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ARTICLE

Homeostasis model assessment of insulin resistance and the risk of cardiovascular events in middle-aged non-diabetic Japanese men

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Abstract
Aims/hypothesis Little is known about the relationship between the HOMA of insulin resistance (HOMA-IR) and the risk of cardiovascular events in Asian populations, which have lower levels of HOMA-IR than Western populations. Accordingly, we determined the predictive value of HOMA-IR for cardiovascular risk in a Japanese population that was apparently free of diabetes, addressing whether insulin resistance itself increases cardiovascular risk independently of other relevant metabolic disorders.
Methods We followed 2,548 non-diabetic men aged 35 to 59 years for 11 years. The hazard ratios for the incidence of cardiovascular events due to increased HOMA-IR were estimated using a Cox proportional hazards model that was adjusted for potential confounding factors.
Results The multivariate-adjusted hazard ratio for cardiovascular events compared with the first quartile of HOMA-IR (≤0.66) was 1.07 (95% CI 0.44–2.64) for the second (HOMA-IR 0.67–1.01), 1.36 (0.56–3.28) for the third (HOMA-IR 1.02–1.51) and 2.50 (1.02–6.10) for the fourth quartile (HOMA-IR ≥1.52). The hazard ratio associated with a one SD (0.61) increment in log-transformed HOMA-IR was 1.51 (1.13–2.02). A similar positive relationship was observed for coronary events and stroke. In addition, the relationship between HOMA-IR and cardiovascular risk was broadly similar in participants with and without hypertension, dyslipidaemia (elevated triacylglycerol and/or reduced HDL-cholesterol), abdominal obesity and current smoking.
Conclusions/interpretation Increased HOMA-IR predicted subsequent cardiovascular events in non-diabetic Japanese men. The association was independent of traditional cardiovascular risk factors and other relevant metabolic disorders.

Keywords Cardiovascular diseases · Coronary heart disease · Epidemiology · Homeostasis model assessment · Insulin resistance · Stroke

Abbreviation
HOMA-IR HOMA of insulin resistance

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Introduction

Insulin resistance characterised by decreased sensitivity of tissue to insulin and compensatory elevation in fasting plasma insulin leads not only to abnormal glucose metabolism [1, 2], but also to elevated blood pressure and abnormal lipid profiles such as elevated triacylglycerol and reduced HDL-cholesterol [3–6]. Some investigators have suggested that insulin resistance with compensatory hyperinsulinaemia plays a key role in the clustering of relevant metabolic disorders in the same individual (the metabolic syndrome) [7–10] and that this clustering is a high-risk state for the development of cardiovascular disease [11–14]. However, the contribution of insulin resistance with compensatory hyperinsulinaemia to the development of cardiovascular disease is likely to be independent of abnormal glucose metabolism and other relevant metabolic disorders [1, 6, 15–22]. Since insulin resistance is highly prevalent in the general population [3, 18, 23, 24], it is important to know whether the presence of insulin resistance is an early indicator of increased cardiovascular risk and whether physicians should evaluate insulin resistance to improve overall cardiovascular risk prediction.

The HOMA of insulin resistance (HOMA-IR) is easily available for estimating insulin resistance and is well correlated with estimates of insulin resistance obtained from the euglycaemic–hyperinsulinaemic clamp technique (gold standard) [25, 26]. A number of cohort studies, mainly in Western populations, have examined the relationship between HOMA-IR and the risk of cardiovascular events (including coronary events and stroke) in a general or non-diabetic population [11, 12, 19–21, 27–36]. However, only a few of these studies showed that increased HOMA-IR predicts subsequent cardiovascular events separately from other relevant metabolic disorders [19–21]. In addition, little is known about the relationship between HOMA-IR and the risk of cardiovascular events in Asian populations [35, 36], which have a relatively lower prevalence of obesity and lower levels of HOMA-IR than Western populations [12, 19, 21, 24]. We therefore attempted to determine the predictive value of HOMA-IR for the occurrence of a first-ever cardiovascular event in middle-aged Japanese men who were apparently free of diabetes.

Methods

Study design and participants The study population consisted of Japanese men who worked for a metal products factory in Toyama prefecture, Japan; this factory employed approximately 4,400 men and 2,600 women. The Industrial Safety and Health Law in Japan requires employers to conduct annual health examinations on all employees. Examinations include screening tests for traditional cardiovascular risk factors and questionnaires on medical history and lifestyle. Details of this study population have been reported previously [37, 38]. In 1996, 2,952 male employees aged 35 to 59 years, who accounted for approximately 90% of all male workers of target age, participated in a baseline survey that included a usual health examination and measurement of fasting plasma insulin. The participants were followed-up for 11 years until March 2007. Written informed consent was obtained. The present cohort study was approved by the Institutional Review Committee of Kanazawa Medical University for Ethical Issues.

Of the 2,952 participants, 59 were excluded due to a history of previous cardiovascular events (n=11), missing information at the time of the baseline survey (n=15) or failure to obtain information in the follow-up survey (n=33). To evaluate the true effect of insulin resistance on the occurrence of cardiovascular events independently of abnormal glucose metabolism and to diminish the possibility of inaccurate estimates of insulin resistance from HOMA-IR [39, 40], participation in the study was restricted to individuals who were apparently free of diabetes at baseline in order. Thus, 345 additional participants were excluded due to abnormal glucose metabolism defined as fasting glucose ≥6.11 mmol/l, HbA_{1c} ≥5.8% and/or taking medication for diabetes [41]. The remaining 2,548 participants were included in the analyses.

Baseline examination Data collected at study entry included age, medical history, smoking and alcohol drinking habits, leisure-time physical activity and anthropometric indices including waist circumference, blood pressure, serum total cholesterol, HDL-cholesterol, triacylglycerol, fasting plasma glucose, insulin and HbA_{1c}. Fasting blood samples were obtained by cubital venipuncture and then shipped to a single laboratory (BML, Toyama, Japan) for analysis. Plasma fasting glucose levels were measured enzymatically using an automatic analyser (GA1140; Kyoto Daiichi Kagaku, Kyoto, Japan). Fasting plasma insulin was measured by radioimmunoassay (Gamma Counter ARC-950; Aloka, Tokyo, Japan). HOMA-IR was calculated using a previously published formula [25]. Other blood chemical markers were also measured using widely accepted methods. Measurements of anthropometric indices and blood pressure were carried out by trained staff. Information on medical history and lifestyle was obtained using a self-administered questionnaire.

Follow-up survey Vital status and the incidence of cardiovascular events were ascertained in March 2007, representing a follow-up period of over 11 years. Questionnaires on medical history in the annual health check-ups and medical certifications for absence due to illness were used to obtain

information on cardiovascular event history for participants who remained employed at the target factory. Similar questionnaires were sent by mail once a year to retired participants. The medical records of all participants who were thought to have a cardiovascular event were reviewed to confirm the diagnosis.

The diagnostic criteria for myocardial infarction were modified on the basis of those of the Monitoring trends and determinants of cardiovascular disease (MONICA) project conducted by the World Health Organization [42]. Myocardial infarction was defined as typical chest pain with abnormal and persistent Q or QS waves in the electrocardiogram and/or changes in cardiac enzyme activity. Sudden cardiac death was defined as death within 1 h of onset, a witnessed cardiac arrest or abrupt collapse. Angina pectoris was also included as a coronary event when patients underwent coronary artery angioplasty or bypass surgery. Stroke was defined as a focal neurological disorder with rapid onset, which persisted for at least 24 h or until death, with supporting evidence from examinations such as computed tomography or magnetic resonance imaging.

The primary outcome in the present study was the incidence of a first-ever cardiovascular event. All such events were classified into two categories: coronary events and stroke. The former included myocardial infarction, sudden cardiac death and angina pectoris requiring an intervention, whereas the latter included cerebral infarction, cerebral haemorrhage, subarachnoid haemorrhage and unspecified stroke.

Statistical analysis Initially, hazard ratios and their corresponding 95% CIs for the outcomes of interest were calculated for each quartile of HOMA-IR at baseline, with the first quartile serving as the reference. A Cox proportional hazards regression model was used that incorporated the following variables as covariates: age (years), waist circumference (cm), smoking habits (current, former or never smoking), drinking habits (heavy, light, occasional or no drinking), leisure-time physical activity (hard, moderate, light or no activity), systolic blood pressure (mmHg), medication for hypertension (yes or no), log-transformed triacylglycerol (mmol/l), HDL-cholesterol (mmol/l), non-HDL-cholesterol (mmol/l), medication for hypercholesterolaemia (yes or no) and HbA_{1c} (%). Non-HDL-cholesterol was calculated as total cholesterol minus HDL-cholesterol and used as a covariate instead of LDL-cholesterol [43]. Values for triacylglycerol were logarithmically transformed due to their skewed distribution. In addition, the trend between HOMA-IR and the risk of cardiovascular events was explored in a multivariate Cox model with a continuous term for log-transformed HOMA-IR (due to their skewed distribution) instead of HOMA-IR category. We also conducted a similar analysis, in which the reference was the combination of the first and second quartiles of HOMA-IR. Hazard ratios associated with a one SD increment in log-transformed HOMA-IR were also estimated in the Cox model. This approach was applied to fasting insulin, as well as to HOMA-IR, to see whether the association with cardiovascular risk was similar for these two indices.

An analysis was also performed based on previous evidence of the association between HOMA-IR and insulin resistance in a Japanese population. Oimatsu et al. [44] reported that when setting the cut-off value for HOMA-IR at 1.73 in a Japanese population, the sensitivity and specificity for the presence of insulin resistance evaluated by the euglycaemic–hyperinsulinaemic clamp technique were 64.3% and 78.9%, respectively. Using this evidence as a landmark for grouping HOMA-IR, we divided the participants in our study into the following five groups: (1) HOMA-IR<1.00; (2) 1.00≤HOMA-IR<1.50; (3) 1.50≤HOMA-IR<2.00; (4) 2.00≤HOMA-IR<2.50; and (5) 2.50≤HOMA-IR. Hazard ratios in each HOMA-IR group were calculated in a multivariate Cox model, with the HOMA-IR<1.00 group serving as reference.

Finally, analyses were repeated after study participants had been stratified by the presence or absence of: (1) hypertension (defined as systolic blood pressure ≥130 mmHg, diastolic blood pressure ≥85 mmHg and/or taking medication for hypertension); (2) dyslipidaemia (defined as triacylglycerol ≥1.69 mmol/l and/or HDL-cholesterol <1.03 mmol/l); and (3) abdominal obesity (defined as waist circumference ≥85 cm). The above are based on the Japanese criteria for metabolic syndrome [45] and are all closely linked with insulin resistance with compensatory hyperinsulinaemia [3–6, 24, 46]. This stratification was done to avoid the potential confounding effect of other relevant disorders on cardiovascular risk prediction and to determine whether there was an interaction between each disorder and insulin resistance with regard to risk of cardiovascular events. Similar stratified analyses were also conducted on the basis of smoking status (current smoking or not), because smoking remains a major cardiovascular risk factor in Japanese men [47] and is known to influence plasma insulin levels [48]. The significance of the interaction between increased HOMA-IR and each of the four factors (hypertension, dyslipidaemia, abdominal obesity and smoking) for the risk of cardiovascular events was tested using an interaction term for the categorical variables in the multivariate Cox model.

