Hiroshima females comprised the largest group (45%), followed by Hiroshima males (26%), Nagasaki females (17%), and Nagasaki

TABLE 1
International Classification of Diseases (ICD) Codes for the Indexed Diseases

Disease	ICD edition		
	7	8	9
Malignant tumor	140–205	140–209	140–208
Chronic liver disease and cirrhosis	581, 583	571, 573	571
Renal failure	593	583, 593	583, 593
Collagen disease	456, 710, 722	446, 712, 716, 734	446, 710, 711, 714
Ulcer	540–542	531–534	531–534, 578

males (12%). The exposed group consisted of 7,465 subjects with radiation dose estimates greater than zero, and the unexposed group consisted of 4,858 subjects with dose estimates of zero.

Cigarette Smoking

Information on cigarette smoking was obtained from mail surveys and questionnaires administered in person to selected AHS participants in 1965, 1969, 1979 and 1991. The data from the different questionnaires with varying designs were collated to classify subjects simply as ever-smokers if they smoked any amount of cigarettes at any time, nonsmokers if they never smoked, or missing smoking information. In men, 11% were nonsmokers, 76% were smokers, and 13% had no data. In women, 70% were nonsmokers, 18% were smokers, and 12% had no data.

Diseases

Disease information was included in the analysis to control for secondary anemia, including cancer and some liver diseases shown to be associated with atomic bomb radiation (*7, 8*). The disease classes included (1) malignant tumor, (2) chronic liver disease and cirrhosis, (3) renal failure, (4) collagen disease, and (5) ulcer. Disease occurrence in individuals was determined by scanning the computerized database containing the AHS clinical diagnoses stored as the International Classification of Diseases (ICD) codes (Table 1). The disease onset time was not considered. A total of 3,433 subjects (27.9%) had incidences of the indexed diseases, 1,442 (38.1%) in men and 1,991 (26.1%) in women; 26.7% were in the unexposed group and 28.6% were in the exposed group. For men, 26% of the diseases were malignant tumor, 44% chronic liver disease and cirrhosis, 5% renal failure, 11% collagen disease, and 14% ulcer. For women, 36% were malignant tumor, 28% chronic liver disease and cirrhosis, 6% renal failure, 24% collagen disease, and 6% ulcer. Subjects with multiple diagnoses were classified into the incident disease category.

Radiation Dosimetry

The Dosimetry System 86 (DS86) organ dose estimates for bone marrow were used. The estimates take into account the effects of shielding from terrain and structures at each survivor's location as well as the orientation of the body and the shielding of the internal organs at the time of the bombing. The doses comprise the sum of γ rays and neutrons, or total shielded kerma. The dose components were truncated to 4 Gy in consideration of the imprecision in dose assessment for the high-dose subjects (*8*). The neutron component was multiplied by 10 before taking the sum to account for its greater biological effect per unit dose (*9*). An adjustment for random dosimetry error was also made to account for the uncertainty in the factors related to dose computation (*10, 11*).

Laboratory Determinations

Hemoglobin concentration was measured by a manual procedure before 1973 in Hiroshima and before 1977 in Nagasaki and by automated procedures after those times. Five machines were used in Hiroshima (Corman spectrophotometer, Hycel counter, Hemalog 8, Toa medical CC-180, Coulter MAXM) and six machines in Nagasaki (Corman spectrophotometer, Hycel counter, Sysmex CC-170, Sysmex CC-180, Coulter T-660, Coulter MAXM). Quality control procedures were used in both laboratories to maintain reproducibility and consistency of laboratory results. However, standardization among the different machines was not conducted.

Statistical Methods

The longitudinal relationship of Hb with age was estimated by the mixed-effects model, which accounts for correlations among measurements within an individual and variations across subjects (*12, 13*). The program MlwiN was used for maximum likelihood estimation of the model parameters using convergence criteria of less than 0.01 relative change between iterations for each parameter estimate (*14*). We assumed that the expected Hb level in an individual varied as a cubic function of age: $E(\mathrm{Hb}\,|\,\beta_0, \ldots, \beta_3) = \beta_0 + \beta_1(\mathrm{age}) + \beta_2(\mathrm{age}^2) + \beta_3(\mathrm{age}^3)$. The set of coefficients $[\beta_0, \beta_1, \beta_2, \beta_3]$ is considered unique to each individual but varies randomly across subjects according to multivariate normal distribution with a set of means that are linear functions of the covariates with a 4×4 unstructured covariance matrix and an error variance that is a quadratic function of age for each sex.

The mixed-effects model allows for missing data. It does not limit analyses to subjects with a full set of responses. As long as data are "missing at random" (*15*), that is, missingness is related to observed responses but not to missing ones, then the maximum likelihood estimates are valid and fully efficient under the correctly specified model and error distribution. In this analysis, if the Hb value was available, then all covariates, except cigarette smoking as described above, were available at the corresponding examination. If missingness in Hb, most likely due to nonparticipation in the AHS examinations, is related to radiation dose, then the results on radiation effects may be biased. However, we have no evidence that nonparticipation is related to DS86 doses.

The covariates included sex; indicator variables of age-in-1945 categories 0–10, 11–20, 21–30, 31–40, 41–50, 51 and older, which is equivalent to birth-year categories of 1935–1945, 1925–1934, 1915–1924, 1905–1914, 1895–1904, <1895; disease (absent, present); smoking status (nonsmoker, smoker, missing data); DS86 bone marrow doses in grays; and the indicators of the 20 examination cycles for each city. The most recent examination period (July 1, 1996–June 30, 1998) of both cities combined was set as the referent cycle. Age in 1945 as a quantitative variable was used to examine differential trends in radiation effects among birth cohorts. The indicators of the examination cycles were included to account for systematic variations in Hb measurements over time. Although a preliminary analysis showed Hb to be slightly but significantly higher in Nagasaki than in Hiroshima, the difference is more likely due to interlaboratory variation than to a biological difference between the cities. Since the city by dose interaction was not significant, we omitted city as a covariate in our presentation.

Each covariate was included as a set of four terms, multiplying itself by [1, age, age^2, and age^3]. The significance of the covariate was evaluated initially by the 4 *df* likelihood ratio test that the parameters associated with these terms were simultaneously equal to zero. If the significance level was less than 0.40, the least significant term, by Wald's test criteria

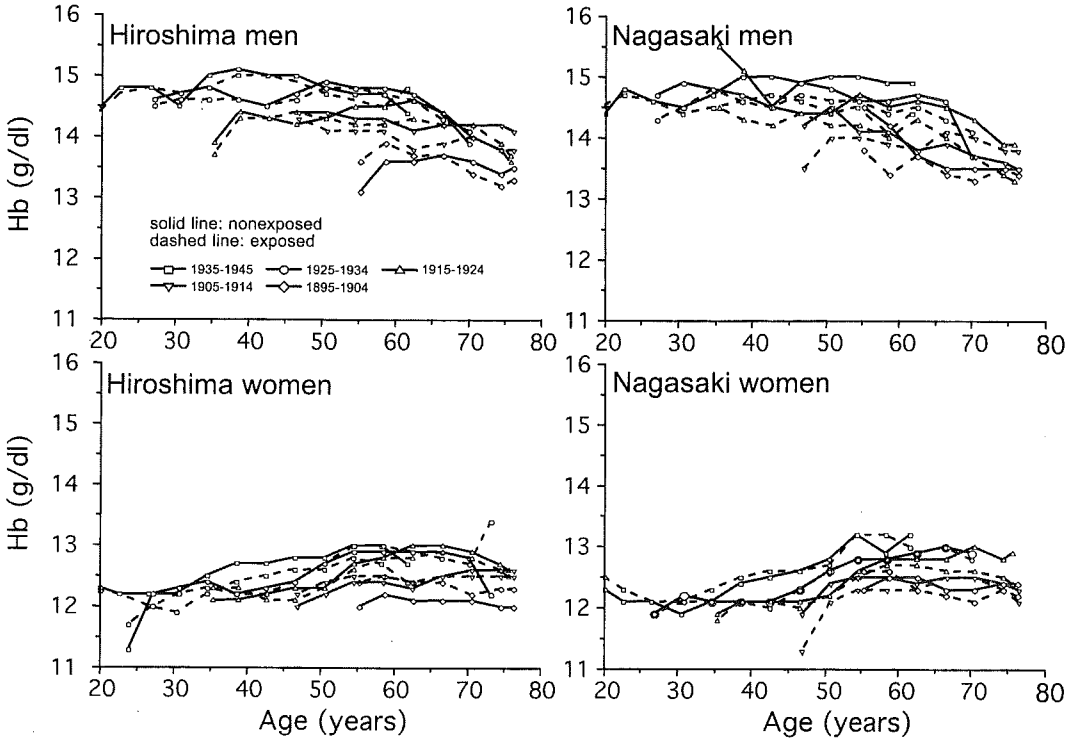

FIG. 1. Mean Hb and mean age in 5-year age intervals for men and women of Hiroshima and Nagasaki by birth cohort.

and type I error of 0.05, was removed successively until only the individually significant terms remained or all terms were removed. The indicators of examination cycles were retained regardless of statistical significance. Interactions of the age terms with all the between-subject covariates were allowed, but not with the indicators of examination cycles.