Statistical analyses were performed using the Statistical Package for the Social Sciences version 12.0J for Windows (SPSS Japan, Tokyo, Japan). All probability values were two-tailed and the significance level was set at $p<0.05$.

Results

Characteristics of the study population The baseline characteristics of the 2,548 study participants (mean age 45.0 years) grouped by quartile of HOMA-IR are summarised in Table 1. The mean age decreased slightly with increasing HOMA-IR. The mean values for body mass index, waist circumference, systolic and diastolic blood pressure, and serum total and non-HDL-cholesterol, as well as the median values for triacylglycerol, fasting plasma glucose and fasting plasma insulin increased with increasing HOMA-IR, whereas the mean value for HDL-cholesterol and the rates of current smoking, light-to-heavy alcohol drinking and moderate-to-hard activity decreased with increasing HOMA-IR.

HOMA-IR and the risk of cardiovascular events The study involved 25,506 person-years of follow-up in 2,548 study participants. The mean overall follow-up period was 10.0 years. During follow-up, 58 first-ever cardiovascular events were recorded, including 25 myocardial infarctions, three sudden cardiac deaths, five cases of angina pectoris with coronary intervention, 13 cerebral infarctions, eight cerebral haemorrhages and four subarachnoid haemorrhages. The crude incidence rate of a first cardiovascular event in the study population was 2.27 per 1,000 person-years.

Compared with the first quartile of HOMA-IR, the second quartile showed little increase in the risk of cardiovascular events, but the third and fourth quartiles showed a gradual trend towards increased risk. The age-adjusted hazard ratio (95% CI) was 1.09 (0.45–2.62) for the second, 1.50 (0.66–3.43) for the third and 2.95 (1.41–6.14) for the fourth quartile. After further adjustment for traditional cardiovascular risk factors and other metabolic disorders relevant to insulin resistance, the hazard ratio was 1.07 (0.44–2.64), 1.36 (0.56–3.28) and 2.50 (1.02–6.10), respectively (Fig. 1a). When cardiovascular events were divided into coronary events and stroke, a similar pattern was observed for both event subtypes; the multivariate-adjusted hazard ratio comparing the fourth with the first quartile of HOMA-IR was 2.03 (0.61–6.75) for coronary events and 3.23 (0.82–12.79) for stroke (Fig. 1b, c). When the first and second quartiles were combined as reference, the multivariate-adjusted hazard ratio comparing the fourth with the first and second quartiles combined was 2.40 (1.16–4.94) for cardiovascular events (Table 2), 2.27 (0.86–6.00) for coronary events and 2.64 (0.89–7.85) for stroke.

The trend was significant for all the outcomes, with $p<0.01$ for trend for cardiovascular events, $p=0.04$ for coronary events and $p=0.05$ for stroke. The hazard ratio associated with a one SD (0.61) increment in log-transformed HOMA-IR was 1.51 (1.13–2.02) for cardiovascular events (Table 2), 1.48 (1.02–2.14) for coronary events and 1.59 (1.00–2.54) for stroke.

The observed patterns were quite similar between HOMA-IR and fasting insulin (pmol/l) for all the outcomes. The multivariate-adjusted hazard ratio for cardiovascular events was 0.91 (0.40–2.05) for the second (20.85–34.73 pmo/l), 1.43 (0.62–3.34) for the third (34.74–48.62 pmol/l) and 2.60 (1.10–6.15) for the fourth (48.63–506.99 pmol/l) quartile, with the first quartile of fasting insulin (6.95–20.84 pmo/l) serving as the reference. The multivariate-adjusted hazard ratio comparing the fourth with the first quartile of fasting insulin was 1.85 (0.57–5.93) for coronary events and 4.01 (1.10–14.67) for stroke. The trend was of definite significance or borderline significance for each outcome, with $p<0.01$ for trend for cardiovascular events, $p=0.09$ for coronary events and $p=0.04$ for stroke. The hazard ratio associated with a one SD (0.58 pmol/l) increment in log-transformed fasting insulin was 1.47 (1.10–1.96) for cardiovascular events, 1.39 (0.95–2.02) for coronary events and 1.62 (1.03–2.57) for stroke.

In the second approach, the crude incidence rate per 1,000 person-years was 1.56 for HOMA-IR<1.00 (n=1,265), 1.62 for 1.00≤HOMA-IR<1.50 (n=620), 2.92 for 1.50≤HOMA-IR<2.00 (n=349), 3.37 for 2.00≤HOMA-IR<2.50 (n=151) and 8.15 for 2.50≤HOMA-IR (n=163), with each group having 20, 10, 10, 5 and 13 cardiovascular events, respectively. The age-adjusted hazard ratio for cardiovascular events compared with HOMA-IR<1.00 was 1.10 (0.52–2.36) for 1.00≤HOMA-IR<1.50, 2.07 (0.97–4.43) for 1.50≤HOMA-IR<2.00, 2.37 (0.89–6.32) for 2.00≤HOMA-IR<2.50 and 5.83 (2.90–11.74) for 2.50≤HOMA-IR; the multivariate-adjusted hazard ratio was 1.07 (0.48–2.36), 1.95 (0.84–4.53), 2.51 (0.85–7.48) and 5.54 (2.33–13.15), respectively.

HOMA-IR and the risk of cardiovascular events in patients grouped according to blood pressure, lipids, abdominal obesity or smoking status The associations observed in the overall population were broadly similar in participants with and without hypertension, dyslipidaemia, abdominal obesity or current smoking (Table 2). There was no significant interaction between increased HOMA-IR and any of these four factors with regard to the risk of cardiovascular events (p values for interaction, see Table 2).

Discussion

The present cohort study demonstrated a positive relationship between HOMA-IR and the risk of a first-ever cardiovascular event in middle-aged Japanese men who were apparently free of diabetes, adjusting for major cardiovascular risk factors.

Table 1 Baseline risk characteristics of the 2,548 non-diabetic men participants in Toyama, Japan (1996) grouped by quartile of HOMA-IR

Characteristic	HOMA-IR				*p* value for difference[b]
	1st quartile (0.18–0.66)	2nd quartile (0.67–1.01)	3rd quartile (1.02–1.51)	4th quartile (1.52–18.73)	
Participants (*n*)	649	629	624	646	
Age (years)	45.7±6.5	45.3±6.2	44.9±6.5	44.3±6.5	<0.01
HOMA-IR[a]	0.48 (0.42–0.62)	0.84 (0.76–0.91)	1.24 (1.12–1.38)	1.98 (1.71–2.52)	
Height (cm)	166.9±6.3	167.7±6.2	168.2±5.7	168.4±5.7	<0.01
Weight (kg)	60.3±7.3	63.9±7.4	67.1±7.7	71.0±8.5	<0.01
BMI (kg/m^2)	21.6±2.2	22.7±2.3	23.7±2.4	25.0±2.6	<0.01
Waist circumference (cm)	75.3±6.3	78.5±6.6	81.4±6.6	84.7±7.0	<0.01
Cigarette smoking habits (%)					
Never	22.5	30.2	33.2	32.8	<0.01
Former	7.9	12.1	11.5	14.2	
Current	69.6	57.7	55.3	52.9	
Alcohol drinking habits (%)					
None	20.3	20.7	22.1	27.2	0.01
Occasional	28.0	32.6	31.9	31.1	
Light	28.8	28.5	28.5	25.5	
Heavy	22.8	18.3	17.5	16.1	
Leisure-time physical activity (%)					
None	65.5	64.1	65.7	69.7	0.02
Light	16.5	21.1	21.3	19.2	
Moderate	12.0	10.7	9.6	7.6	
Hard	6.0	4.1	3.4	3.6	
Systolic BP (mmHg)	118.8±13.1	120.6±13.4	122.5±14.0	124.9±14.3	<0.01
Diastolic BP (mmHg)	74.6±9.9	76.2±10.2	77.0±10.6	78.8±10.4	<0.01
Medication for hypertension (%)	2.9	4.3	4.5	7.9	<0.01
Serum total cholesterol (mmol/l)	5.07±0.79	5.32±0.84	5.33±0.89	5.43±0.85	<0.01
Serum non-HDL-cholesterol (mmol/l)	3.51±0.82	3.81±0.85	3.96±0.91	4.16±0.86	<0.01
Hypercholesterolaemia medication (%)	0.5	0.8	2.1	1.4	0.04
Serum triacylglycerol (mmol/l)[a]	0.90 (0.68–1.24)	1.02 (0.77–1.42)	1.22 (0.89–1.70)	1.53 (1.07–2.15)	<0.01
Serum HDL-cholesterol (mmol/l)	1.56±0.42	1.51±0.40	1.38±0.35	1.27±0.33	<0.01
Fasting plasma glucose (mmol/l)[a]	4.77 (4.50–4.94)	5.00 (4.61–5.27)	5.00 (4.77–5.27)	5.16 (4.88–5.55)	<0.01
Fasting plasma insulin (pmol/l)[a]	13.89 (13.89–20.84)	27.78 (20.84–27.78)	41.67 (34.73–41.67)	62.51 (48.62–76.40)	<0.01
HbA_{1c} (%)	4.99±0.33	5.01±0.32	5.00±0.34	5.03±0.33	0.12

Values are expressed as mean ± SD, median (interquartile range) or per cent of participants in the respective category

[a] Median is presented due to a skewed distribution

[b] One-way analysis of variance, Kruskal–Wallis test or χ^2 test to compare each risk characteristic among the quartiles of HOMA-IR

Similar positive relationships were observed for coronary events and stroke. This pattern was broadly similar regardless of the presence or absence of other relevant metabolic disorders (hypertension and dyslipidaemia), abdominal obesity and smoking, with no evidence of an interaction effect between increased HOMA-IR and each of these four factors on the risk of cardiovascular events. To the best of our knowledge, this is the first prospective survey that shows a

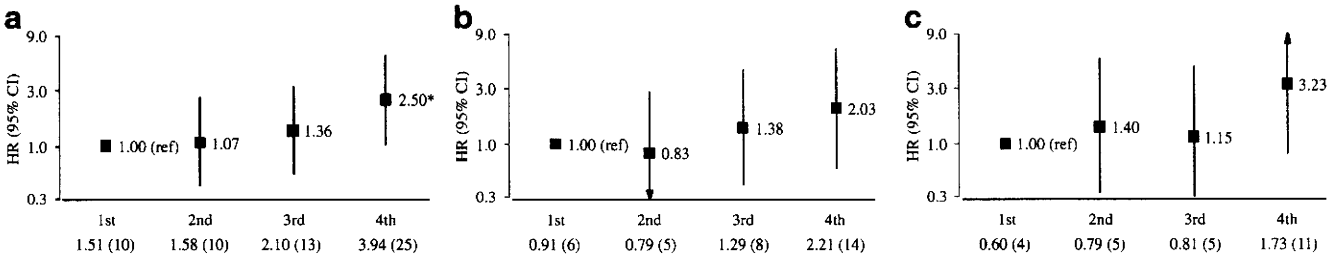

Fig. 1 Hazard ratios for the incidence of (**a**) cardiovascular events, (**b**) coronary events and (**c**) stroke in each quartile of HOMA-IR in 2,548 men over 11 years of follow-up (1996–2007). A Cox proportional hazards regression model was used with adjustment for age, waist circumference, smoking habits, drinking habits, leisure-time physical activity, systolic blood pressure, medication for hypertension, serum non-HDL-cholesterol, medication for hypercholesterolaemia, log-serum triacylglycerol, serum HDL-cholesterol and HbA_{1c}. The ranges of the first (n=649), second (n=629), third (n=624) and fourth (n=646) quartiles of HOMA-IR were 0.18–0.66, 0.67–1.01, 1.02–1.51 and 1.52–18.73, respectively. Values, x-axes are crude incidence rates per 1,000 person-years (n events). y-Axes are $\log_3$ scale. *$p<0.05$. ref, reference

significantly positive relationship between HOMA-IR and the risk of coronary events and stroke in an Asian population, avoiding the potential confounding effect of other relevant metabolic disorders on the risk of cardiovascular events. Although a previous Chinese study examined the relationship between HOMA-IR and the risk of cardiovascular events, that study reported a positive trend, which did not reach statistical significance [36].

Hedblad et al. [19] reported that non-diabetic individuals with the 75th percentile value of the distribution of HOMA-IR (≥2.12 for men, ≥1.80 for women) of their study population had a significantly higher risk of myocardial infarction than those without these HOMA-IR values, after adjustment for traditional risk factors including fasting glucose. In addition, the Bruneck study [20] reported a similar relationship between HOMA-IR and the risk of cardiovascular events. Furthermore, the San Antonio Heart Study [21] reported that non-diabetic individuals with HOMA-IR ≥2 (which was close to the median value) were at increased risk of coronary artery disease and stroke compared with those with HOMA-IR <2. Our results are consistent with the findings of these previous Western studies. An important finding of our study was that increased HOMA-IR can predict subsequent coronary events and stroke in Asians, in whom stroke is the predominant subtype of cardiovascular event and the ratio of ischaemic stroke:haemorrhagic stroke differs from that in Whites [49]. In addition, our data suggest an apparent increase in the risk of cardiovascular events with an HOMA-IR of about 1.5, although the cardiovascular risk remains unchanged below 1.5. Interestingly, our findings support a previous Japanese study, which suggested that a HOMA-IR value of 1.73 was the appropriate cut-off level for insulin resistance [44]. However, further studies are required to provide more detailed information on this issue.

Our stratified analyses further emphasise that insulin resistance with compensatory hyperinsulinaemia has an effect on development of the diseases studied that is distinct from that of other relevant metabolic disorders. In theory, even isolated insulin resistance without any other relevant metabolic disorders may predict subsequent coronary events and stroke. Consequently, measures to reverse insulin resistance in addition to the management of traditional cardiovascular risk factors may improve the overall cardiovascular risk profile, particularly in non-diabetic individuals. In addition, insulin resistance and abdominal obesity may play independent roles, at least in part, in the development of cardiovascular disease, although obesity is closely associated with insulin resistance [24, 46]. Our observations are consistent with the findings of the San Antonio Heart Study [21]. However, the present study did not elucidate the underlying mechanism for the possible causal relationship between insulin resistance with compensatory hyperinsulinaemia and cardiovascular events. It is also unlikely that smoking and insulin resistance have a synergistic effect on the development of cardiovascular disease.

Our study has several limitations. First, as our study participants consisted solely of male workers in one factory, caution should be exercised when generalising our results. Second, only participants who were apparently free of diabetes at baseline were included in the analyses. This inclusion was based on fasting glucose <6.11 mmol/l and HbA_{1c} <5.8% [41], because we had no data on plasma glucose and insulin after glucose loading. Third, no information was available on other factors that affect fasting insulin, e.g. the presence of an insulin-producing tumour. Finally, coronary events included only cases of angina pectoris requiring coronary intervention; medication-managed cases of angina pectoris were excluded. Furthermore, we were not able to divide stroke into ischaemic and haemorrhagic types in our study due to the relatively small numbers of each event.

In conclusion, our data suggest that HOMA-IR is a useful index for prediction of subsequent coronary events and stroke in a non-diabetic Japanese male population. In addition, insulin resistance with compensatory hyperinsulinaemia is

Table 2 Hazard ratios for the incidence of cardiovascular events in the third and fourth quartiles of HOMA-IR compared with the combination of the first and second quartiles in 2,548 non-diabetic men over 11 years of follow-up (1996–2007)

Variable	HOMA-IR by quartile			Log-HOMA-IR	*p* value for interaction[c]
	1st+2nd (0.18–1.01)	3rd (1.02–1.51)	4th (1.52–18.73)	1 SD (0.61) increment	
Overall					
Events/participants (*n*/*n*)	20/1,278	13/624	25/646		
Crude rate per 1,000 person-years	1.54	2.10	3.94		
Multivariate-adjusted HR (95% CI)[a]	1.00 (reference)	1.31 (0.63–2.73)	2.40 (1.16–4.94)	1.51 (1.13–2.02)	
Absence of hypertension					
Events/participants (*n*/*n*)	9/886	3/393	14/362		
Crude rate per 1,000 person-years	1.00	0.77	3.91		
Multivariate-adjusted HR (95% CI)[a]	1.00 (reference)	0.68 (0.18–2.61)	3.09 (1.11–8.62)	1.34 (0.90–1.99)	
Presence of hypertension[b]					0.42
Events/participants (*n*/*n*)	11/392	10/231	11/284		
Crude rate per 1,000 person-years	2.80	4.37	3.98		
Multivariate-adjusted HR (95% CI)[a]	1.00 (reference)	1.84 (0.73–4.64)	1.80 (0.64–5.08)	1.69 (1.08–2.64)	
Absence of dyslipidaemia					
Events/participants (*n*/*n*)	15/1,061	6/426	14/322		
Crude rate per 1,000 person-years	1.39	1.40	4.43		
Multivariate-adjusted HR (95% CI)[a]	1.00 (reference)	0.81 (0.29–2.23)	3.02 (1.22–7.44)	1.69 (1.16–2.47)	
Presence of dyslipidaemia[b]					0.26
Events/participants (*n*/*n*)	5/217	7/198	11/324		
Crude rate per 1,000 person-years	2.33	3.66	3.45		
Multivariate-adjusted HR (95% CI)[a]	1.00 (reference)	1.89 (0.58–6.20)	1.65 (0.51–5.35)	1.19 (0.76–1.87)	
Absence of abdominal obesity					
Events/participants (*n*/*n*)	17/1,114	9/433	11/331		
Crude rate per 1,000 person-years	1.50	2.08	3.38		
Multivariate-adjusted HR (95% CI)[a]	1.00 (reference)	1.26 (0.54–2.97)	1.96 (0.82–4.66)	1.47 (1.01–2.13)	
Presence of abdominal obesity[b]					0.91
Events/participants (*n*/*n*)	3/164	4/191	14/315		
Crude rate per 1,000 person-years	1.88	2.14	4.54		
Multivariate-adjusted HR (95% CI)[a]	1.00 (reference)	1.21 (0.26–5.58)	3.85 (0.93–15.93)	1.72 (1.04–2.84)	
Absence of current smoking					
Events/participants (*n*/*n*)	4/463	1/279	10/304		
Crude rate per 1,000 person-years	0.85	0.36	3.22		
Multivariate-adjusted HR (95% CI)[a]	1.00 (reference)	0.48 (0.05–4.58)	4.60 (1.11–19.17)	2.06 (1.20–3.54)	
Presence of current smoking					0.76
Events/participants (*n*/*n*)	16/815	12/345	15/342		
Crude rate per 1,000 person-years	1.94	3.52	4.51		
Multivariate-adjusted HR (95% CI)[a]	1.00 (reference)	1.56 (0.70–3.46)	1.79 (0.76–4.22)	1.38 (0.97–1.94)	

Data are presented for the total study population (overall) and also grouped according to characteristics as indicated

[a] Cox proportional hazards regression model with multivariate adjustment for age, waist circumference, smoking habits, drinking habits, leisure-time physical activity, systolic blood pressure, medication for hypertension, serum non-HDL-cholesterol, medication for hypercholesterolaemia, log-serum triacylglycerol, serum HDL-cholesterol and HbA_{1c}

[b] Definitions based on the Japanese criteria for metabolic syndrome; hypertension was defined as systolic blood pressure ≥130 mmHg, diastolic blood pressure ≥85 mmHg and/or taking medication for hypertension; dyslipidaemia was defined as triacylglycerol ≥1.69 mmol/l and/or HDL-cholesterol <1.03 mmol/l; abdominal obesity was defined as waist circumference ≥85 cm [45]

[c] The significance of the interaction effect between increased HOMA-IR and each of the four factors on the risk of cardiovascular events was tested using an interaction term for the categorical variables in the Cox model

likely to have an effect on the development of cardiovascular disease separately from other relevant metabolic disorders. Given that HOMA-IR is calculated after the assessment of traditional cardiovascular risk factors and the measurement of fasting insulin, HOMA-IR could provide additional information that could improve overall prediction of cardiovascular risk.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