RESULTS

Sixty-two percent of the study subjects were women and 71% were Hiroshima residents. More than half of the cohort had 10 or more Hb determinations (range: 1–20). Although Hb was available for ages between 13 and 99 years, the data were truncated at age 80 because of the low participation rate among the very elderly, which may bias the results. The participation rate under age 80 years was high, ranging from 77% to 90% throughout the examinations. Hb levels were completely missing for less than 1% of AHS participants. The mean age for men at the first examination (1958–1960) was 44.4 years (range: 13–79), which increased to 65.5 years (range: 51–79) at the 20th examination (1996–1998). For women, the corresponding ages were 43.6 years (range: 13–80) and 68.0 years (range: 51–80). The unexposed group included 4,858 subjects with DS86 bone marrow dose estimates of zero. Among the 7,465 exposed subjects, the mean dose was 0.68 ± 0.70 Gy and the median dose was 0.43 Gy. Mean Hb levels at 5-year age intervals are plotted in Fig. 1 by cohort groups for the exposed and unexposed men and women of each city. No adjustment was made to account for variation in Hb measurements with time.

The baseline longitudinal model of Hb was estimated for the exposed and unexposed subjects combined, as a function of age, sex, cohort group, disease and smoking status. To test for radiation effects, dose as a continuous variable was included as a constant and its interaction with age, age^2 and age^3 and their significance tested. The modifying effects of sex, cohort (or age in 1945), disease and smoking were examined by testing the significance of their interaction with dose, age, age^2 and age^3. The individual results are presented below.

Radiation Effects

The parameter estimates of dose, dose · age^2, and dose · age^3 were significant ($\chi^2_3 = 56.8$, $P < 0.0001$). Because the results for radiation were similar regardless of adjustment for disease and smoking, the unadjusted results are presented. The top panel of Fig. 2 shows the estimated longitudinal trends of Hb for men and women at 0 Gy and 1 Gy, and the bottom panel shows the difference in the expected Hb values between the two exposure levels. An age-dependent decrease in mean Hb with radiation exposure was evident. At 1 Gy, the mean difference ranged from 0.10 g/dl (95% CI: 0.04 to 0.16), or 0.67%, at 40 years of age to more than 0.24 g/dl (95% CI: 0.08 to 0.40), or 1.8%, at 80 years of age. The difference increased slightly from the late teens to mid-30s, where it reached a plateau until about age 65, when the difference increased with further aging.

As a measure of goodness of fit, we calculated the con-

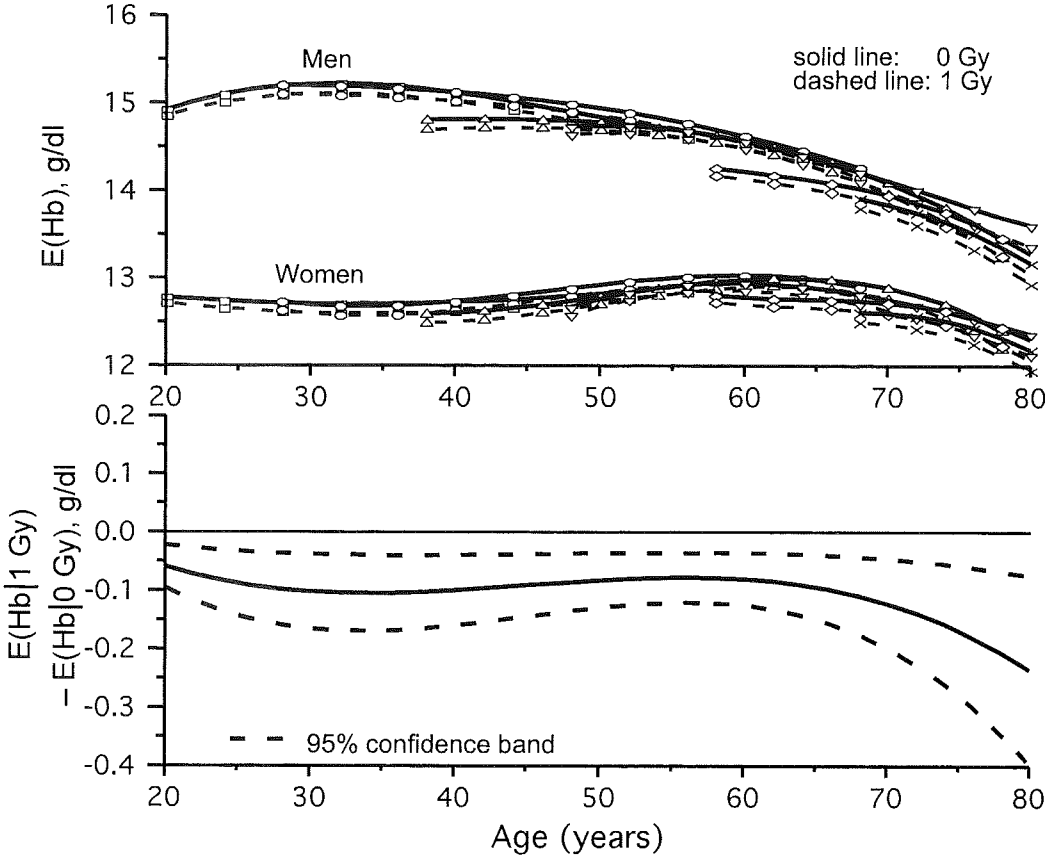

FIG. 2. Estimated longitudinal model of Hb at 1 Gy and 0 Gy exposure by sex and birth cohort (top panel). Difference in expected Hb level at 1 Gy (E[Hb|1 Gy]) and at 0 Gy (E[Hb|0 Gy]) by age (bottom panel).

ditional model coefficient of determination (R^2) for the above model (*16*). R^2 is the percentage of the total variation in Hb accounted for by the fitted model, which includes intra- and intersubject variations, compared to that explained by the null model, which is defined here as the subject-specific mean of all Hb measurements plus the random error. The calculated R^2 was 0.33, or 33% of the variation in Hb could be explained by the fitted model over that accounted for by fitting only the individuals' means.

Radiation effects were also examined by dose categories (0, 0.001–0.500, 0.501–1.000, 1.001–2.000, 2.001–3.0, >3.0 Gy). No significant differences in Hb levels were found among the first three dose categories ($\chi^2_2 = 3.3$, $P = 0.19$), suggesting that a decrease in Hb may not occur at less than 1 Gy exposure. Mean Hb levels for the three highest dose categories above 1.0 Gy differed significantly from that at 0 to 1.0 Gy combined ($\chi^2_3 = 61.8$, $P < 0.0001$), with an additional indication that relative to 0–1.0 Gy, Hb decreased by 0.9% or 0.14 g/dl (95% CI: 0.08 to 0.19) at 1.001 to 2.0 Gy exposure and by 1.8% or 0.27 g/dl (95% CI: 0.18 to 0.35) at >2 Gy exposure.

Sex Difference in Radiation Effects

There was suggestive evidence that the radiation-associated reduction in Hb was greater for men than for women ($\chi^2_1 = 3.3$, $P = 0.07$, for sex · dose). However, the difference became nonsignificant after adjusting for smoking ($\chi^2_1 = 2.3$, $P = 0.13$).

Birth Cohort Difference in Radiation Effects

To test for birth cohort differences in radiation effects, the interaction of age in 1945 and dose by [1, age, age^2 and age^3] terms were included, which were not significant ($0.08 \leq P \leq 0.30$, $df = 1$, for age in 1945 · dose · age^2 as the only remaining term; P value depended on whether smoking and disease effects were included in the baseline model).