References

1. Facchini FS, Hua N, Abbasi F, Reaven GM (2001) Insulin resistance as a predictor of age-related diseases. J Clin Endocrinol Metab 86:3574–3578
2. Bonora E, Kiechl S, Willeit J et al (2004) Population-based incidence rates and risk factors for type 2 diabetes in white individuals: the Bruneck study. Diabetes 53:1782–1789
3. Zavaroni I, Bonora E, Pagliara M et al (1989) Risk factors for coronary artery disease in healthy persons with hyperinsulinemia and normal glucose tolerance. N Engl J Med 320:702–706
4. Mitchell BD, Haffner SM, Hazuda HP, Valdez R, Stern MP (1992) The relation between serum insulin levels and 8-year changes in lipid, lipoprotein, and blood pressure levels. Am J Epidemiol 136:12–22
5. Haffner SM, Valdez RA, Hazuda HP, Mitchell BD, Morales PA, Stern MP (1992) Prospective analysis of the insulin-resistance syndrome (syndrome X). Diabetes 41:715–722
6. Yip J, Facchini FS, Reaven GM (1998) Resistance to insulin-mediated glucose disposal as a predictor of cardiovascular disease. J Clin Endocrinol Metab 83:2773–2776
7. Reaven GM (1988) Banting lecture 1988. Role of insulin resistance in human disease. Diabetes 37:1595–1607
8. DeFronzo RA, Ferrannini E (1991) Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Diab Care 14:173–194
9. Alberti KG, Zimmet PZ (1998) Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation. Diabet Med 15:539–553
10. Balkau B, Charles MA (1999) Comment on the provisional report from the WHO consultation. European Group for the Study of Insulin Resistance (EGIR). Diabet Med 16:442–443
11. Isomaa B, Almgren P, Tuomi T et al (2001) Cardiovascular morbidity and mortality associated with the metabolic syndrome. Diab Care 24:683–689
12. Meigs JB, Rutter MK, Sullivan LM, Fox CS, D'Agostino RB Sr, Wilson PW (2007) Impact of insulin resistance on risk of type 2 diabetes and cardiovascular disease in people with metabolic syndrome. Diab Care 30:1219–1225
13. Lakka HM, Laaksonen DE, Lakka TA et al (2002) The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. JAMA 288:2709–2716
14. Ninomiya T, Kubo M, Doi Y et al (2007) Impact of metabolic syndrome on the development of cardiovascular disease in a general Japanese population: the Hisayama study. Stroke 38:2063–2069
15. Zethelius B, Lithell H, Hales CN, Berne C (2005) Insulin sensitivity, proinsulin and insulin as predictors of coronary heart disease. A population-based 10-year, follow-up study in 70-year old men using the euglycaemic insulin clamp. Diabetologia 48:862–867
16. Pyörälä M, Miettinen H, Laakso M, Pyörälä K (2000) Plasma insulin and all-cause, cardiovascular, and noncardiovascular mortality: the 22-year follow-up results of the Helsinki Policemen Study. Diab Care 23:1097–1102
17. Kiyohara Y, Fujishima M (1997) Insulin resistance and cardiovascular disease incidence in a elderly Japanese population: the Hisayama study. Jpn J Geriatr 34:360–364 (article in Japanese)
18. Fujiwara T, Saitoh S, Takagi S et al (2005) Development and progression of atherosclerotic disease in relation to insulin resistance and hyperinsulinemia. Hypertens Res 28:665–670
19. Hedblad B, Nilsson P, Engström G, Berglund G, Janzon L (2002) Insulin resistance in non-diabetic subjects is associated with increased incidence of myocardial infarction and death. Diabet Med 19:470–475
20. Bonora E, Kiechl S, Willeit J et al (2007) Insulin resistance as estimated by homeostasis model assessment predicts incident symptomatic cardiovascular disease in Caucasian subjects from the general population: the Bruneck study. Diab Care 30:318–324
21. Hanley AJG, Williams K, Stern MP, Haffner SM (2002) Homeostasis model assessment of insulin resistance in relation to the incidence of cardiovascular disease: the San Antonio Heart Study. Diab Care 25:1177–1184
22. Barr EL, Cameron AJ, Balkau B et al (2010) HOMA insulin sensitivity index and the risk of all-cause mortality and cardiovascular disease events in the general population: the Australian Diabetes, Obesity and Lifestyle Study (AusDiab) study. Diabetologia 53:79–88
23. Bonora E, Kiechl S, Willeit J et al (1998) Prevalence of insulin resistance in metabolic disorders: the Bruneck Study. Diabetes 47:1643–1649
24. Ohnishi H, Saitoh S, Ura N et al (2002) Relationship between insulin resistance and accumulation of coronary risk factors. Diabetes Obes Metab 4:388–393
25. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28:412–419
26. Bonora E, Targher G, Alberiche M et al (2000) Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. Diab Care 23:57–63
27. Resnick HE, Jones K, Ruotolo G et al (2003) Insulin resistance, the metabolic syndrome, and risk of incident cardiovascular disease in nondiabetic American Indians: the Strong Heart Study. Diab Care 26:861–867
28. Robins SJ, Rubins HB, Faas FH et al (2003) Insulin resistance and cardiovascular events with low HDL cholesterol: the Veterans Affairs HDL Intervention Trial (VA-HIT). Diab Care 26:1513–1517
29. Alssema M, Dekker JM, Nijpels G, Stehouwer CD, Bouter LM, Heine RJ (2005) Proinsulin concentration is an independent predictor of all-cause and cardiovascular mortality: an 11-year follow-up of the Hoorn Study. Diab Care 28:860–865

30. Rutter MK, Meigs JB, Sullivan LM, D'Agostino RB Sr, Wilson PW (2005) Insulin resistance, the metabolic syndrome, and incident cardiovascular events in the Framingham Offspring Study. Diabetes 54:3252–3257
31. Dekker JM, Girman C, Rhodes T et al (2005) Metabolic syndrome and 10-year cardiovascular disease risk in the Hoorn Study. Circulation 112:666–673
32. Onat A, Hergenç G, Türkmen S, Yazici M, Sari I, Can G (2006) Discordance between insulin resistance and metabolic syndrome: features and associated cardiovascular risk in adults with normal glucose regulation. Metabolism 55:445–452
33. Jeppesen J, Hansen TW, Rasmussen S, Ibsen H, Torp-Pedersen C, Madsbad S (2007) Insulin resistance, the metabolic syndrome, and risk of incident cardiovascular disease: a population-based study. J Am Coll Cardiol 49:2112–2119
34. Jeppesen J, Hansen TW, Olsen MH et al (2008) C-reactive protein, insulin resistance and risk of cardiovascular disease: a population-based study. Eur J Cardiovasc Prev Rehabil 15:594–598
35. Adachi H, Hirai Y, Tsuruta M, Fujiura Y, Imaizuml T (2001) Is insulin resistance or diabetes mellitus associated with stroke? An 18-year follow-up study. Diabetes Res Clin Pract 51:215–223
36. Chien KL, Hsu HC, Su TC, Chen MF, Lee YT, Hu FB (2008) Fasting and postchallenge hyperglycemia and risk of cardiovascular disease in Chinese: the Chin-Shan Community Cardiovascular Cohort study. Am Heart J 156:996–1002
37. Sakurai M, Miura K, Takamura T et al (2006) Gender differences in the association between anthropometric indices of obesity and blood pressure in Japanese. Hypertens Res 29:75–80
38. Sakurai M, Miura K, Nakamura K et al (2009) Relationship between abdominal obesity, accumulation of metabolic abnormalities and risk of cardiovascular disease: an 11-year follow-up of middle-aged Japanese men. Jpn J Cardiovasc Dis Prev 44:1–9 (article in Japanese)
39. Kang ES, Yun YS, Park SW et al (2005) Limitation of the validity of the homeostasis model assessment as an index of insulin resistance in Korea. Metabolism 54:206–211
40. Sakai K, Nakamaru K, Miyagawa K, Yamaguchi K (2007) Simple indices of insulin resistance and their limits in clinical application. J Japan Diab Soc 50:725–730 (article in Japanese)
41. The Japan Diabetes Society (2008) Guideline for diabetes treatment 2008–2009. Bunkodo, Tokyo (book in Japanese)
42. Tunstall-Pedoe H, Kuulasmaa K, Amouyel P, Arveiler D, Rajakangas AM, Pajak A (1994) Myocardial infarction and coronary deaths in the World Health Organization MONICA Project. Registration procedures, event rates, and case-fatality rates in 38 populations from 21 countries in four continents. Circulation 90:583–612
43. Friedewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18:499–502
44. Oimatsu H, Saitoh S, Ura N, Shimamoto K (2000) A practical index for evaluation of insulin resistance. J Japan Diab Soc 43:205–213 (article in Japanese)
45. No authors listed (2005) Definition and the diagnostic standard for metabolic syndrome—Committee to Evaluate Diagnostic Standards for Metabolic Syndrome. J Jpn Soc Int Med 94:794–809 (article in Japanese)
46. Ludvik B, Nolan JJ, Baloga J, Sacks D, Olefsky J (1995) Effect of obesity on insulin resistance in normal subjects and patients with NIDDM. Diabetes 44:1121–1125
47. Hozawa A, Okamura T, Murakami Y et al (2007) Joint impact of smoking and hypertension on cardiovascular disease and all-cause mortality in Japan: NIPPON DATA80, a 19-year follow-up. Hypertens Res 30:1169–1175
48. Rönnemaa T, Rönnemaa EM, Puukka P, Pyörälä K, Laakso M (1996) Smoking is independently associated with high plasma insulin levels in nondiabetic men. Diab Care 19:1229–1232
49. Ueshima H, Sekikawa A, Miura K et al (2008) Cardiovascular disease and risk factors in Asia: a selected review. Circulation 118:2702–2709

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Original Article

Occupational Class and Incidence Rates of Cardiovascular Events in Middle Aged Men in Japan

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Abstract: We investigated whether occupational class affects the incidence of cardiovascular events in Japanese factory workers. We prospectively evaluated 1,794 male workers aged 40–59, including 632 non-manual and 1,162 manual workers, employed in a metal products factory in Japan. The hazard ratios of stroke, myocardial infarction (MI) and cardiovascular events (combined stroke, MI and sudden cardiac death) for manual workers were compared with non-manual workers as estimated by the Cox proportional hazards regression model. Among the 1,794 workers, there were 60 cardiovascular events (32 cases of stroke, 23 cases of MI and 5 cases of sudden cardiac death) with an incidence rate of 3.14 per 1,000 person-years for cardiovascular events; 1.68 for stroke and 1.20 for MI. Blood pressure and HbA1c significantly increased the risk of stroke. Body mass index and total cholesterol significantly increased the risk of MI. However, occupational class was determined not to be a risk factor for cardiovascular events. The hazard ratios of stroke, MI and cardiovascular events for manual workers compared with non-manual workers were 0.97 (95%CI, 0.45–2.08), 0.73 (95%CI, 0.30–1.79) and 0.92 (95%CI, 0.53–1.61), respectively. Our study did not reveal significant occupational class inequalities in the rate of cardiovascular events. These findings are not in accordance with studies from other industrialized countries.

Key words: Stroke, Myocardial infarction, Cardiovascular events, Occupational class, Prospective study

Introduction

Studies in European countries[1–9] have revealed inequalities in mortality and incidence rate of cardiovascular disease by occupational class, with a higher rate found in manual workers compared to non-manual workers. Widening relative health inequalities among occupational classes were also reported[3, 9]. Moreover, results from the Whitehall study showed a clear inverse relationship between employment grade and coronary heart disease mortality and morbidity in civil servants with rel-

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atively stable employment[10–12]. The inequalities of traditional cardiovascular risk factors, such as smoking and high blood pressure, and psychosocial factors were considered to be mediating factors for such health inequalities[6–8, 10–12].

In Japan, much effort was been expended to establish biological and behavioral risk factors for stroke and myocardial infarction[13–16]. Risk factors such as blood pressure, metabolic factors and smoking are not different from those of European countries and the US. However, little information on how occupational class inequalities relate to cardiovascular disease is available in Japan. A multi-center, community-based cohort study from Japan did not show significant differences in mortality from cardiovascular disease between office workers and manual workers, although there was a significant difference in mortality from stroke in the self-employed versus the employed[17].

In the present study, we analysed incidence rates of cardiovascular events including stroke, MI and sudden cardiac death in a Japanese cohort of middle-aged employees over a 12-yr period comparing manual and non-manual workers. The aim of this study was to investigate whether occupational class (manual versus non-manual workers) affected the incidence rate of cardiovascular events in Japan.

Methods

Study population

Ethics approval was obtained from the Kanazawa Medical University Epidemiological Research Ethics Committee, Ishikawa, Japan. The study population consisted of Japanese men employed by a light metal factory in Toyama Prefecture, Japan. This factory employed 4,440 male and 2,776 female employees. Among the 1,967 male workers aged 40–59, 1,920 workers who underwent a medical checkup and a questionnaire in 1994 (participation rate, 97.6%), were enrolled as study participants. Of the 1,920 participants, 126 workers who had a history of cardiovascular events or who provided insufficient information at baseline were excluded. The cohort consisted of 1,794 men. Subjects were followed annually until they were diagnosed with a cardiovascular event or until the end of 2006.

Occupational category

Subjects were categorized as non-manual workers or manual workers according to their occupation. Information on occupation at baseline was sorted into seven categories based on the company's own classification; i.e., managers, engineers, clerks, salesmen, laborers, transport workers, and others. This was compatible with the Japanese Standard Occupational Classification[18]. Employees classified as managers included workers who acted as general supervisors. Engineers were system engineers and workers engaged in developing new products. The transport workers were mainly engaged in picking up and delivering employees, driving relatively short distances to deliver materials and goods, and operating cranes. Laborers were involved in the operation of machines and the construction or processing of aluminum products. The 'others' category included guards, gardeners, shop-persons at the branch factory, and individuals engaged in managing dormitories and catering. Non-manual workers consisted of managers, engineers, clerks, and salesmen, with the remaining individuals (laborers, transport workers, and workers in the 'others' category) considered manual workers.

Baseline examination

Anthropometric data and biomarkers were obtained from all subjects in 1994. Body weight was measured with the subject wearing light clothing without shoes using a standard scale and height and was measured to the nearest 0.1 cm, without socks, using a stadiometer. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured with a mercury sphygmomanometer on the right arm of the subject while he was seated after 5 min of rest. Mean blood pressure (MBP) was obtained by the formula: $MBP = (SBP + DBP \times 2)/3$. A fasting blood sample was taken from each subject at least 10 h after his last meal. Laboratory tests were performed by a laboratory test center. Serum total cholesterol (TCh) and glycated hemoglobin A1c (HbA1c) were measured by enzyme assay and latex agglutination method, respectively.

A self-administered questionnaire was used to collect information concerning working hours, mental and physical work load, and lifestyle, including smoking habits, drinking habits, and leisure time physical activity. Working hours were classified into two groups: ≥10 h and <10 h per day. Physical and mental work load were assessed using a single questionnaire and classified into either heavy work or others. Smoking habit was classified into either smoking or non-smoking. Drinking habit was measured by the frequency of drinking per week and classified into two categories: <5 times/wk or ≥5 times/wk. Leisure-time physical activity was classified into either lack of regular leisure time physical activity (participation <1 time per week) or regular leisure-time physical activity.

Follow-up and endpoint determination

Incidence of cardiovascular events were ascertained

until December 2006. Among 1,794 subjects, 1,331 remained in the company and 463 retired or left the factory before the end of the follow-up period. For subjects who stayed with the target company, histories taken at the annual health checkup and medical certification for sickness absence were used for the follow-up. For the 463 retired participants, questionnaires regarding history of cardiovascular events were sent annually by mail. Thirty-one subjects (6.7%) were lost from the 463 retired workers or from those who left the factory before the end of follow-up period, including 8.0% for manual workers and 3.6% for non-manual workers. In the case of deceased subjects, information was obtained from the subjects' families. To confirm the diagnosis, hospital medical records were reviewed with subjects' consent. For some deceased subjects, death certificates were referenced.

The criteria for MI were modified from those of the WHO (MONICA Project)[19]. Definite MI was indicated by typical chest pain, with the appearance of abnormal and persistent Q or QS waves, changes in cardiac enzyme activity, or both. Probable MI was indicated by typical chest pain when the findings of electrocardiograms or enzyme activity were not available. Sudden cardiac death was defined as death within 1 h of onset, a witnessed cardiac arrest, or abrupt collapse.

Stroke was defined as a focal neurological disorder with rapid onset, which persisted at least 24 h or until death. Stroke events were classified as cerebral hemorrhage, cerebral infarction, or subarachnoid hemorrhage based on computed tomography (CT) and magnetic resonance imaging (MRI). Stroke cases without imaging studies were subclassified according to clinical criteria. The proportion of stroke cases confirmed by CT and MRI was 91.7%.

Cardiovascular events included stroke, definite or probable MI and sudden cardiac death.

Statistical analysis

The means of age, BMI, BP, HbA1c, and TCh at the baseline examination were calculated and tested by t test according to occupational category (manual *vs.* non-manual). Smoking habit, drinking habit, and leisure time physical activity were tested by χ^2 test according to occupational category. The incidence rates of stroke, MI and cardiovascular events were expressed per 1,000 person-years. The incidence rate and its 95% confidence interval (CI) were computed by the approximate Poisson Method[20]. Age-adjusted rates were calculated with indirect methods of standardization using all workers as a standard population. In the multivariate analysis, the hazard ratios and 95%CI for stroke, MI and cardiovascular events were estimated by the Cox's proportional hazards regression model, after adjusting for confounding factors, including age, BMI, MBP, HbA1c, TCh, smoking, drinking habit, and leisure time physical activity. In the analysis, biological confounding factors were used as continuous variables, irrespective of medication. The analyses were performed using SPSS 16.0 software (SPSS Inc, Chicago, IL, USA) with $p<0.05$ considered statistically significant.

Results

The total number of subjects and their baseline characteristics by occupational class are shown in Table 1. There were 632 non-manual workers and 1,162 manual workers. The mean age of the non-manual workers was significantly younger than that of the manual workers. The baseline BMI, SBP, DBP and TCh were similar between the two groups, but the HbA1c level of the manual workers was significantly higher. The difference between non-manual and manual workers with regards to behavioral risk factors, such as smoking and drinking habits, was not significant. The prevalence of lack of regular leisure time physical activity was higher for manual workers than for non-manual workers. The prevalence of long work hours and heavy mental work load were higher for non-manual workers than manual workers.

Table 2 shows the incidence rates of stroke, MI and cardiovascular events combined stroke, MI and sudden cardiac death according to occupational class. In the 12-yr follow-up period, there were 60 cardiovascular events, with an incidence rate of 3.14 per 1,000 person-years. The mean follow-up time to an event was 5.6 yr. Among these 60 cases, 23 were classified as definite MI and 5 sudden cardiac deaths, and 32 were classified as stroke, including 25 cases of cerebral infarction, 5 cases of cerebral hemorrhage and 2 cases of subarachnoid hemorrhage. There were 23 events (11 stroke, 10 MI and 2 sudden cardiac deaths) in non-manual workers and 37 events (21 stroke, 13 MI and 3 sudden cardiac deaths) in manual workers. For the manual workers, the incidence rate of stroke was similar to non-manual workers, but the incidence rate of MI was lower. All age-adjusted incidence rates of cardiovascular events, stroke, and MI between the two groups were not significantly different.

In Table 3 we show the hazard ratios of the biological factors, health-related behavioral factors and work related factors for stroke, MI and cardiovascular events using the Cox proportional hazards regression model. In the Cox model, age, MBP and HbA1c significantly increased the risk of stroke. BMI and Tch significantly increased the risk of MI. Smoking was suggested as a risk factor for MI. Age, MBP and HbA1c increased the risk of cardiovascular events, and habitual drinking significantly decreased the risk of cardiovascular events and MI.

Table 1. Baseline characteristics of 1,794 male subjects according to occupational class

Characteristics	All subjects	Occupational category		$p^{\dagger}$
		Non-manual workers	Manual workers	
No. of subjects	1,794	632	1,162	
Age (yr)	47.6 (4.9)	47.0 (4.8)	47.9 (5.0)	0.001
Body mass index (kg/m^2)	22.9 (2.7)	23.0 (2.6)	22.8 (2.7)	0.190
Diastolic blood pressure (mm Hg)	76.5 (11.3)	76.1 (11.5)	76.7 (11.2)	0.307
Systolic blood pressure (mm Hg)	120.4 (15.5)	120.2 (15.2)	120.4 (15.7)	0.788
Mean blood pressure (mm Hg)	91.1 (11.8)	90.8 (11.9)	91.3 (11.8)	0.443
Glycosylated hemoglobin A1c (%)	5.18 (0.62)	5.14 (0.61)	5.20 (0.63)	0.043
Total cholesterol (mg/dl)	204.7 (34.5)	202.8 (33.7)	205.7 (35.0)	0.098
Smoking habit (smokers, %)	58.4	58.7	58.3	0.856
Drinking habit (≥ 5 times/wk, %)	54.1	54.7	53.7	0.684
Physical activity (<1 time/wk, %)	57.2	51.5	60.1	0.001
Working hours (more than 10 h, %)	6.3	14.1	2.3	0.000
Physical working load (heavy, %)	11.3	4.6	14.8	0.000
Mental working load (heavy, %)	21.8	27.7	18.7	0.000

*Values are the means (standard deviations) for continuous variables and percentages for categorical variables.
†Significant difference according to Student's t test for means or χ^2 test for frequencies.

Table 2. Incidence rate per 1,000 person-years of cardiovascular events, stroke and myocardial infarction during 12-yr follow-up by occupational class

	N	Person-years	Cases	Crude incidence rate	Adjusted incidence rate†	95%CI
Cardiovascular events						
All subjects	1,794	19,093	60	3.14	—	—
Non-manual workers	632	6,600	23	3.48	3.63	2.46–5.88
Manual workers	1,162	12,493	37	2.96	2.90	1.81–3.53
Stroke						
All subjects	1,794	19,093	32	1.68	—	—
Non-manual workers	632	6,600	11	1.67	1.73	0.73–2.85
Manual workers	1,162	12,493	21	1.68	1.65	0.92–2.31
Myocardial infarction						
All subjects	1,794	19,093	23	1.20	—	—
Non-manual workers	632	6,600	10	1.52	1.58	0.79–3.35
Manual workers	1,162	12,493	13	1.04	1.02	0.39–1.33

CI; confidence interval.
Cardiovascular events: combined stroke, myocardial infarction and sudden cardiac death.
†Adjusted for age, calculated by the indirect method of standardization using all workers as a standard population.