Disease as Modifier of Radiation Effects

Although A-bomb exposure is associated with two of the disease classes considered, malignant tumor (*8*) and chronic liver disease and cirrhosis (*17*), both of which are associated with secondary anemia (*18*), we found no evidence that radiation effects on Hb varied for those with and without the diseases. The evidence was suggestive ($\chi^2_1 = 3.3$, $P = 0.07$, for disease · dose as the remaining term) when adjustment was made only for disease status in the baseline model; the Hb levels of the exposed subjects with diseases appeared to be more greatly reduced than those of the non-diseased exposed subjects. However, the interaction became

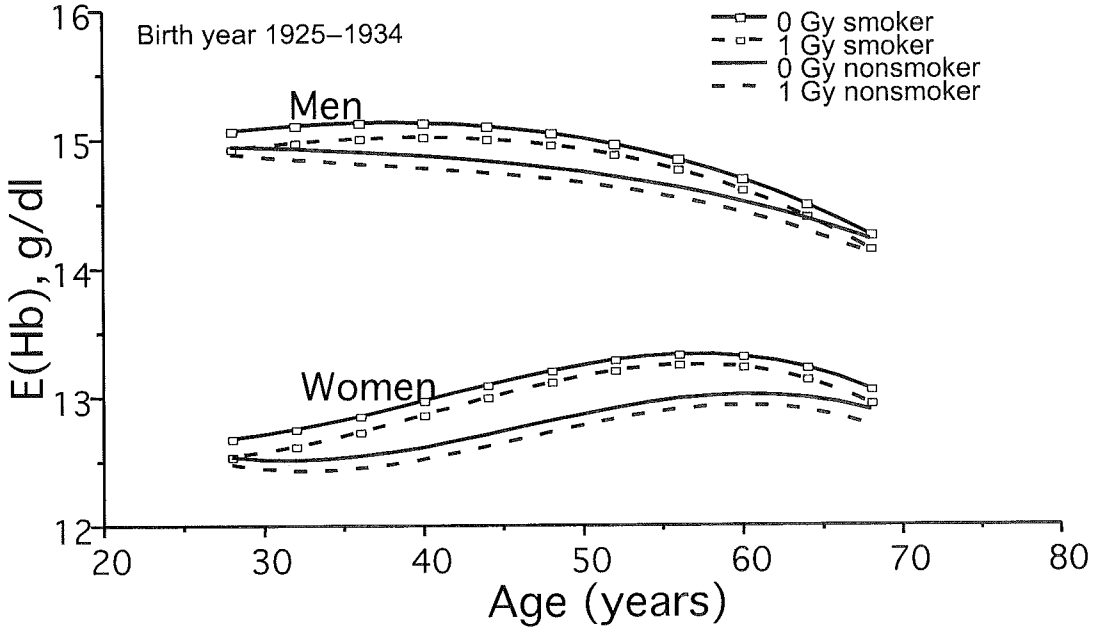

FIG. 3. Difference in expected Hb level at 1 Gy (E[Hb | 1 Gy]) and at 0 Gy (E [Hb | 0 Gy]) by age for nonsmokers and smokers of 1925–1934 birth cohort.

nonsignificant ($\chi_1^2 = 2.2$, $P = 0.14$, for disease · dose) when the effects of both smoking and disease were controlled for in the baseline model. Thus Hb reduction occurs with increasing radiation dose regardless of disease and smoking status.

Smoking as Modifier of Radiation Effects

Differences in radiation effects between smokers and nonsmokers were examined by restricting the analysis to 10,750 AHS subjects for whom smoking information was available. The baseline model was re-estimated for these subjects, adjusting for smoking status. The mean Hb of smokers was significantly elevated compared to nonsmokers, more so for female than for male smokers and more so in the older cohort than in the younger cohort of smokers. Since the results for radiation effects were similar whether adjusted for disease or not, we report the unadjusted results. In addition to radiation effects ($\chi_3^2 = 73.4$, $P < 0.0001$, for dose, dose · age^2, and dose · age^3), the interaction of smoking with dose was also significant ($\chi_1^2 = 7.3$, $P = 0.007$ for smoke · dose · age^3). Figure 3 shows the fitted Hb level by smoking and exposure status (0 or 1 Gy) for men and women born in 1925–1934. The Hb levels increased with smoking and decreased with radiation exposure. The lowest to the highest expected levels of Hb occurred, respectively, for exposed nonsmokers, unexposed nonsmokers, exposed smokers, and unexposed smokers. Moreover, the magnitude of radiation effects on lowering Hb was greater for smokers than for nonsmokers at younger ages. Figure 4 shows the estimated level of reduction in Hb at 1 Gy for smokers and nonsmokers. The amount of reduction at 1 Gy exposure was larger for smokers than for nonsmokers before age 35 years but was essentially the same for smokers and nonsmokers after that age.

DISCUSSION

Bone marrow is one of the most radiosensitive organs in the body (*19*). The dose (D_0) that reduces the cell surviving fraction to about 37% for hematopoietic stem cells was reported to be 1.0–1.4 Gy for granulocyte macrophage colony-forming units and 0.93–1.3 Gy for erythropoietic progenitor cells (*20, 21*). Over 1 Gy of acute irradiation causes transient or permanent bone marrow suppression due to damage to hematopoietic stem cells (*22–26*). Accumulating evidence has demonstrated that, although hematopoietic stem cells are intact, radiation exposure at more than 10 Gy, as in bone marrow transplantation and central lymphatic irradiation, causes long-lasting anemia in humans and animals (*27–32*). Radiation nephropathy and decreased levels of circulating erythropoietin have also been linked to anemia occurrence in such patients (*27, 29, 30, 33, 34*). However, whether whole-body irradiation at the level of a few grays can cause chronic anemia has not been determined. In the present study, we demonstrate that a radiation-induced reduction in Hb levels occurs without apparent renal dysfunction 40–50 years after A-bomb exposure.

Evidence from the Chernobyl nuclear accident is inconclusive (*35–38*). A study of the peripheral blood erythrocytes in 150 persons working constantly in the zone of rigid radiation control after the Chernobyl nuclear accident reported significantly reduced hemoglobin concentration compared to 50 blood donors (*35*). Pregnant women residing in and neonates born in a geographic area heavily ex-

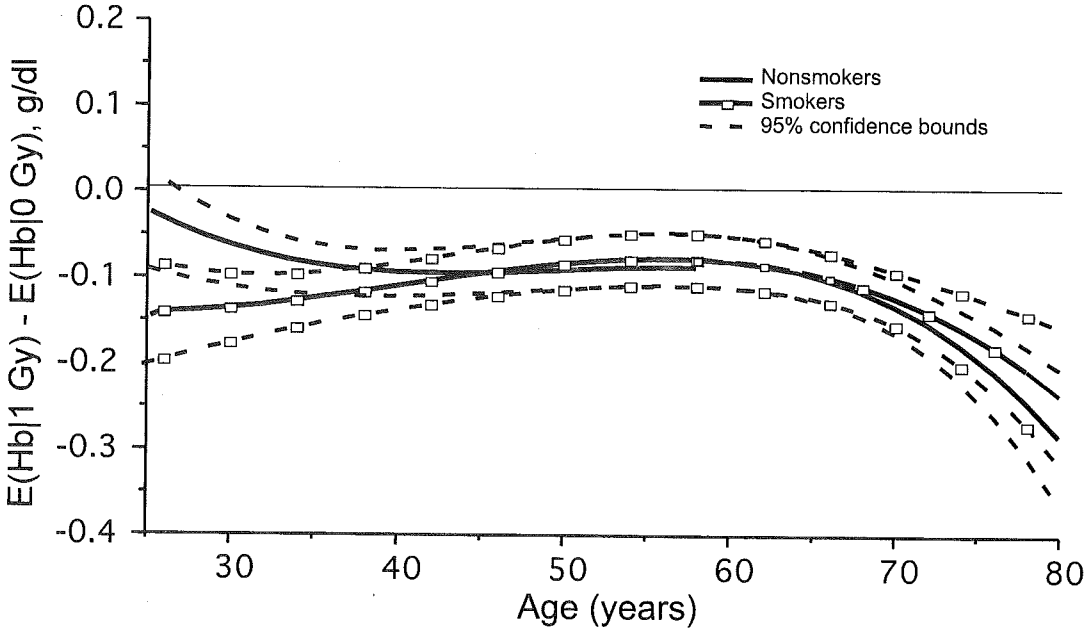

FIG. 4. Difference in expected Hb level at 1 Gy (E[Hb | 1 Gy]) and at 0 Gy (E[Hb | 0 Gy]) and 95% confidence bands by age for nonsmokers and smokers.

posed to ionizing radiation after the Chernobyl accident also appeared to have a greater risk of anemia compared to those in lightly exposed areas (*36, 39*), although malnutrition has been considered as a likely cause in one study (*36*).

Early studies of the A-bomb survivors showed that damage to the bone marrow was evident soon after A-bomb exposure, resulting in anemia, thrombocytopenia and leukopenia (*40*). Evidence for long-term effects so far has been less definitive. A 1947–1948 cross-sectional study comparing mostly school children in Hiroshima with epilation symptoms, indicative of high-dose exposure, with a similar control group in the city of Kure (*41*) showed that the mean hemoglobin concentration of the Hiroshima subjects was depressed slightly (2.3%) but significantly. Although the turmoil after the war had great effects, radiation was also considered to be responsible to some extent. A comparison of the Hb levels in over 4000 exposed and nonexposed Hiroshima subjects in the mid-1950s failed to show any biologically significant difference (*4*). Finch and Finch (*42*) noted a somewhat lower Hb concentration in Hiroshima males aged 0–19 years ATB with T65D (an older method for individual dose estimation) kerma of 1 Gy or more during the 1966–1968 AHS examination. In the subsequent examinations in 1968–1980, the cross-sectional Hb means tended to decrease significantly with higher T65D doses, which was more pronounced in the younger subjects (*5*).

In this analysis of Hb collected over a 40-year period in the AHS commencing 13 years after the bombings, we found that Hb levels of the exposed men and women were depressed compared to unexposed subjects, and that this difference increased with age. The highest dose for our study subjects was nearly 4 Gy. This would be considered too low to induce radiation nephropathy, since doses that caused functional impairment of the kidney in animal experiments usually exceeded 7 Gy (*19*). Previous analyses in the AHS did not show radiation-related increases in the prevalence of renal disease (*5*) or proteinuria (*43*), although effects on creatinine and erythropoietin have not been examined. The reduction in Hb with radiation dose regardless of the presence of certain diseases, including renal failure, observed in our analysis suggests that the decreases in Hb among the irradiated survivors was caused by factors other than radiation-induced renal disorder.

A decreasing trend for Hb with age was reported previously for the unirradiated elderly AHS men and women (*6*). An age-related functional decline in hematopoietic stem cells was also observed in animal experiments (*44*) and was associated with anemia in aged mice (*45*). These animal experiments support the hypothesis that accumulated damage in the DNA repair system in hematopoietic stem cells and bone marrow stroma cells and changes in cell cycle regulation with age could cause decreased functional capacities of aged hematopoietic stem cells. The observation that both Hb and naïve CD4 and CD8 T cells in the peripheral blood lymphocyte population decreased with age and radiation dose 40–45 years after the atomic bombings (*46, 47*) could suggest that a functional decline in hematopoietic stem cells was induced by A-bomb exposure. Our finding of increased radiation effects on Hb levels after age 65 years is consistent with the hypothesis that pre-existing DNA damage in hematopoietic stem cells and stroma cells from radiation exposure accelerated the aging process in the elderly subjects. Longitudinal analysis of other blood cells such as neutrophils and platelets will be useful for investigating this hypothesis.

A small increase in microcytic anemia observed in sub-

groups of AHS subjects in a previous cross-sectional analysis of Hb (*5*) might have been due to an increase in secondary anemia from radiation-related diseases. The decreased level of Hb among exposed subjects in the present study was not due to secondary anemia brought on by certain radiation-related disease such as cancer and liver disease since the decrease was observed among subjects without these diseases as well. Clinical characterization of the anemia type in high-dose subjects was not done in this study; thus a detailed analysis of the anemia type will be useful.

Cigarette smoking was associated with elevated Hb levels, which was also shown in a previous study of the unirradiated AHS subjects (*6*) and is consistent with other study results (*48, 49*). We further showed here that the effects of radiation and smoking on Hb are opposite of one another. Smoking effects raising Hb are greater than radiation effects lowering Hb. Moreover, before age 35 years, radiation effects are greater for smokers than for nonsmokers. Generally, the lowest fitted Hb is found for exposed nonsmokers and the highest for unexposed smokers.

In summary, this longitudinal analysis showed that the Hb levels of the exposed AHS subjects were depressed compared to those of the unexposed subjects over a 40-year period and could not be explained by the presence of certain anemia-associated diseases.

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Smoking and Alcohol Habits as Risk Factors for Benign Digestive Diseases in a Japanese Population: The Radiation Effects Research Foundation Adult Health Study

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Key Words
Smoking · Alcohol · Gastric ulcer · Duodenal ulcer · Chronic liver diseases · Cholelithiasis

Abstract
Background: Although an association between benign digestive diseases and smoking or drinking habits was reported, consistent results have not been obtained either in European, American or Japanese populations. ***Methods:*** Smoking and alcohol habits as risk factors for the incidence of gastric ulcer, duodenal ulcer, chronic liver disease and cirrhosis as well as cholelithiasis were examined using the longitudinal data of the Adult Health Study collected biennially between 1958 and 1998. During 1958–1998, 1,093 gastric ulcers, 437 duodenal ulcers, 2,054 chronic liver diseases and cirrhoses, and 1,136 cholelithiasis cases were newly detected based on medical history, fluoroscopy or endoscopy and ultrasonography. Smoking and drinking histories were obtained from five and three questionnaires, respectively, administered during different periods. The relative risks (RRs) for ever smoked to never smoked and that for ever drank to never drank were estimated after adjustment for city, sex, age, birth cohort, calendar time and radiation dose. ***Results:*** The analysis showed a positive association of smoking with gastric ulcer (RR: 2.03, 95% CI: 1.71–2.41), duodenal ulcer (RR: 1.31, 95% CI: 0.99–1.72), chronic liver disease and cirrhosis (RR: 1.23, 95% CI: 1.08–1.39) and cholelithiasis (RR: 1.19, 95% CI: 1.02–1.40), and a positive association of drinking with chronic liver disease and cirrhosis (RR: 1.10, 95% CI: 0.99–1.23). ***Conclusions:*** The peptic ulcer, chronic liver disease and cholelithiasis incidence increased significantly with smoking, and the chronic liver disease incidence increased significantly with drinking simultaneously in a prospective study of a Japanese population.

Introduction

Gastric ulcer, duodenal ulcer, chronic liver disease and cirrhosis as well as cholelithiasis are common diseases in the Japanese population, as they are in other countries. The consistent results that cigarette smoking is associated with an increased risk for both gastric and duodenal ulcers [1–5] and chronic liver disease [6] have been obtained in past epidemiological studies. On the other hand, the relationship between cigarette smoking and cholelithiasis was inconsistent [7–11]. Harmful effects of alcohol on chronic liver disease [6, 12] have been shown, but the effects on other diseases were controversial [1, 2, 9–11].

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In the 1990s, the importance of the pathogenesis of *Helicobacter pylori* in gastritis, peptic ulcer and gastric cancer [13], and of hepatitis B virus (HBV) and hepatitis C virus (HCV) in chronic liver disease and cirrhosis and hepatocellular carcinoma [14] was recognized. The effects of smoking and drinking on such diseases acting synergistically with HBV or HCV infection have also become clear [15–21].

Previous investigations on the relationship between benign digestive diseases and smoking or drinking included various study designs (cross-sectional and prospective studies) and study populations (gender, age, general population and outpatient). Incidence studies are critical to assessing causal associations with potential risk factors. Although gastric ulcer, duodenal ulcer, chronic liver disease and cirrhosis, and cholelithiasis are common diseases, and *H. pylori,* HBV and HCV infection show a higher prevalence in Asia [22–24], few epidemiological studies have examined the relationship between benign digestive disease and smoking or drinking in this region, and the design of most Japanese studies was of a cross-sectional nature [3, 5].

In this study, we examined the relationship between peptic ulcer, chronic liver disease and cholelithiasis with smoking and alcohol intake. The data are from the prospective cohort study of Japanese men and women who participated in the Radiation Effects Research Foundation (RERF) Adult Health Study (AHS) conducted from 1958 to 1998.

Subjects and Methods

Subjects

The AHS was begun in 1958 by the Atomic Bomb Casualty Commission, which was succeeded in 1975 by the RERF, as a long-term biennial clinical examination of the late effects of exposure to atomic-bomb radiation among the atomic-bomb survivors and their controls in Hiroshima and Nagasaki [25]. Two thirds of AHS subjects were within 2,000 m of the hypocenter, and one third of AHS subjects were beyond 3,000 m at the time of the atomic bombings. Subjects in the latter group were exposed minimally to the atomic-bomb radiation (dose = 0). The study subjects in this analysis are the 11,982 who attended at least 2 examinations between July 1, 1958, and June 30, 1998.