Table 4 shows the hazard ratios of stroke, MI and cardiovascular events for manual workers compared to non-manual workers using the Cox's proportional hazards regression model. In the model, the hazard ratio of cardiovascular events for manual workers compared to non-manual workers was 0.80 (95%CI, 0.48–1.35) after adjusting for age alone and 0.92 (95%CI, 0.53–1.61) after adjusting for age, BMI, MBP, TCh, and HbA1c and for all confounding factors, including health-related behaviors (smoking, drinking, and leisure time physical activity). The hazard ratio of stroke for manual workers compared to non-manual workers was 0.97 (95%CI, 0.45–2.08) after adjusting for all confounding factors, including biological factors and health-related behaviors. The hazard ratio of MI for manual workers compared to non-manual workers was 0.73 (95%CI, 0.30–1.79) after adjusting for all confounding factors, including biological factors and health-related behaviors. These results did not change after con-

Table 3. Relative risk of the biological factors, health-related behavior and work related characteristics for cardiovascular events, stroke and myocardial infarction according to Cox's Proportional Hazards Regression model

Factor	Category	Cardiovascular events			Stroke			MI		
		HR	95%CI		HR	95%CI		HR	95%CI	
Age (yr)	Linear (each 10 increase)	2.49	1.43–4.34	*	3.50	1.58–7.77	*	1.48	0.61–3.54	
BMI	Linear (each 1 increase)	1.04	0.95–1.15		1.00	0.87–1.14		1.17	1.00–1.27	*
MBP (mmHg)	Linear (each 10 increase)	1.51	1.21–1.88	*	1.82	1.36–2.42	*	1.03	0.70–1.50	
TCh (mg/dl)	Linear (each 10 increase)	1.02	0.95–1.10		0.98	0.88–1.08		1.14	1.02–1.27	*
HbA1c (%)	Linear (each 1 increase)	1.53	1.19–1.97	*	1.66	1.26–2.19	*	1.34	0.77–2.33	
Smoking habit	smokers vs. nonsmokers	1.60	0.90–2.82		1.10	0.51–2.37		2.74	0.98–7.69	+
Drinking habit	≥5 times/wk vs. others	0.51	0.29–0.89	*	0.59	0.28–1.25		0.34	0.13–0.89	*
Physical activity	<1 time/wk vs. others	0.96	0.56–1.66		0.99	0.47–2.07		0.86	0.35–2.10	
Work hours	≥10 h vs. others	1.04	0.32–3.40		1.73	0.23–13.01		0.51	0.11–2.30	
Physical workload	Heavy vs. others	1.25	0.47–3.30		2.04	0.45–9.25		1.80	0.23–14.4	
Mental workload	Heavy vs. others	0.69	0.35–1.34		0.46	0.19–1.11		1.53	0.42–5.57	
Occupational class	Non-manual vs. manual	1.00	0.56–1.79		0.88	0.39–1.95		0.78	0.32–1.94	

All factors were put into the model together. *$p<0.01$, +$p<0.10$.
HR; hazard ratio, CI; confidence interval, HbA1c; glycated hemoglobin A1c, BMI; body mass index, MBP; mean blood pressure, MI; myocardial events, TCh; total cholesterol.
Cardiovascular events: combined stroke, myocardial infarction and sudden cardiac death.

Table 4. Hazard ratios of cardiovascular events, stroke and myocardial infarction by occupational class according to Cox's proportional hazards regression model

	Model 1		Model 2		Model 3	
	HR	95%CI	HR	95%CI	HR	95%CI
Cardiovascular events						
Non-manual workers	1.00	—	1.00	—	1.00	—
Manual workers	0.80	0.48–1.35	0.79	0.47–1.33	0.92	0.53–1.61
Stroke						
Non-manual workers	1.00	—	1.00		1.00	—
Manual workers	0.94	0.45–1.96	0.92	0.44–1.93	0.97	0.45–2.08
Myocardial infarction						
Non-manual workers	1.00	—	1.00	—	1.00	—
Manual workers	0.68	0.32–14.3	0.66	0.29–1.51	0.73	0.30–1.79

HR; relative risk, CI; confidence interval.
Cardiovascular events: combined stroke, myocardial infarction and sudden cardiac death.
Model 1: adjusted for age.
Model 2: adjusted for age (linear), body mass index (linear), mean blood pressure (linear), total cholesterol (linear) and glycated hemoglobin A1c (linear).
Model 3: adjusted for age (linear), body mass index (linear), mean blood pressure (linear), total cholesterol (linear), glycated hemoglobin A1c (linear), and health-related behavior [smoking habit (smokers versus nonsmokers), drinking habit (≥5 times/wk versus others), leisure time physical activity (<1 time/wk versus others)].

trolling for work related factors, including working hours and mental and physical work load.

Discussion

To investigate whether a relationship between occupational class and cardiovascular events exists in Japan as compared to other industrialized nations, we conducted a prospective, 12-yr follow-up study of a group of male factory employees. Incidence rates were 1.68/1,000 person-year for stroke and 1.20/1,000 person-year for MI among all subjects. These rates approximated the rates from other Japanese study populations, such as men in Osaka aged 40–69 (stroke 1.18/1,000 person-year; MI 0.9/1,000 person-year) and men in Akita aged 40–69 (stroke 2.31/1,000 person-year; MI 0.51/1,000 person-year)[21]. If we compare the data for the same age group, the incidence rates in our subjects are higher compared to these

other two geographic areas.

In the present study, manual workers did not show a significant increase in the risk of cardiovascular events compared to non-manual workers. The incidence rate of stroke in both groups was almost identical. The incidence rate of MI in manual workers was lower compared to non-manual workers, but no significant difference was found. Also, the results did not differ after adjusting for behavioral or biological risk factors. Analysis was also performed after sub-categorization of the non-manual workers into two groups; that is, either managers and engineers (n=411) or clerical and sales workers (n=221). We did not find a significantly higher risk of cardiovascular events for manual workers compared with managers and engineers or clerical and sales workers (data not shown). These findings are in disagreement with many studies from other industrialized countries. Stroke mortality and incidence rate of manual workers were significantly higher than those of non-manual workers in European countries and US[1, 2, 4, 7, 9], although a north-south gradient was observed for ischemic heart disease; mortality from ischemic heart disease was strongly related to occupational class in England and Wales, Ireland, Finland, Sweden, Norway and Denmark, but not in France, Switzerland, Spain and Portugal[1].

Other studies have attempted to explain the health inequalities regarding cardiovascular diseases by differences in health behaviors or biological risk factors[6, 8, 10, 11]. These studies showed that members of the manual class had higher blood pressure levels, higher BMI, and increased prevalence of current cigarette smoking. However, we did not find significant differences in those risk factors at baseline between the two groups, except for HbA1c and leisure time physical activity. In a comparative study on socioeconomic differences in behavioral and biomedical risk factors in a Japanese and English cohort of employed men, it was found that higher employment grades in Japan had higher BMI and waist-to-hip-ratios and lower high-density lipoprotein cholesterol levels, while the opposite associations were found among men in England[22, 23]. These results were validated by another study in Japan, using educational background as a socioeconomic factor in the study[24]. A health management system, including health check-ups and a follow-up system to prevent work-related diseases, including cardiovascular disease, was developed based on labor laws enacted in Japan[25]. All workers, particularly those in large enterprises, receive equal benefits from this system regardless of their occupational class. This might partially contribute to the lack of inequality among the traditional risk factors and incidence of cardiovascular events by occupational classes in our subjects.

Psychosocial factors were also considered as contributing to the inequalities between occupational classes and employment grades as they relate to cardiovascular diseases[7, 12]. Researchers found that the lower employment grades suffered more psychosocial distress. We used a simple questionnaire asking for a self-estimation of mental load and work hours instead of the established questionnaires normally used to detect job psychosocial distress. Non-manual workers worked more hours and felt more mental load compared with manual workers. It appeared that the psychosocial distress of manual workers was not higher than non-manual workers.

Our study has several limitations. Our subjects were recruited in one workplace. Our results may therefore reflect the tendency of subjects in a stable and favorable workplace environment. Since there has been little previous research on this topic, more studies are required. In addition, the problem of statistical power due to small sample size cannot be ignored. In addition, our follow-up period may have been too short to acquire an adequate number of subjects in the age range of 40–59. However, the traditional cardiovascular risk factors were determined as significant risks in this study. Therefore, we can say that occupational class did not affect the onset of cardiovascular events to the same degree as the traditional risk factors. Since the mean age of our non-manual workers was younger than that of our manual workers, we may have underestimated the incidence rates of cardiovascular events in non-manual workers even after age adjustment. This could potentially mask inequalities in cardiovascular events between occupational classes, which is opposite to the findings of other industrialized countries. Finally, some might query whether the difference in occupational class contained socioeconomic aspects. We analysed the employment grade after 6 yr from baseline for 1,783 subjects. Our data revealed that there was a significant difference in employment grade between the two groups; 42.7% of non-manual workers were of managerial or administrative rank, compared to only 5.1% of manual workers. Therefore, the occupational category was considered to reflect employment grade.

In conclusion, our findings do not indicate an elevated risk for the development of cardiovascular events in manual workers when compared to non-manual workers in a large-scale factory in Japan. These findings are not in accordance with many studies from other industrialized countries.