Women comprised 62% of the study participants, and 69% of the cohort were Hiroshima residents (table 1). About 50% of the AHS subjects had died by the end of June 1998. A high participation rate (75–90%) has been maintained in the AHS for subjects living in Hiroshima and Nagasaki cities and their neighboring towns. More than half of the study participants underwent 11 or more biennial clinical examinations.

A subject's follow-up began at the initial AHS visit and ended on the earlier of either the date of the last disease-free visit or the date of the disease onset. The disease onset date was estimated as the midpoint between the first disease diagnosis date and the date of the previous disease-free examination. For each disease, prevalent cases at the initial visit were excluded. The person-years of follow-up for each disease are shown in table 2.

Clinical Procedures and Selection of Diseases for Study

The biennial health examinations, conducted with informed consent, consist of history taking, physical examination and laboratory tests. A detailed description of the clinical procedures is available elsewhere [25, 26]. The disease diagnosis code(s) was(were) entered in the AHS database using the International Classification of Diseases (ICD) codes [27]. The first three digits of the ICD codes, up to 6 diagnoses per person, were stored until June 1986; the four-digit codes, up to 12 per person, were stored thereafter. The Appendix shows the respective ICD codes for each disease. Gastric ulcer was diagnosed by medical history and fluoroscopy or endoscopy optionally conducted for subjects whose stool occult blood test was positive at the AHS routine examinations. Chronic liver disease and cirrhosis, and cholelithiasis were diagnosed by medical history, liver function test and ultrasonography. Ultrasonography was used optionally from 1981 to 1990 in Hiroshima and from 1984 to 1990 in Nagasaki. Since 1991, it has been performed routinely.

Cigarette Smoking and Alcohol Consumption Data

Cigarette smoking history was obtained from five self-administered questionnaires for participants during certain periods (administered to men in 1965, to women in 1969–1970, to both men and women in 1965–1966, in 1979–1980 and in 1991). The response rate was over 95% for all surveys. Due to differences in the questionnaires and the composition of the survey participants, the subjects were classified simply as 'never smoked', 'ever smoked' or 'missing smoking information'. Ever smoked individuals were indicated to have smoked any quantity of cigarettes in any of the surveys. Never smoked subjects were indicated to have not smoked at any time in all the surveys in which they participated. Nonparticipants of the surveys were put in the 'missing information' group.

Information on alcohol intake was obtained from three self-administered questionnaires (administered to both sexes in 1965–1966, in 1979–1980 and in 1991). The same classification for smoking was applied to define 'never drank', 'ever drank' and those with 'missing data'. Nondrinkers were subjects who did not drink any quantity of beer, whiskey, sake (Japanese rice wine), shochu (Japanese distilled liquor), wine or other liquor. It is likely that the never smoked and never drank groups included actual smokers and drinkers due to a lack of timely information.

Statistical Methods

Poisson regression methods for the longitudinal analysis of incidence data were applied, using Amfit of the Epicure package of programs [28]. The disease incidence rates were stratified by city (Hiroshima, Nagasaki), sex (male, female), age at examination in years (0–39, 40–49, 50–59, 60–69, 70+), age at atomic bombings in years (0–9, 10–19, 20–29, 30–39, 40+), calendar time (July 1, 1958, to June 30, 1978; July 1, 1978, to June 30, 1998), cigarette smoking (never smoked, ever smoked, missing data) and alcohol consumption status (never drank, ever drank, missing data). Atomic-bomb radiation dose based on DS86 total organ dose information in gray (missing, 0, 0.001–0.49, 0.5–1.49, 1.50+) was also included in disease rate stratification, especially since a small but significant posi-

Table 1. Characteristics of study subjects

	Hiroshima		Nagasaki	
	men	women	men	women
Total	2,984	5,265	1,561	2,172
Age in 1945, years				
Mean	31.0	30.0	25.5	23.6
SD	15.9	14.6	14.7	13.0
Age at examination				
Range, years	13–92	13–98	14–98	14–97
Radiation dose				
Dose = 0	1,003 (34)	1,746 (33)	514 (33)	691 (32)
Mean, Gy	0.38	0.33	0.41	0.40
Missing	286 (10)	388 (7)	450 (29)	519 (24)
Smoking				
Never smoked	300 (10)	3,709 (70)	205 (13)	1,658 (76)
Ever smoked	2,366 (79)	996 (19)	1,258 (80)	364 (17)
Missing data	318 (11)	560 (11)	98 (6)	150 (7)
Drinking				
Never drank	472 (16)	3,180 (60)	272 (17)	1,530 (70)
Ever drank	2,076 (70)	1,440 (27)	1,132 (73)	464 (21)
Missing data	436 (15)	645 (12)	157 (10)	178 (8)
Number of cases				
Gastric ulcer				
Before 6/1978	262 (66)	215 (55)	114 (63)	67 (54)
After 7/1978	132 (34)	179 (45)	68 (37)	56 (46)
Duodenal ulcer				
Before 6/1978	114 (67)	66 (57)	71 (76)	34 (58)
After 7/1978	55 (33)	49 (43)	23 (24)	25 (42)
Chronic liver disease, cirrhosis				
Before 6/1978	311 (52)	237 (31)	180 (55)	125 (34)
After 7/1978	286 (48)	531 (69)	145 (45)	239 (66)
Cholelithiasis				
Before 6/1978	44 (21)	154 (29)	27 (21)	71 (27)
After 7/1978	165 (79)	380 (71)	99 (79)	196 (73)

Figures in parentheses indicate percentages.

tive dose-response relationship was detected for chronic liver disease and cirrhosis (estimated relative risk, RR, at 1 Gy = 1.15, 95% confidence interval, CI: 1.06–1.25) in the previous AHS report [26]. Weighted liver dose (calculated as the sum of gamma rays and the neutron component given a weight of 10) was used for chronic liver disease and cirrhosis, and weighted stomach dose was used for all other diseases. A detailed description of the dose estimation is available elsewhere [26]. The number of disease cases in each stratum was assumed to be an independent Poisson variate with mean $PY_{ij}\gamma_{ij}$, where PY_{ij} is the person-years and γ_{ij} is the disease incidence rate in the ij-th stratum. γ_{ij} may also be represented by $\gamma_{ij} = \gamma_{i0}\, RR_{ij}$, where γ_{i0} is the incidence rate in the referent stratum, which is the first level of each covariate mentioned above, and RR_{ij} is the relative risk in the j-th level. An additive linear model was assumed for RR_{ij}: $RR_{ij} = 1 + \beta x_{ij}$, where x_{ij} is the j-th level of a covariate in stratum i, and β is the excess risk associated with that stratum relative to the referent level.

Generally, the covariates were treated as categorical except for age at the time of the bombings and radiation dose for which the cell-specific means were used. The likelihood ratio method was used for testing the significance of the coefficient of continuous variables and, for heterogeneity of the RR among covariate categories, for computing the 95% CI.

Results

The characteristics of study subjects are shown in table 1. Eleven percent of males never smoked, 80% ever smoked and 9% had no smoking information. Among females, 72% never smoked, 18% ever smoked and 10% had no smoking information. Among males, 16% never drank,

70% ever drank and 13% had missing drinking data. Among females, 63% never drank, 26% ever drank and 11% had missing drinking data. The distribution of smokers by atomic-bomb exposure did not vary significantly for males ($p = 0.14$), but there were significantly more smokers among the exposed females than among the nonexposed females (23 vs. 17%; $p < 0.0001$). The distribution of drinkers did not vary significantly by radiation exposure for males or females (data not shown).

The numbers of incidence cases of gastric ulcer, duodenal ulcer, chronic liver disease and cirrhosis, and cholelithiasis during 1958–1998 were 1,093, 437, 2,054 and 1,136, respectively. Chronic liver disease ascertained using the three-digit ICD code (571) includes alcoholic liver disease (571.0–571.3), chronic hepatitis (571.4), cirrhosis without mention of alcohol (571.5), biliary cirrhosis (571.6) and other chronic nonalcoholic liver disease (571.8). The dramatic increase in the incidence of chronic liver disease and cirrhosis, and cholelithiasis in the latter period (after July 1978) was due to the use of ultrasonography in examinations. Nonalcoholic fatty liver detected by ultrasonography comprised about 70% of the incident cases of chronic liver disease and cirrhosis in the same period [26]. The crude incidence rates per 10,000 person-years for gastric ulcer, duodenal ulcer, chronic liver disease and cirrhosis, and cholelithiasis were 69.7, 26.8, 100.2 and 33.6 in men and 28.0, 9.2, 62.7 and 43.8, in women, respectively.

The RR estimates are shown in table 2. For each risk factor, the first category was treated as the reference group. For analysis, the background rates were stratified by all the variables except the one for which the risk was being estimated.

Increased risk for ever smoked compared with never smoked was detected for all diseases. The RRs for gastric ulcer, duodenal ulcer, chronic liver disease and cirrhosis, and cholelithiasis were 2.03 (95% CI: 1.71–2.41), 1.31 (95% CI: 0.99–1.72), 1.23 (95% CI: 1.08–1.39) and 1.19 (95% CI: 1.02–1.40), respectively. An increased risk among ever drank compared with never drank was detected for chronic liver disease and cirrhosis (RR: 1.10, 95% CI: 0.99–1.23). Gastric ulcer, duodenal ulcer and cholelithiasis were not associated with alcohol intake.

We examined the effects of smoking and drinking for chronic hepatitis and cirrhosis after June 1986 by excluding nonalcoholic fatty liver (571.8). An increased RR of 1.77 (95% CI: 1.24–2.52) was observed for ever smoked compared to never smoked. However, the risk for ever drank was not increased relative to never drank (RR: 0.99, 95% CI: 0.72–1.35).

In addition to smoking, the incidence of gastric ulcer was significantly higher for Hiroshima residence, men, younger birth cohort and older age. The incidence of duodenal ulcer was also higher in men, younger birth cohort and younger age. Significant period and birth cohort effects were shown for chronic liver disease and cirrhosis, and cholelithiasis. Use of ultrasonography may have increased the detection rate in the latter period and in the younger birth cohort. Age and sex effects were statistically significant for these diseases. A significant positive dose-response relationship for chronic liver disease and cirrhosis was detected, the same as in the previous AHS report [25, 26].

Discussion

Participants in the present analysis do not fully represent the general Japanese population because they comprise atomic-bomb survivors and their controls. However, the results from this analysis can be extrapolated to the general population, since one third of the participants were minimally exposed, with a radiation dose estimate of zero, and, even though radiation effects were found for chronic liver diseases and not for peptic ulcer and cholelithiasis [26], the effects were accounted for by including radiation dose as a covariate in the analysis. Higher incidence among men for peptic ulcer and chronic liver disease, and higher incidence among women for cholelithiasis were found in this study, as observed in previous studies [1, 3, 6, 29]. The patterns of the age effects were similar to those reported by other cohort studies [3, 7, 30]. The fact that the effects of demographic variables, such as age and gender, observed in this incidence analysis were consistent with the results of other studies conducted in the general population [1, 6, 7, 29, 30] shows the validity of this study.

Smoking and drinking information in this study were obtained from multiple questionnaires administered over some time. Although such data are capable of reflecting the changing habits over time compared to a one-time questionnaire, the lack of detailed temporal information necessitated the use of the ever smoked or drank categories rather than the current or past smoker/drinker classifications. The never smoked or drank group could include actual smokers or drinkers who adopted the habits after the last questionnaire or changed habits in between questionnaires. Since the amounts of cigarette or alcohol intake were not available in this study, it was not possible to perform dose response analysis either.

Table 2. Multivariate adjusted relative risks for gastric ulcer, duodenal ulcer, liver disease and cholelithiasis

Disease	Gastric ulcer	Duodenal ulcer	Liver disease	Cholelithiasis
Number of cases	1,093	437	2,054	1,136
Person-years	279,650	287,700	272,632	282,585
Crude incidence rate (×10,000 PY)	39.1	15.2	75.3	40.2
Relative risk				
City (Nagasaki/Hiroshima)	0.73	0.98	0.92	1.04
p value	<0.01	0.84	0.09	0.61
Sex (female/male)	0.69	0.39	0.71	1.35
p value	<0.01	<0.01	<0.01	<0.01
Period (late/early)	1.18	1.13	1.51	2.88
p value	0.13	0.48	<0.01	<0.01
Age in 1945				
0–9	1.00	1.00	1.00	1.00
10–19	1.01	1.01	0.74	1.49
20–29	1.03	0.75	0.48	1.57
30–39	0.69	0.51	0.29	1.70
40+	0.53	0.36	0.19	0.80
p value	<0.01	0.02	<0.01	<0.01
Age at examination				
15–39	1.00	1.00	1.00	1.00
40–49	1.94	1.87	1.76	2.70
50–59	2.37	1.51	3.04	4.46
60–69	2.32	0.92	4.17	4.35
70+	2.28	0.75	4.04	3.99
p value	<0.01	<0.01	<0.01	<0.01
Radiation dose				
0	1.00	1.00	1.00	1.00
0.001–0.49 Gy	1.06	1.08	1.10	0.98
0.50–1.49 Gy	1.06	1.25	1.14	0.95
1.50+ Gy	0.97	0.90	1.21	1.00
p value	0.88	0.41	<0.01	0.98
Smoking				
Never	1.00	1.00	1.00	1.00
Ever	2.03	1.31	1.23	1.19
95% CI	1.71, 2.41	0.99, 1.72	1.08, 1.39	1.02, 1.40
Missing	1.48	0.25	0.71	0.46
p value	<0.01	0.03	<0.01	0.02
Drinking				
Never	1.00	1.00	1.00	1.00
Ever	1.04	1.08	1.10	1.02
95% CI	0.89, 1.20	0.85, 1.38	0.99, 1.23	0.89, 1.17
Missing	0.69	0.64	1.41	0.83
p value	0.27	0.40	0.04	0.74

Liver disease = Chronic liver disease and cirrhosis; PY = person-years. Early period = July 1958 to June 1978; late period = July 1978 to June 1998.

In spite of these limitations, this study is one of the few prospective epidemiological studies that examined the relationship between benign digestive disease and smoking or drinking among Asians. The follow-up duration here is the longest.

This study showed that the incidence of peptic ulcer, chronic liver disease and cholelithiasis increased significantly with smoking, and that of chronic liver disease increased significantly with drinking in a Japanese population.

The crude incidence of gastric ulcer was higher than that of duodenal ulcer in this population, which is consistent with previous reports from other Japanese populations [3, 5] and Japanese-Americans [2], but contradicts those from European countries [17, 31]. The incidence of peptic ulcer for the ever smoked group in this study was significantly higher than that for the never smoked group, although the RR estimates (2.0 for gastric and 1.3 for duodenal ulcer) were lower than those for current smoker versus nonsmoker in other Japanese reports (3.1–3.7 for gastric ulcer and 1.9–3.0 for duodenal ulcer) [2, 3, 5], since ex-smokers were included in the ever smoked group in this study. The RR for ever smoked to never smoked was higher in gastric ulcer than that in duodenal ulcer, the same finding as in studies of other Japanese [3, 5] and Japanese-Americans in Hawaii [2], and in a report by the US Surgeon General [32]. On the other hand, the RR was slightly greater for duodenal ulcer than for gastric ulcer among European countries [4, 17]. Participants in our study were born before 1945. Since the prevalence of *H. pylori* infection (70–80%) was high for those born before 1950 in Japan [33], smoking may affect peptic ulcer not only as an independent risk factor but also as an accelerator of the risk from *H. pylori* infection [17]. After adjusting for smoking history, alcohol was not related to incidence of peptic ulcer, a finding similar to those in previous studies [1, 2].

As reported in previous studies [6, 12], smoking and drinking were found to have statistically significant effects on chronic liver disease and cirrhosis. Inclusion of nonalcoholic fatty liver detected by ultrasonography under chronic liver disease and cirrhosis in the latter period due to the use of three-digit ICD codes for case detection might have affected the RR estimates. Analysis of the period after June 1986 excluding nonalcoholic fatty liver showed a positive association of smoking but no association of drinking. The prevalence rate of HBV surface antigen (1.1–2.0%) and that of HCV antibody (7.2–10.0%) in the previous AHS reports [34–36] was similar to that of other Japanese reports and higher than that of US or European countries [22, 24]. Since synergistic effects of cigarette smoking or alcohol intake with HBV or HCV infections on liver cirrhosis development were detected in some studies [18–21], subjects with HBV or HCV infection should be advised to reduce or stop smoking and drinking.