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References

1) Kunst AE, Groenhof F, Mackenbach JP, the EU Working Group on Socioeconomic Inequalities in Health (1998) Occupational class and cause specific mortality in middle aged men in 11 European countries: comparison of population based studies. BMJ **316**, 1636–42.
2) Kunst AE, del Rios M, Groenhof F, Mackenbach JP for the European Union Group on Socioeconomic Inequalities in Health (1998) Socioeconomic inequalities in stroke mortality among middle-aged men: an international overview. Stroke **29**, 2285–91.
3) Hallqvist J, Lundberg M, Diderichsen F, Ahlbom A (1998) Socioeconomic differences in risk of myocardial infarction 1971–1994 in Sweden: time trends, relative risks and population attributable risks. Int J Epidemiol **27**, 410–5.
4) Hart CL, Hole DJ, Smith GD (2000) Influence of socioeconomic circumstances in early and later life on stroke risk among men in a Scottish cohort study. Stroke **31**, 2093–7.
5) Smith GD, Hart C, Blane D, Gillis C, Hawthorne V (1997) Lifetime socioeconomic position and mortality: prospective observational study. BMJ **314**, 547–52.
6) Suadicani P, Hein HO, Gyntelberg F (2001) Socioeconomic status and ischaemic heart disease mortality in middle-aged men: importance of the duration of follow-up. The Copenhagen Male Study. Int J Epidemiol **30**, 248–55.
7) Virtanen SV, Notkola V (2002) Socioeconomic inequalities in cardiovascular mortality and the role of work: a register study of Finnish men. Int J Epidemiol **31**, 614–21.
8) Emberson JR, Whincup PH, Morris RW, Morris RW, Walker (2004) Social class differences in coronary heart disease in middle-aged British men: implications for prevention. Int J Epidemiol **33**, 289–96.
9) Mackenbach JP, Bos V, Andersen O, Cardano M, Costa G, Harding S, Reid A, Hemström Ö, Valkonen T, Kunst AE (2003) Widening socioeconomic inequalities in mortality in six Western European countries. Int J Epidemiol **32**, 830–7.
10) Marmot MG, Rose G, Shipley M, Hamilton PJS (1978) Employment grade and coronary heart disease in British civil servants. JECH **32**, 244–9.
11) Marmot MG, Smith GD, Patel C, Stansfeld S, North F, Head J, White I, Brunner E, Feeney A (1991) Health inequalities among British civil servants: the Whitehall II study. Lancet **337**, 1387–93.
12) Marmot MG, Bosma H, Hemingway H, Brunner E, Stansfeld S (1997) Contribution of job control and other risk factors to social variations in coronary heart disease incidence. Lancet **350**, 235–9.
13) NIPPON DATA80 Research Group (2003) Impact of elevated blood pressure on mortality from all causes, cardiovascular diseases, heart disease and stroke among Japanese: 14 year follow-up randomly selected population from Japanese (NIPPON Data80). J Hum Hypertens **17**, 851–7.
14) Okamura T, Kadowaki T, Hayakawa T, Kita Y, Okayama A, Ueshima H, NIPPON DATA80 Research Group (2003) What cause of mortality can we predict by cholesterol screening in the Japanese general population? J Intern Med **253**, 169–80.
15) Ueshima H, Choudhury SR, Okayama A, Hayakawa T, Kita Y, Kadowaki T, Okamura T, Minowa M, Iimura O, NIPPON DATA80 Research Group (2004) Cigarette smoking as a risk factor for stroke death in Japan. Stroke **35**, 1836–41.
16) Iso H, Imano H, Kitamura A, Sato S, Naito Y, Tanigawa T, Ohira T, Yamaguchi K, Iida M, Shimamoto T (2004) Type 2 diabetes and risk of non-embolic ischaemic stroke in Japanese men and women. Diabetologia **47**, 2137–44.
17) Fujino Y, Iso H, Tamakoshi A, Inaba Y, Koizumi A, Kubo T, Yoshimura T for the JACC Study Group (2005) A prospective cohort study of employment status and mortality from circulatory disorders among Japanese workers. J Occup Health **47**, 510–7.
18) Statistics Bureau, Director-general for policy planning & Statistical Research and Training Institute, Ministry of Internal Affairs and Communications. Japan Standard Occupational Classification. http://www.stat.go.jp/english/index/seido/shokgyou. Accessed January 28, 2009.
19) WHO MONICA project (1990) MONICA Manual, revised Ed., Cardiovascular Diseases Unit, WHO, Geneva.
20) Kahn HA, Sempos CT (1989) Statistical methods in Epidemiology. Oxford University Press, New York.
21) Kitamura A, Sato S, Kiyama M, Imano H, Iso H, Okada T, Ohira T, Tanigawa T, Yamagishi K, Nakamura M, Konishi M, Shimamoto T, Iida M, Komachi Y (2008) Trends in the incidence of coronary heart disease and stroke and their risk factors in Japan, 1964 to 2003. JACC **52**, 71–9.
22) Martikainen P, Ishizaki M, Marmot MG, Nakagawa H, Kagamimori S (2001) Socioeconomic differences in behavioural and biological risk factors: a comparison of a Japanese and an English cohort of employed men. Int J Epidemiol **30**, 833–8.
23) Ishizaki M, Yamada Y, Morikawa Y, Noborizaka Y, Ishida M, Miura K, Nakagawa H (1999) The relationship between waist-to-hip ratio and occupational status and life-style factors among middle-aged male and female Japanese workers. Occup Med **49**, 177–82.
24) Anzai Y, Ohkubo T, Nishino Y, Tsuji I, Hisamichi S (2000) Relationship between health practices and education level in the rural Japanese population. J Epidemiol **10**, 149–56.
25) Ministry of Health, Labour and Welfare, Japan. Industrial Safety and Health Act (Act No. 57 of 1972), Ministry of Health, Labour and Welfare, Tokyo.

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Regular Paper

Longitudinal Trends of Total White Blood Cell and Differential White Blood Cell Counts of Atomic Bomb Survivors

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White blood cell/Longitudinal trend/Radiation exposure/Dose-response.

In studying the late health effects of atomic-bomb (A-bomb) survivors, earlier findings were that white blood cell (WBC) count increased with radiation dose in cross-sectional studies. However, a persistent effect of radiation on WBC count and other risk factors has yet to be confirmed. The objectives of the present study were 1) to examine the longitudinal relationship between A-bomb radiation dose and WBC and differential WBC counts among A-bomb survivors and 2) to investigate the potential confounding risk factors (such as age at exposure and smoking status) as well as modification of the radiation dose-response. A total of 7,562 A-bomb survivors in Hiroshima and Nagasaki were included in this study from 1964–2004. A linear mixed model was applied using the repeated WBC measurements. During the study period, a secular downward trend of WBC count was observed. Radiation exposure was a significant risk factor for elevated WBC and differential WBC counts over time. A significant increase of WBC counts among survivors with high radiation dose (> 2 Gy) was detected in men exposed below the age of 20 and in women regardless of age at exposure. Effects on WBC of low dose radiation remain unclear, however. Cigarette smoking produced the most pronounced effect on WBC counts and its impact was much larger than that of radiation exposure.

INTRODUCTION

In epidemiological and clinical studies, white blood cell (WBC) count has been widely used as an indicator for inflammation. The previous studies on the late health effects of A-bomb survivors have shown that WBC count has a tendency to increase with radiation dose, based on cross-sectional data from 1958 to 1980.[1,2] Neriishi and Nakashima[3] also later reported observing a positive association between radiation dose and WBC count among A-bomb survivors in a special inflammatory test study conducted during 1988–92. All the aforementioned are cross-sectional studies and a persistent effect of radiation on WBC count has not been confirmed.

With mounting evidence of an association between elevated WBC count and hypertension,[4,5] cardiovascular disease,[6–10] or cancer mortality,[11–13] attention has focused on risk factors that lead to a chronic increase in WBC level. It is well documented that smoking causes an elevation in WBC count and that the level of WBC count gradually declines after smoking cessation.[14–19] Body mass index (BMI) or obesity was shown to be positively correlated with higher WBC levels.[20–22] The relationship between WBC count, an indicator for inflammation, and smoking or BMI was reasonable, since smoking and BMI are strongly related to inflammatory processes. However, limited data exist on longitudinal trends of WBC count in relation to those risk factors.

WBC counts measured biennially since 1958 in the Adult Health Study (AHS) provided an opportunity to examine the WBC trend in the Japanese cohort. The primary objective of this longitudinal analysis was to apply modern statistical methods accounting for mixed (fixed and random) effects to evaluate the influence of radiation exposure on WBC over time. In addition, potential confounding risk factors (such as smoking and BMI) and modification of radiation dose-response were investigated. The findings elucidate the overall trend of WBC due to aging and the association between WBC and radiation dose. Furthermore, they provide evidence as to whether chronic inflammation persists years after radiation exposure.

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MATERIALS AND METHODS

Study population and measurements

The Adult Health Study (AHS) is a program of biennial clinical health examinations started in 1958 by the Atomic Bomb Casualty Commission (ABCC) in Hiroshima and Nagasaki, and succeeded by the Radiation Effects Research Foundation (RERF) in 1975, to investigate the long-term health effects of A-bomb radiation in humans.[23] The AHS originally contained about 20,000 individuals, which is a subset of the 120,321 member Life Span Study (LSS) cohort. The AHS includes all of the higher-dose survivors and samples of controls and persons with lower doses. As part of each biennial physical examination, blood pressure, blood cell counts, blood chemistries, urinalysis, chest X-ray, electrocardiogram, and special tests to confirm suspected diagnoses are carried out. WBC counts in whole blood were manually counted until 1968 in Hiroshima and until 1977 in Nagasaki, after which automated machines were used. Neutrophils, lymphocytes, and monocytes (hereafter named "differential WBC counts") were the primary components of WBC. The differential WBC counts were recorded as percentages of total WBC. WBC counts by each hematology autoanalyzer were compared by the Melangeur method and good correlation was confirmed. Body mass index (BMI) was calculated as body weight divided by the square of the standing height (kg/m^2). Cigarette smoking information was obtained from an epidemiologic questionnaire in 1965. Clinical diagnoses were classified according to the International Classification of Diseases (ICD) system in effect at the time (7^{th}–10^{th} revisions).[24]

At examination cycle 4 (1964–66), 12,140 participants underwent the health examination. After excluding individuals who were not in the cities at the time of the bombings (n = 2,911), without estimated Dosimetry System 2002 (DS02)[25] radiation dose (n = 1,308), missing hematological or smoking information at baseline (n = 324), or age at examination greater or equal to 80 (n = 35), there were 7,562 participants (2,741 males and 4,821 females) available for analysis. Each individual was examined at least once and the repeated measurements were collected until 2004. Missing data due to absence from health examinations was rare. Thus, the impact of missing data on our analysis should be minor. We assumed that missing data is missing completely at random and not related to either WBC or the risk factors considered.

Statistical methods

Longitudinal trends in WBC and differential WBC counts are assumed to vary with age. To account for correlations among observations within subjects collected over time, the linear mixed effects model described by Laird and Ware[26,27] was used. The model for the i^{th} individual is

$$y_i = X_i\beta + Z_i b_i + \varepsilon_i$$

with $$b_i \sim N_q(0, \Psi)$$

$$\varepsilon_i \sim N_{ni}(0, \sigma^2\Lambda_i)$$

where y_i is the n_i x 1 response vector for observations in the i^{th} individual; X_i is the n_i x p model matrix for the p fixed effects (including gender, city, age, smoking status, BMI, an indicator for inflammation-related diseases[a], radiation dose, and birth cohort) for observations in individual i; β is the p x 1 vector of fixed-effect coefficients; Z_i is the n_i x q model matrix for the q random effects for individual i that allows the intercepts and slopes for each individual to differ; b_i is the q x 1 vector of random-effect coefficients for individual i following a normal distribution; ε_i is the n_i x 1 vector of errors for observations in individual i following a normal distribution; Ψ is the q x q variance-covariance matrix for the random effects; and $\sigma^2\Lambda_i$ is the n_i x n_i covariance matrix for the errors in individual i. The fixed effect estimates, β, give the population average intercept and slope(s); these parameters model the systematic variation within an individual due to age, gender, etc. The random effects, b_i, account for heterogeneity among the individuals in terms of how their changes in WBC count over time differ from the population average.