We found smoking to be associated with an increased risk for cholelithiasis, but drinking was not in the AHS. This study was a long-term population study, and cholelithiasis was detected by not only history but also by ultrasonography. The varying results on the relationship between smoking or drinking and cholelithiasis [7–11] may be due to differences in study population, study design, and method of case detection. Although the biological mechanisms of the relationship between smoking and cholelithiasis are unclear [11], increased liver diseases among the smoker group in this study may have caused increased cholelithiasis since some reports show a close relation between cirrhosis and gallstones [37, 38]. Kono et al. [11] reported a significant decrease in the prevalence of gallstone and postcholecystectomy status in moderate and heavy drinkers among Japanese men, but the present study showed no association of alcohol with cholelithiasis. The lack of information on the amount of alcohol intake might have led to undetected effects of alcohol in this study.

Previous investigations have reported an association between individual benign digestive disease and smoking habits. This long-term study of a Japanese cohort showed that smoking is associated with an increased risk for multiple benign digestive diseases. Since smoking was shown as the most important risk factor for digestive cancers [39–42] and increased risks of gastric cancer with gastric ulcer [43], hepatocellular carcinoma with cirrhosis [39] and gallbladder cancer with gallstone [40] were observed, future investigation on the role of smoking in the pathogenesis of benign and malignant digestive diseases in this cohort should be useful.

In conclusion, the incidence of peptic ulcer, chronic liver disease and cholelithiasis incidence were increased significantly with smoking, and the incidence of chronic liver disease increased significantly with drinking in a prospective study of a Japanese population.

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Serum Uric Acid Concentration as a Risk Factor for Cardiovascular Mortality: A Longterm Cohort Study of Atomic Bomb Survivors

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ABSTRACT. ***Objective.*** To elucidate the association of serum uric acid concentration with cardiovascular mortality risk.

Methods. Serum uric acid level measured from 1966 through 1970 in 10,615 Japanese individuals from a cohort of atomic bomb survivors was analyzed for association with subsequent cardiovascular and all-cause mortality until 1999 using the Cox proportional hazard model.

Results. During an average followup of 24.9 years, 5225 deaths occurred, of which 1984 were ascribed to cardiovascular disease. In men, after adjustment for age, elevated serum uric acid level was associated with both cardiovascular and all-cause mortality. After additional adjustment for potential cardiovascular disease risk factors including body mass index, smoking status, alcohol consumption, systolic blood pressure, cholesterol level, and histories of hypertension, diabetes and cardiovascular disease, elevated serum uric acid level in men was associated with all-cause mortality but not with cardiovascular mortality. In women, even after these adjustments, elevated serum uric acid level was significantly associated with cardiovascular and all-cause mortality.

Conclusion. Increased serum uric acid level is a significant and independent risk factor for cardiovascular mortality in women and for all-cause mortality in both men and women. (J Rheumatol 2005;32:906–12)

Key Indexing Terms:
URIC ACID CARDIOVASCULAR DISEASE MORTALITY COHORT

Besides the well known causal relationship between uric acid and clinical manifestations of gout, an association of increased serum uric acid concentration with cardiovascular disease was first suggested about 50 years ago[1]. Since serum uric acid level is closely linked to other cardiovascular disease risk factors such as hypertension, hyperlipidemia, and obesity, numerous studies have debated whether the suggested association is independent from these other risk factors[2-14]. Among recent large-scale prospective studies, a report from the Framingham Heart Study noted that the apparent relationship of uric acid to cardiovascular or all-cause mortality did not remain significant after adjustments for other cardiovascular disease risk factors[9]. On the other hand, a report from the First National Health and Nutrition Examination Survey (NHANES I) showed a significant and independent association of uric acid concentration with cardiovascular and all-cause mortality in both men and women[11]. These 2 studies had similar population sizes and followup periods and therefore the source of the discrepancy in the results is unclear. Among individuals at higher risk for cardiovascular events, such as those with hypertension[15-17], prevalent cardiovascular disease[18], and diabetes[19], more consistent results have been obtained for the association of serum uric acid level with future risk for cardiovascular event and cardiovascular mortality.

To investigate this unresolved relationship between serum uric acid level and cardiovascular disease, we utilized a Japanese cohort that has been followed over many years. The Adult Health Study cohort was established in 1958 in the cities of Hiroshima and Nagasaki, Japan, to explore the longterm effects of ionizing radiation from the atomic bombs[20,21]. The participants of this cohort are invited to receive clinical examinations every 2 years, and nearly complete death information has been continuously obtained for this population. We analyzed the relationship between serum uric acid level, measured in more than 10,000 individuals from 1966 through 1970, and subsequent death until 1999, making it the longest and the largest of the studies on the association of uric acid level with subsequent risk for

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cardiovascular and all-cause mortality. We show here that serum uric acid level is significantly and independently associated with cardiovascular mortality in women and with all-cause mortality in both men and women.

MATERIALS AND METHODS

Subjects. The study population comprised the participants of biennial clinical examinations in the Adult Health Study, conducted since 1958 at the Radiation Effects Research Foundation in Hiroshima and Nagasaki, Japan, to evaluate the longterm effects of ionizing radiation from the atomic bombs on human health[20,21]. The original Adult Health Study cohort consisted of 19,961 individuals, about half of whom were exposed to the bomb proximally (< 2000 m from the hypocenter) and the other half who either were exposed distally (≥ 3000 m from the hypocenter) or were not in the city at the time of the bombings. The people making up this latter half were not substantially exposed to radiation from the bombs. The detailed study design of the Adult Health Study has been described[21]. Those persons selected underwent clinical examination at our institute only if they accepted our invitation to do so. The participation rate of subjects was actually about 75%. Serum uric acid level was measured in the examinations conducted from 1966 through 1970 (examination cycles 5–6). The number of participants during this period was 13,591, and the number of those who underwent serum uric acid measurement in this period was 13,559. Among this total, 10,615 participants (3860 men, 6755 women; mean age 48.6 yrs; age range 20–89 yrs) with available lifestyle information (smoking status and drinking habits), disease history, blood pressure, body mass index (BMI), and serum cholesterol level were the subjects for this analysis.

Baseline measurements. Participants were interviewed by nurses to obtain disease histories and lifestyle information including smoking status and drinking habits. Serum uric acid level was measured by a phosphotungstic acid procedure using an autoanalyzer (Technicon Instruments, Tarrytown, NY, USA). Total cholesterol and blood glucose were determined by the Abell-Kendall method and the Folin-Malmros microtechnique, respectively, with an autoanalyzer. Diagnosis of hypertension was based on a systolic blood pressure ≥ 140 mm Hg, a diastolic blood pressure ≥ 90 mm Hg, or current treatment with antihypertensive drugs. Diabetes was defined on the basis of a fasting blood glucose level ≥ 140 mg/dl, a blood glucose level ≥ 180 mg/dl at the 2 h point of the 50 g glucose tolerance test, or the use of oral hypoglycemic agents or insulin.

Outcome measures. Primary outcome measures were death from cardiovascular disease (coronary heart disease, stroke, or other cardiovascular disease) and death from all causes. Deaths were identified through checks on the status of all surviving cohort members, using the Japanese family registration system (*koseki*). No individual was lost during the followup. Information on the underlying cause of death was obtained from death certificates, and was coded according to the *International Classification of Diseases* (ICD). Four ICD revisions were used depending on the time of death. Thus, ICD 7, ICD 8, ICD 9, and ICD 10 were used for deaths during 1966-67, 1968-78, 1979-97, and 1998-99, respectively (Table 1).

Table 1. International Classification of Diseases (ICD) codes for cause of death.

Period of Death	ICD Revision	ICD Codes: Total Cardiovascular Disease	ICD Codes: Coronary Heart Disease	ICD Codes: Stroke
1966–67	7th	400–468 330–334	420	330–334
1968–78	8th	390–458	410–414	430–438
1979–97	9th	390–459	410–414	430–438
1998–99	10th	100–199	120–125	160–169

Statistical analysis. Since uric acid level differs substantially between men and women, results of the 2 sex groups were analyzed separately. To evaluate uric acid level as a risk factor for cardiovascular and all-cause mortality, the subjects were stratified into 5 groups by sex depending on baseline serum uric acid level. For men, the dividing points were 5.0, 6.0, 7.0, and 8.0 mg/dl (297.4, 356.9, 416.4, 475.8 mmol/l, respectively), and for women, the points were 4.0, 5.0, 6.0, and 7.0 mg/dl (237.9, 297.4, 356.9, 416.4 mmol/l, respectively). Cox proportional hazard regression models were used to examine the relationship of serum uric acid level to death from all causes, total cardiovascular disease, coronary heart disease, or stroke. Mortality hazard ratio for each uric acid category was calculated using as reference the lowest uric acid categories, < 5.0 mg/dl (297.4 mmol/l) in men and < 4.0 mg/dl (237.9 mmol/l) in women. These analyses were adjusted for baseline characteristics, including age, BMI (kg/m^2), systolic blood pressure (mm Hg), total cholesterol level (mg/dl), smoking status (non-smoker, ex-smoker, or current smoker), alcohol consumption (g/week), histories (yes/no) of hypertension, coronary heart disease, stroke, diabetes, kidney disease and malignant tumor, and radiation dose (Gray) from the atomic bombings.