We first examined various models for longitudinal patterns of WBC counts for the subjects who were exposed to ≤ 5 mGy radiation or not exposed, and selected the best background model. Three types of age patterns—linear, quadratic, and cubic functions of age—were estimated for each gender separately using the maximum likelihood method. Akaike's Information Criterion (AIC) was used to choose the best fitting pattern among competing models. The AIC is defined as $-2L_m + 2k$, where L_m is the maximized log likelihood and k is the number of parameters. It takes into account both the statistical goodness of fit and the model complexity (number of parameters estimated) by imposing a penalty for increasing the number of parameters. That penalty accounts for the fact that adding additional parameters will always increase goodness of fit, but with diminishing returns in terms of the predictive value of a model; the model with smallest AIC is the model of choice. Once the age pattern was selected, various covariance structures were tested. Selected variance-covariance structures, such as unstructured, compound-symmetry, and first-order autoregressive matrices were examined. All the analyses were conducted using SAS software version 9.1 (SAS Institute, Inc., Cary, NC, USA).

Backward elimination was then used to trim the number

[a] The indicator for inflammation-related disease was defined by the existence of the following conditions: cancer, pneumonia, tonsillitis, and bronchitis.

Table 1. Baseline characteristics by gender, radiation exposure category, and age at exposure.

	Males				Females			
	≤ 5 mGy	5 mGy–1Gy	1 Gy-2 Gy	> 2 Gy	≤ 5 mGy	5 mGy–1Gy	1 Gy-2 Gy	> 2 Gy
Age at exposure < 20								
Number	441	400	152	134	662	589	223	149
Age at examination	32.2 ± 5.0	32.3 ± 5.2	32.8 ± 5.3	32.2 ± 5.9	32.9 ± 5.2	32.6 ± 5.5	33.4 ± 5.2	31.6 ± 5.6
In Hiroshima	60%	70%	58%	77%	59%	72%	43%	72%
Never smoker	11%	22%	14%	15%	91%	87%	96%	84%
Ever smoker	89%	78%	86%	85%	9%	13%	4%	16%
BMI (kg/m^2)	20.9 ± 2.5	20.9 ± 2.6	20.6 ± 2.4	20.2 ± 2.4	21.4 ± 3.01	21.5 ± 3.1	21.6 ± 3.1	21.3 ± 2.7
WBC (1,000/mm^3)	6.82 ± 1.76	6.99 ± 1.92	6.96 ± 1.87	7.21 ± 1.89	6.18 ± 1.76	6.33 ± 1.69	6.16 ± 1.62	6.35 ± 1.77
Neutrophil (1,000/mm^3)	3.84 ± 1.35	3.92 ± 1.49	3.94 ± 1.43	4.08 ± 1.32	3.62 ± 1.41	3.68 ± 1.36	3.57 ± 1.30	3.73 ± 1.45
Lymphocyte (1,000/mm^3)	2.23 ± 0.72	2.29 ± 0.75	2.23 ± 0.77	2.30 ± 0.78	1.90 ± 0.61	1.96 ± 0.61	1.94 ± 0.64	1.93 ± 0.70
Monocyte (1,000/mm^3)	0.50 ± 0.22	0.51 ± 0.23	0.52 ± 0.22	0.54 ± 0.25	0.46 ± 0.21	0.46 ± 0.21	0.44 ± 0.19	0.47 ± 0.23
Age at exposure ≥ 20								
Number	670	607	205	132	1,169	1,440	355	234
Age at examination	57.7 ± 9.0	58.2 ± 9.5	58.1 ± 8.8	58.5 ± 9.6	54.6 ± 10.1	55.0 ± 9.8	54.0 ± 10.0	54.1 ± 9.7
In Hiroshima	66%	82%	65%	73%	77%	84%	66%	79%
Never smoker	15%	15%	18%	13%	84%	78%	77%	76%
Ever smoker	85%	85%	82%	87%	16%	22%	23%	24%
BMI (kg/m^2)	21.0 ± 2.8	20.9 ± 2.8	20.7 ± 3.2	21.0 ± 2.9	22.0 ± 3.4	22.0 ± 3.4	22.0 ± 3.5	22.4 ± 3.6
WBC (1,000/mm^3)	6.70 ± 1.85	6.61 ± 1.87	6.48 ± 1.82	6.47 ± 1.78	5.97 ± 1.69	5.93 ± 1.63	5.97 ± 1.55	6.20 ± 1.63
Neutrophil (1,000/mm^3)	3.82 ± 1.34	3.72 ± 1.35	3.68 ± 1.37	3.60 ± 1.32	3.38 ± 1.29	3.35 ± 1.25	3.39 ± 1.19	3.48 ± 1.29
Lymphocyte (1,000/mm^3)	2.13 ± 0.81	2.14 ± 0.82	2.05 ± 0.79	2.15 ± 0.74	1.96 ± 0.66	1.96 ± 0.69	1.96 ± 0.65	2.08 ± 0.66
Monocyte (1,000/mm^3)	0.50 ± 0.23	0.51 ± 0.23	0.51 ± 0.28	0.51 ± 0.23	0.42 ± 0.19	0.42 ± 0.20	0.42 ± 0.20	0.43 ± 0.18

of covariates beginning with the most insignificant factor, using the likelihood ratio (LR) test with one degree of freedom ($\chi^2 = 3.84$, $df = 1$). The full model first included the covariates: age, age squared, age cubed, city (Hiroshima or Nagasaki), ever smoker (no or yes), continuous BMI (kg/m^2), and an indicator of inflammation-related diseases (no or yes). Age, BMI, and the indicator of inflammation-related diseases were time-dependent variables and updated at the times when WBC measurements were collected at each health examination cycle. For the full model including exposed subjects, various dose response models were investigated. Linear, pure quadratic, linear quadratic, threshold, and categorical (5 mGy - ≤ 0.5 Gy, 0.5 Gy - ≤ 1 Gy, 1 Gy - ≤ 1.5 Gy, 1.5 Gy - ≤ 2 Gy, 2 Gy - ≤ 2.5 Gy, and > 2 Gy, with ≤ 5 mGy as reference) models were tested and the best fitted model was selected based on AIC. In addition, an indicator of dichotomized age at exposure (less than 20) was included to examine whether there is a significant difference in the effect of radiation exposure on WBC among survivors who were exposed at a young age. The effect modification of dose-response was also examined.

RESULTS

Population characteristics and average WBC counts at baseline are shown in Table 1. About 40% of the subjects

Table 2. Values of Akaike's Information Criterion for age and covariance structure selection

Parameter estimates	Compound symmetry	Unstructured	1st order autoregressive
Males:			
Age	42,944	41,981	43,772
Age + age^2	42,897	41,962	43,756
Age + age^2 + age^3	42,873	41,945	43,747
Females:			
Age	71,908	70,440	74,068
Age + age^2	71,821	70,401	74,053
Age + age^2 + age^3	71,823	70,404	74,055

constitute the background group. Females accounted for about 64% of the study subjects and more than two-thirds resided in Hiroshima at the time of bombing. Among male participants, more than 84% were ever smokers. On the other hand, the majority of females never smoked. Also, male participants appeared to have lower BMI than females

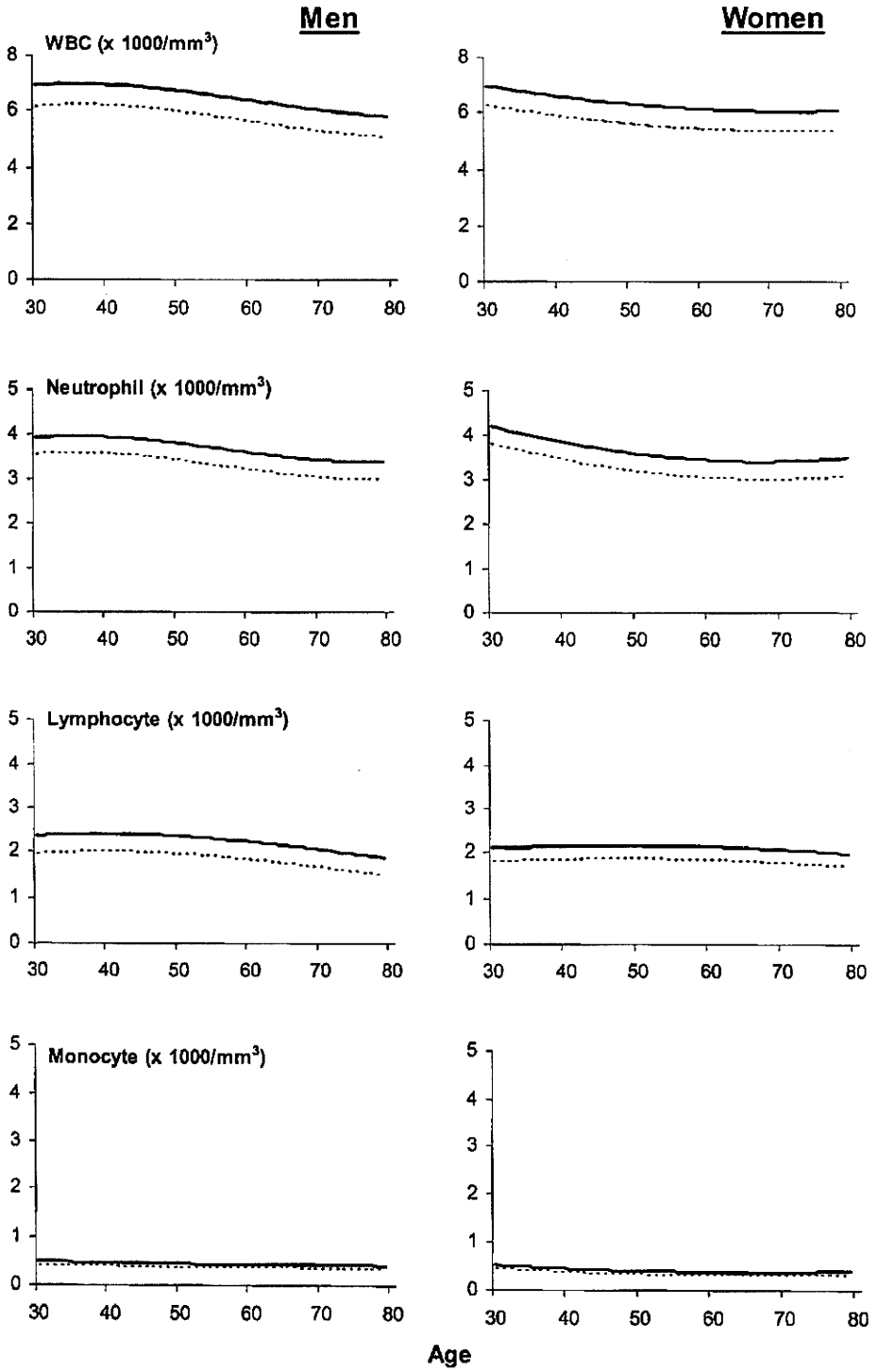

Fig. 1. Longitudinal trends in WBC and differential WBC counts by smoking status for males and females who had radiation exposure less than or equal to 5 mGy or not exposed. The longitudinal trends of WBC and differential WBC counts for ever smokers were plotted in a solid line and that for never smokers was in a dotted line. The models were estimated by gender and adjusted for city, body mass index, and an indicator for infectious disease.