RESULTS

Mean (SD) age of the subjects at the time of uric acid measurement was 49.0 (14.8) years for men and 48.6 (13.5) years for women. Mean (SD) uric acid concentration was 5.4 (1.5) mg/dl [321.2 (89.2) μmol/l] in men and 4.2 (1.1) mg/dl [249.8 (65.4) μmol/l] in women, a statistically significant difference ($p < 0.001$). The 90th and 95th percentiles for uric acid distribution were 7.6 and 8.4 mg/dl for men and 5.6 and 6.3 mg/dl for women, respectively. Uric acid level was significantly and positively associated with other cardiovascular disease risk factors in both sexes including BMI, total cholesterol level, and presence of hypertension (Table 2). Uric acid level was also associated with alcohol use in both sexes (Table 2).

During an average followup of 24.9 years (22.9 yrs for men, 26.0 yrs for women), 5225 subjects (49.2%) of a total of 10,615 subjects died [2266 (58.7%) of 3860 men, 2959 (43.8%) of 6755 women]. Among these deaths, 1984 (38.0%) were attributed to cardiovascular disease (coronary heart disease, 427; stroke, 931; other cardiovascular disease, 626). Crude all-cause and cardiovascular mortality rates were 19.8 (25.6 for men, 16.8 for women) and 7.5 (8.5 for men, 7.0 for women) per 1000 person-years, respectively.

In men, age-adjusted hazard ratio for all-cause mortality was significantly increased in subjects with uric acid level ≥ 8.0 mg/dl compared with those in the lowest uric acid category (< 5.0 mg/dl; Table 3). This increase in risk for all-cause mortality remained significant after adjustment for other cardiovascular disease risk factors. In women, age-adjusted hazard ratio for all-cause mortality increased significantly in all uric acid categories compared with the lowest uric acid category (Table 3). After full adjustment, the hazard ratio for all-cause mortality remained significant in the uric acid categories 6.0–6.9 and ≥ 7.0 mg/dl, and a higher hazard ratio was observed for uric acid category ≥ 7.0 mg/dl compared with the 6.0–6.9 mg/dl category (Table 3).

The hazard ratio for cardiovascular mortality in men was

Table 2. Baseline characteristics by sex and uric acid level. Values are mean (SD).

Uric Acid Level, mg/dl	No. of Subjects	Age, yrs	Body Mass Index, kg/m^2	Total Cholesterol, mg/dl	Hypertension, %	Diabetes, %	Current Smoking, %	Alcohol Use, g†/wk	Radiation Dose, Gy
Men									
Total	3860	49.0 (14.8)	21.1 (2.8)	181.8 (38.7)	30.4	13.0	74.1	107 (140)	0.39 (0.82)
< 5.0	1184	51.8 (14.4)	20.5 (2.6)	178.1 (36.7)	27.8	14.4	75.4	92 (126)	0.38 (0.78)
5.0–5.9	1127	48.4 (14.5)	20.8 (2.8)	180.6 (37.0)	25.9	12.6	75.3	101 (144)	0.40 (0.83)
6.0–6.9	866	46.8 (14.8)	21.3 (2.8)	183.1 (39.7)	30.4	11.1	73.7	116 (141)	0.39 (0.85)
7.0–7.9	390	46.6 (15.1)	22.1 (3.1)	187.5 (41.8)	36.9	12.8	73.3	119 (143)	0.36 (0.77)
≥ 8.0	293	49.0 (15.2)	22.5 (3.0)	189.5 (43.3)	49.5	15.0	66.9	141 (156)	0.39 (0.84)
p for trend		< 0.001	< 0.001	< 0.001	< 0.001	> 0.5	0.007	< 0.001	> 0.5
Women									
Total	6755	48.6 (13.5)	22.1 (3.4)	191.8 (41.2)	26.4	6.6	14.4	8 (41)	0.36 (0.75)
< 4.0	3015	47.2 (13.0)	21.7 (3.0)	185.1 (39.6)	19.4	5.1	12.6	5 (26)	0.35 (0.70)
4.0–4.9	2261	48.6 (13.5)	22.1 (3.4)	191.9 (39.6)	27.1	5.9	14.6	9 (42)	0.36 (0.76)
5.0–5.9	1005	50.5 (14.0)	23.1 (3.7)	202.6 (42.3)	34.5	9.4	16.2	11 (50)	0.40 (0.86)
6.0–6.9	339	52.8 (14.1)	23.9 (4.0)	209.2 (46.2)	46.0	12.7	21.2	20 (78)	0.37 (0.78)
≥ 7.0	135	56.9 (13.8)	23.9 (4.0)	212.3 (45.1)	60.0	15.6	21.5	20 (60)	0.40 (0.76)
p for trend		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.07

† Ethanol weight.

Table 3. Relation of serum uric acid level to all-cause mortality.

Uric Acid Level, mg/dl	Person-yrs	No. of Deaths	Mortality Rate*	Age Adjusted Hazard Ratio	95% CI	Fully Adjusted† Hazard Ratio	95% CI
Men							
< 5.0	25,811	754	29.2	1.0		1.0	
5.0–5.9	26,259	649	24.7	1.00	0.90, 1.11	0.99	0.89, 1.10
6.0–6.9	20,871	461	22.1	0.95	0.85, 1.07	0.94	0.83, 1.06
7.0–7.9	9243	214	23.2	1.05	0.90, 1.22	1.05	0.90, 1.23
≥ 8.0	6182	188	30.4	1.38	1.17, 1.61	1.22	1.03, 1.44
Women							
< 4.0	81,405	1130	13.9	1.0		1.0	
4.0–4.9	58,786	980	16.7	1.10	1.01, 1.20	1.07	0.98, 1.16
5.0–5.9	25,226	525	21.0	1.14	1.02, 1.26	1.08	0.97, 1.20
6.0–6.9	7458	221	29.6	1.59	1.37, 1.84	1.44	1.24, 1.67
≥ 7.0	2538	103	40.6	1.90	1.54, 2.31	1.63	1.32, 2.00

* Values are expressed per 1000 person-years. † In addition to age, adjusted for BMI, smoking status, alcohol consumption, systolic blood pressure, total cholesterol level, histories of hypertension, diabetes, coronary heart disease, kidney disease and malignant tumor, and estimated radiation dose from the atomic bombs.

significantly increased in the highest uric acid category (≥ 8.0 mg/dl) compared with subjects in the lowest uric acid category when adjustment was made only for age. However, this hazard ratio increase did not remain significant after full adjustment (Table 4). When cardiovascular disease was restricted to coronary heart disease, age-adjusted hazard ratio for mortality was significantly increased in the uric acid category ≥ 8.0 mg/dl, but it was no longer significant after full adjustment (Table 4). For stroke mortality, no significant increase in hazard ratio was observed in any of the uric acid categories in men.

In women, a significant increase in the hazard ratio for cardiovascular mortality was observed in the uric acid categories 6.0–6.9 and ≥ 7.0 mg/dl compared with the lowest uric acid category (< 4.0 mg/dl) even after full adjustment, and a higher hazard ratio was observed in the uric acid category ≥ 7.0 mg/dl compared with the 6.0–6.9 mg/dl category (Table 4). A significant increase in coronary heart disease mortality was observed for the 6.0–6.9 mg/dl category, but the increase was not significant in the ≥ 7.0 mg/dl category (Table 4). This may result from the small number of cases in this category (n = 12). For stroke mortality, a significant increase in hazard ratio was found for the ≥ 7.0 mg/dl category (Table 4).

Since menopausal status has substantial effects on both uric acid level and cardiovascular disease occurrence, the relation between uric acid level and mortality risk was examined in different age groups in women. Thus, women